Probe compounds to assess the photochemical activity

of dissolved organic matter

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Abstract

The photochemical properties of dissolved organic matter (DOM) have been of interest to scientists and engineers since the 1970s. Upon light absorption, chromophoric DOM (CDOM) can sensitize the formation of different short-lived reactive intermediates (RIs), including hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$) and superoxide radical anion ($O_2^{-\bullet}$). In addition, a fraction of the excited singlet states in CDOM decays into excited triplet states ($^3CDOM^*$), which are also important photochemical transients in environmental systems. These RIs have a significant impact on different processes in sunlit waters, including degradation of organic contaminants and the inactivation of pathogens. Due to their transient nature and low steady-state concentrations, the use of common analytical techniques for the direct measurement of these species is impractical. Therefore specific probe compounds (PCs) are used. PCs include furfuryl alcohol for $^1O_2$, and terephthalic acid for $^{\bullet}OH$. In this publication, we present a critical review of the use of PCs for the assessment of the formation of photochemically generated RIs. We first introduce the concept of a PC, including the kinetic treatment and necessary assumptions needed to conduct a specific measurement. Afterwards, we present short overviews of the most studied RIs and review relevant issues regarding the use of specific PCs for their measurement. We finalize by offering recommendations regarding the use of PCs in environmental photochemistry.

36   **TOC/Abstract Art**

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Abbreviations

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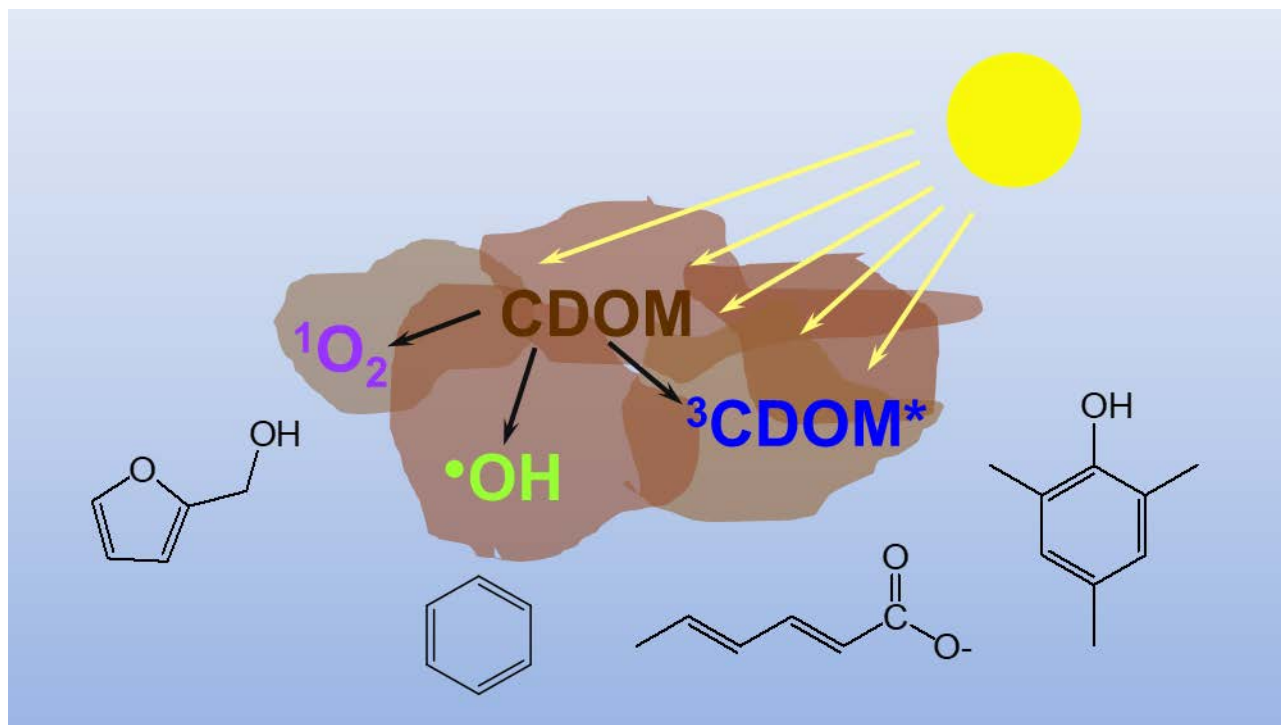
40    $a_{CDOM}(\lambda)$ Decadic absorption coefficient of the CDOM solution at the wavelength λ

41    $CO_3^{\bullet-}$ Carbonate radical

42   CDOM   Chromophoric DOM

43    $^3CDOM^*$ Excited triplet states of CDOM

44   DOM   Dissolved organic matter

45    $E_p^{0\,\prime}(\lambda)$ Spectral photon fluence rate

46   E_{CDOM}^T Energy associated with an excited triplet state of CDOM

47   FFA   Furfuryl alcohol

48   H_2O_2 Hydrogen peroxide

49    $[I]_{ss}$ Steady state concentration of a specific compound I.

50    $[I]$ Instantaneous concentration of a specific compound I

51   IC   Internal conversion

52   ISC   Intersystem crossing

53   k_{RI}^d Pseudo-first-order deactivation rate constant for a particular RI

54    $k_{RI}^{d,0}$ First-order rate constant for the unimolecular deactivation of a particular RI

55   k_{PC}^{obs} Pseudo-first-order transformation rate constant of a PC

56    $k_{RI,PC}^q$ Second-order rate constant for the quenching of an RI by a PC

57   k_{RI,Q_i}^q Second-order rate constants for the quenching of an RI by a Q_i

58
59    $k_{RI,PC}^t$ Second-order rate constant for the transformation of a PC after reaction with an RI

60   MCLA   2-methyl-6-(4-mehoxyhenyl)-3,7-dihydroimidazo[1,2-a]pyrazine-3(7H)-one

61    $^{\bullet}OH$ Hydroxyl radical

62    $^1O_2$ Singlet oxygen

63	$O_2^{\bullet-}$	Superoxide radical anion
64	PC	Probe compound
65	Q	Quencher (either present in solution (Q_i) or controlled as part of experiment
66		(Q_x))
67	r_{CDOM}^{abs}	Rate of light absorption by CDOM
68	r_I^f	Formation rate of a specific compound, I
69	RI	Reactive intermediate
70	ROS	Reactive oxygen species
71	TC	Target compound
72	TMP	2,4,6-trimethylphenol
73	TP	Transformation product
74	VR	Vibrational relaxation
75	$\phi_{CDOM}^{e^-}$	Photoionization quantum yield of CDOM
76	ϕ_{CDOM}^{ISC}	Intersystem crossing quantum yield of CDOM
77	$\phi_{CDOM}^{^1O_2}$	Singlet oxygen quantum yield of CDOM
78	$\gamma_{^1O_2}$	Yield for the production of $^1O_2$ from $^3CDOM^*$, defined as number of $^1O_2$
79		molecules formed per number of quenched $^3CDOM^*$ molecules
80	$\gamma_{RI,TP}$	Yield of the formation of a TP upon the reaction between a PC and an RI

81

Introduction

82

83   The photophysical and photochemical properties of dissolved organic matter (DOM)

84   are of interest due to their control on many important processes in environmental

85   (photo)chemistry, including contaminant fate and transport, carbon cycling and optical

86   properties of natural systems. Since light absorption is a prerequisite for photoprocesses to

87   take place, in this paper we will extensively use the term of chromophoric DOM (CDOM) to

88   indicate the light-absorbing components of DOM. The interaction of CDOM with light has

89   been of interest for many decades, mostly due to the interest in the use of optical properties

90   (i.e., absorbance and fluorescence) as a surrogate for the concentration and reactivity of the

91   DOM present in a particular sample. For example, early studies characterizing DOM focused

92   on its color and electronic absorption spectra. [1,2] Recently, there has been a renewed interest

93   in the study of these properties, as it is discussed below.

94   The formation and fate of CDOM excited states (photophysics) is a very complex

95   process, given its complexity to the varied and mostly unknown chemical composition of

96   CDOM. It is well known that CDOM absorbs light and its electronic absorption spectrum is

97   characterized as an exponential decay towards the longer wavelengths. Upon light

98   absorption, chromophores (defined as sub-units where light is being absorbed) are taken to

99   excited singlet states (S_1, S_2,...Sn), in time frames in the order of femtoseconds (see Figure 1).

100   [3] Although for any given CDOM sample it is impossible to ascertain the specific

101   chromophores, it is known that well-characterized groups are present, including aromatic

102   ketones and aldehydes, quinones, and phenolic compounds. [4-6] As it is common with organic

103   molecules, a fraction of the energy absorbed is quickly dissipated to the medium through

104   vibrational relaxation (VR). After this initial relaxation to the first excited singlet state (with

105   time frames of 10^{-12} seconds), there are other pathways that result in the deactivation of the

106   CDOM. First, it could fluoresce, where a photon is emitted from the lowest singlet state.

107   Fluorescence is indeed a very popular technique to characterize CDOM, [7-12] although only a

108   small percentage of the chromophores in the mixture fluoresce at a significant extent. The

109   quantum yields for this process, which have been reported to be excitation wavelength-

110   dependent, are in the order of 0.4–3% for DOM isolates and wastewater-derived organic

111   matter. [4, 13-15] Consistent with these relatively low quantum yields, fluorescence lifetimes are

112   very short and decrease with increasing emission wavelength, demonstrating that the excited

113   singlet states rapidly deactivate and that the emission is complex.[13] CDOM could also be

114   deactivated from this excited singlet state, and there is interest in understanding the non-

115   radiative deactivation pathways. They comprise internal conversion (IC) to the ground-state

116   and intersystem crossing (ISC) to an excited triplet state. As discussed extensively below,

117   excited triplet states are important species in the photochemical reactions involving CDOM.

118   The aqueous environmental photochemistry of CDOM has received significant

119   attention over the past decades. CDOM can sensitize the degradation of organic contaminants

120   through the formation of a suite of short-lived (lifetimes in the order of seconds or shorter)

121   reactive intermediates (RIs). [16] (It is also worth mentioning that DOM can also inhibit the

122   degradation of organic contaminants through reaction with certain RIs, although this process

123   is not considered here.[17, 18]) The RIs include the reactive oxygen species (ROS) hydroxyl

124   radical ($^{\bullet}OH$), singlet oxygen ($^{1}O_2$) and superoxide radical anion ($O_2^{\bullet-}$)[19], and also excited

125   triplet states of CDOM ($^{3}CDOM^{*}$). The RIs are involved in many important processes, and

126   are therefore of interest to environmental chemists and engineers. In environmental systems,

127   the steady-state concentrations of these RIs are below a level that would make direct

128   quantification feasible. For example, the steady-state concentrations of $^{1}O_2$ and $^{\bullet}OH$ in sunlit

129   waters are in the order of 10^{-12} to 10^{-16} M, respectively. Therefore, a specific probe compound

130   (PC) is used to measure their concentrations.

131   *Probe Compounds in Environmental Photochemistry*

132   For this review, we will define PCs as neutral or charged molecules and stable

133   radicals that can be employed to detect the presence (or identity) of or to quantify short-lived

134   RIs. Quantification of the RIs is largely inaccessible to observation by classical analytical

135   tools such as chromatography, spectroscopic and colorimetric methods, and electrochemical

136   sensors. Typical PCs used to detect RIs in environmental photochemistry undergo a chemical

137   reaction with such RIs, and their depletion or the formation of a specific product are used to

138   confirm the presence and quantify formation rates or steady-state concentrations of individual

139   RIs. PCs also include scavengers and quenchers of RIs. Although these compounds are used

140   in an indirect way, their purpose is to modify the concentration of a specific RI, thus

141   changing the rates of the reactions that are used to detect the RI. Ideally, PCs should be

142   absolutely selective, i.e. give a positive response only in the presence of an individual RI.

143   However, this condition cannot be fulfilled if the RI of interest is concomitantly present with

144   an unselective RI, such as $^{\bullet}$OH. Moreover, in environmental photochemistry, PCs have to be

145   resistant to direct phototransformation and be soluble in water at convenient levels for

146   quantification.

147   *Scope of the Review*

148   Several review articles are available that cover the topic of the photochemistry of

149   surface waters, with particular emphasis on the transformation of dissolved contaminants.

150   Starting from a general overview,[20] these papers were either focused on broad contaminant

151   classes, dealing with the photochemical transformation of pesticides[21, 22] and

152   pharmaceuticals,[23] or aimed at the characterization of photoreactivity in terms of RIs,[24-27] or

153   explicitly treated the photochemical role of CDOM.[28, 29] Absent from these recent

154   publications is a detailed discussion of the use of PCs in the study of RIs and their role in

155   environmental photochemistry, including the kinetic treatment and necessary assumptions

156 that are made in order to derive useful information from their use, as presented for instance in

157 a book chapter from the 1990's.[19] Therefore, the goal of this paper is to review the use of PCs

158 to characterize the formation of RIs derived from photoirradiation of CDOM, under

159 conditions relevant to environmental photochemists, to pinpoint pitfalls and suggest possible

160 solutions for improvement in this area. This review complements the recent article published

161 by Burns *et al.*, [30] which offers a more detailed summary of the universe of methods available

162 to analytical chemists to measure different RIs. This paper focuses on short-lived RI,

163 therefore the quantification of hydrogen peroxide (H_2O_2) is not discussed. We refer the reader

164 to the aforementioned review by Burns *et al.*[30] for a discussion regarding the quantification of

165 this species.

166   After a section on the kinetics and fundamental equations governing the application of

167 PCs, the literature on CDOM-derived RI such as $^3CDOM^*$, $^1O_2$, $^{\bullet}OH$ and further reactive

168 species is reviewed in separate sections. The final section is devoted to a critical discussion of

169 the use of PCs in the simultaneous presence of multiple RIs, as they occur upon

170 photoirradiation of natural waters, and to developing methods that ensure an improved

171 assessment of formation rates and concentrations of RIs in such environmental scenarios.

172 **Kinetic Methods for the Use of Probe Compounds**

173   *Formation Rates and Steady-State Concentrations of RIs*

174   As anticipated in the Introduction, the detection and quantification of a given RI in an

175 environmental system requires the use of a specific PC. In general terms, two main

176 approaches can be taken towards the quantification of an RI using a PC: Either to monitor the

177 direct degradation of the PC or to measure the formation of a by-product formed by the

178 (selective) reaction of an RI. Examples of PCs whose depletion is used to measure a specific

179 RI are furfuryl alcohol (FFA) for $^1O_2$,[31] and 2,4,6-trimethylphenol (TMP) for $^3CDOM^*$.[32] In

180   the cases for which the presence of an RI only causes a minor change in PC concentration,

181   the measurement of product formation may be an adequate alternative that increases the

182   sensitivity of the detection method. A typical example is given by terephthalic acid (1,4-

183   benzenedicarboxylic acid), used as a PC for the $^\bullet$OH: The formed fluorescent product, 2-

184   hydroxyterephthalic acid, is utilized to quantify $^\bullet$OH.[33]

185   Scavengers and quenchers (S/Q) are often used as tools to test the involvement of a

186   specific RI in the transformation of a target compound (TC), defined here as a chemical of

187   interest regarding its photochemical fate in environmental systems. To be able to reach the

188   goal of their application, S/Q are employed at high concentrations relative to that of the TC,

189   and this bears a potential risk for side-reactions, which may arise from the formation of

190   secondary RIs or reaction products that contribute to or additionally hinder the transformation

191   of the TC. The addition of other organic compounds (either added purposely as quenchers,

192   scavengers or inadvertently as a co-solvent) could result in the reduction in concentration of

193   any RI, therefore confounding the observed results.

194   Within the scope of this review two quantities regarding a given RI are fundamental,

195   namely its formation rate, r_{RI}^f, and its steady-state concentration, $[\text{RI}]_{ss}$. They are related by

196   equation 1:

197 $$[\text{RI}]_{ss} = \frac{r_{RI}^f}{k_{RI}^d} \qquad\qquad (1)$$

198   where k_{RI}^d is the pseudo-first-order deactivation rate constant of the RI and includes the

199   contributions of all possible reactions involving the depletion of the RI. Thereby, the

200   fundamental kinetic assumption is made that the reaction channels contributing to the

201   depletion of the RI are either of true first-order, as for the unimolecular deactivation in the

202   case of excited electronic states, or true second-order, first order in [RI] and first order in the

203   concentration of a quencher, Q_i, which is present in large excess with respect to the RI,

204   leading to pseudo-first-order kinetics. Note that when dealing with radicals the concept of

205   *scavenger* is often used instead of *quencher*, but the kinetic treatment is the same. The

206   pseudo-first-order deactivation rate constant may be expressed as:

207    $$k_{RI}^{d} = k_{RI}^{d,0} + \sum_{i} k_{RI,Q_i}^{q} [Q_i] + k_{RI,PC}^{q} [PC] + k_{RI,Q_X}^{q} [Q_X] \qquad (2)$$

208   where $k_{RI}^{d,0}$ indicates the first-order rate constant for the unimolecular deactivation of the RI

209   (including contributions from the solvent) and k_{RI,Q_i}^{q} is the second-order rate constants for

210   the reaction of the RI with the quencher Q_i. The summation term includes all scavengers

211   naturally present in the environmental system under study. In equation 2, two additional

212   terms have been included to account for the reaction of the RI with a PC and a quencher Q_X

213   that can be added on purpose and are not originally present in the environmental system.

214   Alternatively, one can also tune the concentration of a quencher, such as molecular oxygen

215   (which we will simply call *oxygen* from now on) in the case of $^3CDOM^*$ as the RI, that is

216   already present in the environmental system. In such a case, this quencher should be designed

217   as Q_X and excluded from the summation term relevant to Q_i.

218   We now focus on the kinetics of a PC. Its reaction with an RI, leading to the

219   transformation (consumption) of the PC, is described by the second-order rate constant

220    $k_{RI,PC}^{t}$. Note that $k_{RI,PC}^{t}$ is not identical with $k_{RI,PC}^{q}$. A difference is expected in particular

221   when the RI is in an excited electronic state (as for $^3CDOM^*$ or $^1O_2$), because not every

222   quenching event leads to the transformation of the PC. The transformation rate of the PC is

223   given by:

224    $$r_{PC}^{t} = \frac{d[PC]}{dt} = -k_{RI,PC}^{t} [RI]_{ss} [PC] \qquad (3)$$

225   In the low-concentration limit of the PC, in which its contribution to the quenching of

226   the RI can be neglected (i.e., for $k_{RI,PC}^{q} [PC] << k_{RI}^{d,0} + \sum_{i} k_{RI,Q_i}^{q} [Q_i] + k_{RI,Q_X}^{q} [Q_X]$), $[RI]_{ss}$ is

11

227   independent of [PC] and equation 3 is linear with respect to [PC]. Assuming constant $[RI]_{ss}$

228   the following integrated rate law is obtained:

229    $$\ln\left(\frac{[PC]}{[PC]_0}\right) = -k_{RI,PC}^{t}[RI]_{ss} \times t = -k_{PC}^{obs} \times t \quad (4)$$

230   Under these conditions, one can determine the steady-state concentration of the RI

231   from the pseudo-first-order transformation rate constant of the PC, k_{PC}^{obs}, provided that $k_{RI,PC}^{t}$

232   is known:

233    $$[RI]_{ss} = \frac{k_{PC}^{obs}}{k_{RI,PC}^{t}} \quad (5)$$

234   Alternatively, to determine the formation rate of the RI, one has to apply a PC

235   concentration that is sufficiently high to dominate the deactivation of RI, i.e

236    $k_{RI,PC}^{q}[PC] \gg k_{RI}^{d,0} + \sum_i k_{RI,Q_i}^{q}[Q_i] + k_{RI,Q_X}^{q}[Q_X]$, and $k_{RI}^{d} \cong k_{RI,PC}^{q}[PC]$. Making the latter

237   substitution in equation 1 one obtains:

238    $$[RI]_{ss} = \frac{r_{RI}^{f}}{k_{RI,PC}^{q}[PC]} \quad (6)$$

239   Substituting equation 6 into equation 3 yields:

240    $$r_{PC}^{t} = -k_{RI,PC}^{t} \frac{r_{RI}^{f}}{k_{RI,PC}^{q}[PC]}[PC] = -k_{RI,PC}^{t} \frac{r_{RI}^{f}}{k_{RI,PC}^{q}} \quad (7)$$

241   Solving for r_{RI}^{f} leads to equation 8.

242    $$r_{RI}^{f} = -\frac{k_{RI,PC}^{q}}{k_{RI,PC}^{t}} r_{PC}^{t} \quad (8)$$

243   In the case of scavenging of a radical as the RI, such as $^{\bullet}OH$, one can often assume

244    $k_{RI,PC}^{q} \cong k_{RI,PC}^{t}$, meaning that $r_{RI}^{f} \cong -r_{PC}^{t}$. The analysis of the formation rate of a

245   transformation product (TP) of a PC, r_{TP}^{f}, is often more convenient and accurate than the one

246   of the consumption rate of the PC. If such a TP is utilized to quantify r_{RI}^{f}, the yield of its

247   formation from the reaction of the PC with the RI, $\gamma_{RI,TP}$, must be known. The following

248   equation is then obtained:

249 $$r_{RI}^{f} = \frac{k_{RI,PC}^{q}}{k_{RI,PC}^{t}} \times \frac{r_{TP}^{f}}{\gamma_{RI,TP}} \tag{9}$$

250   *Scavengers, Quenchers and Methods to Tune the Concentration of RIs*

251   The concepts of scavenger and quencher have been illustrated above, and in the

252 following we will employ the term quencher as the representative of both types of

253 compounds. We restrict the kinetic treatment to the case of low [PC] and pseudo-first-order

254 kinetics holding for the transformation of a PC. The ratio of the measured rate constants in

255 the presence and absence of a quencher Q_X added on purpose (i.e., $\frac{k_{PC}^{obs}([Q_X])}{k_{PC}^{obs}([Q_X]=0)}$) are

256 compared to the expected ratios of the corresponding $[RI]_{ss}$, which can be expressed as

257 follows:

258 $$\frac{[RI]_{ss}([Q_X])}{[RI]_{ss}([Q_X]=0)} = \frac{k_{RI}^{d,0} + \sum_i k_{RI,Q_i}^{q}[Q_i] + k_{RI,PC}^{q}[PC] + k_{RI,Q_X}^{q}[Q_X]}{k_{RI}^{d,0} + \sum_i k_{RI,Q_i}^{q}[Q_i] + k_{RI,PC}^{q}[PC]} \tag{10}$$

259   Identity between the measured and expected values of the ratios is taken as evidence

260 that the used PC reflects uniquely the reactivity with the RI. In the case for which Q_X is

261 already present in the environmental system (exemplified by O_2 as a quencher of $^3CDOM^*$)

262 and its concentration is experimentally varied, a similar expression as equation 10 can be

263 used, adding a term for the environmental concentration of Q_X in the denominator. Often

264 quenchers are used in very large excess to almost completely suppress the reaction of an RI

265 with a TC, in which case the aforementioned ratios will approach zero.

266   A an example, a kinetic test frequently employed to demonstrate the involvement of

267 $^1O_2$ in the transformation of a TC is to use deuterium oxide (heavy water, D_2O) instead of

268 water as a solvent. In such a case, $k_{^1O_2}^{d}$ is decreased by decreasing $k_{^1O_2}^{d,0}$, which is 13 times

269 lower in D_2O than in H_2O, and $\left[{}^1O_2\right]_{ss}$ is generally increased by a corresponding factor (all

270 the terms in the right-hand term of equation 2 but $k_{{}^1O_2}^{d,0}$ are usually negligible if no quencher

271 Q_X is added).

272   Below, we will now address the different methods used to assess the formation of RI

273 in environmental systems. For each species, we provide background knowledge on its

274 formation and decay and an overview of its quantification methods, and identify potential

275 problems with the current methods. Although not an exhaustive compendium of all of the

276 chemistry associated with these RI, these sections are designed to guide interested

277 researchers in the development of methods to quantify these RI. Lastly, in this publication we

278 constrain ourselves to the discussion of methods associated with short-lived RI, as stated

279 above.

280 CDOM Triplet States (${}^3CDOM^*$)

281   The formation of excited triplet states upon photoexcitation of CDOM is accepted

282 based on the fact that CDOM consists of a complex mixture of organic molecules that are

283 able to absorb photons of terrestrial sunlight and thus generate excited states according to the

284 Jablonski representation (Figure 1). While there is no doubt that ${}^3CDOM^*$ can be formed, the

285 assessment of their formation rates and steady-state concentrations is a complex task, but

286 decisive to establish the relevance of ${}^3CDOM^*$ for the aquatic environment.

287   In the Jablonski formalism (see Figure 1), the formation rate of ${}^3CDOM^*$, $r^f_{{}^3CDOM^*}$,

288 may be obtained as the product of the light absorption rate of CDOM, r^{abs}_{CDOM}, and its

289 intersystem crossing quantum yield, ϕ^{ISC}_{CDOM} (mol einstein^{-1}):

$$r^f_{{}^3CDOM^*} = r^{abs}_{CDOM} \times \phi^{ISC}_{CDOM} \tag{11}$$

291 In the case of optically thin solutions r^{abs}_{CDOM} can be determined using equation 12,

292
$$r_{CDOM}^{abs} = 2.303 \times \int_{\lambda} a_{CDOM}(\lambda) \times E_p^{0}{}'(\lambda) \times d\lambda \tag{12}$$

293 where $a_{CDOM}(\lambda)$ (m^{-1}) is the *decadic* absorption coefficient of the CDOM solution at the

294 wavelength λ (nm), and $E_p^{0}{}'(\lambda)$ is the spectral photon fluence rate (einstein m^{-2} s^{-1} nm^{-1}). For

295 solutions of higher optical density the determination of r_{CDOM}^{abs} becomes more complex, which

296 requires the application of methods summarized in a recent review article.[34] Note that for

297 simplicity, since the unit *einstein* has the meaning of *moles of photons*, from now quantum

298 yield values will be given as dimensionless.

299   In equation 11 photoionization of CDOM (see Figure 1) was neglected, since its

300 quantum yield, $\phi_{CDOM}^{e^-}$, was estimated to lie below $\approx 2\times10^{-4}$.[29, 35] CDOM is considered to be

301 the main light-absorbing component in natural waters and rates of light absorption can be

302 easily evaluated from experimentally determined electronic absorption spectra of any specific

303 DOM,[36] or, in the absence of such data, from a general model equation.[37] The other key

304 parameter, ϕ_{CDOM}^{ISC}, cannot be easily determined especially because of the difficulty in the

305 quantification of ^{3}CDOM*, which consists of a manifold collection of chromophores in their

306 excited triplet state. An indirect approach involving the use of time-resolved photoacoustic

307 spectroscopy yielded ϕ_{CDOM}^{ISC} values in the range of 0.28–0.82 for two fulvic acids in aqueous

308 solution,[38] with the high values obtained at pH 2.0 and the low values at pH 9.5, and

309 intermediate values ($\approx$0.5–0.6) at circumneutral pH. These estimates are a factor of >3 higher

310 than those obtained by steady-state irradiation techniques, which had values between 0.01

311 and 0.1.[39-41] In the absence of accurate measurements, it is convenient to take the readily

312 available and measurable ^{1}O$_2$ formation quantum yields ($\phi_{CDOM}^{{}^1O_2}$)[42-46] (in the range of

313 1.4–4.6% for DOM isolates and wastewaters)[40, 46] as minimum estimates for ϕ_{CDOM}^{ISC}, given

314 that in the presence of oxygen not all ^{3}CDOM* deactivation events will yield ^{1}O$_2$ (see below).

315   Based on the diverse multitude of chromophores expected to be included in DOM, the

316   electronic energy of ^{3}CDOM* (relative to the ground-state of CDOM), E_{CDOM}^T, is likely

317   distributed over a broad range. Two different approaches have been employed to estimate it.

318   From magnetic circular dichroism spectra of two fulvic acids, Bruccoleri and co-workers[38]

319   obtained average E_{CDOM}^T estimates of 1.74 eV (167 kJ mol^{-1}) and 1.92 eV (185 kJ mol^{-1}),

320   respectively. Zepp and co-workers[47] compared photosensitized transformation rates of

321   1,3-pentadiene and 2,5-dimethylfuran and concluded that about half of the DOM triplets

322   capable of producing ^{1}O$_2$ (E_{CDOM}^T >0.98 eV (94 kJ mol^{-1})) had an E_{CDOM}^T greater than 2.6 eV

323   (250 kJ mol^{-1}). Phosphorescence spectra of humic and fulvic acids dissolved in a polymer

324   film and measured using an excitation wavelength of ≈313 nm indicated an upper limit to

325   E_{CDOM}^T of ≈3.1 eV (≈300 kJ mol^{-1}).[48] For comparison, Figure 2 illustrates the distribution of

326   triplet energies compiled using a set of 755 values measured for organic chromophores in

327   polar solvents.[49] The average value for this set of chemicals (254.5 kJ mol^{-1}) corresponds

328   well to the aforementioned estimated median value for ^{3}CDOM*. However, values above

329   ≈300 kJ mol^{-1} were not found for ^{3}CDOM*, which is not surprising because CDOM cannot

330   cover the whole range of chemical structures that can be synthesized anthropogenically.

331   The lifetime of ^{3}CDOM*, or its inverse, $k_{^3CDOM*}^d$ (see equation 2), is a further decisive

332   factor for the impact of ^{3}CDOM* on the transformation of a TC. In brief, the shorter the

333   lifetime of ^{3}CDOM*, the smaller is its steady-state concentration, and the slower the kinetics

334   for the transformation of the TC. Laser flash photolysis has been used to access ^{3}CDOM*,

335   however reliable data on their transient absorption spectra and lifetimes was not obtained

336   owing to the complexity of the transient signals.[50-54] These have been interpreted to consist of

337   a superposition of spectra from various species produced upon laser excitation, including the

338   hydrated electron, DOM radical cations and ^{3}CDOM*. When applying equation 2 to the case

339   of ^{3}CDOM* in the absence of a PC and Q$_x$, and considering that for surface water conditions
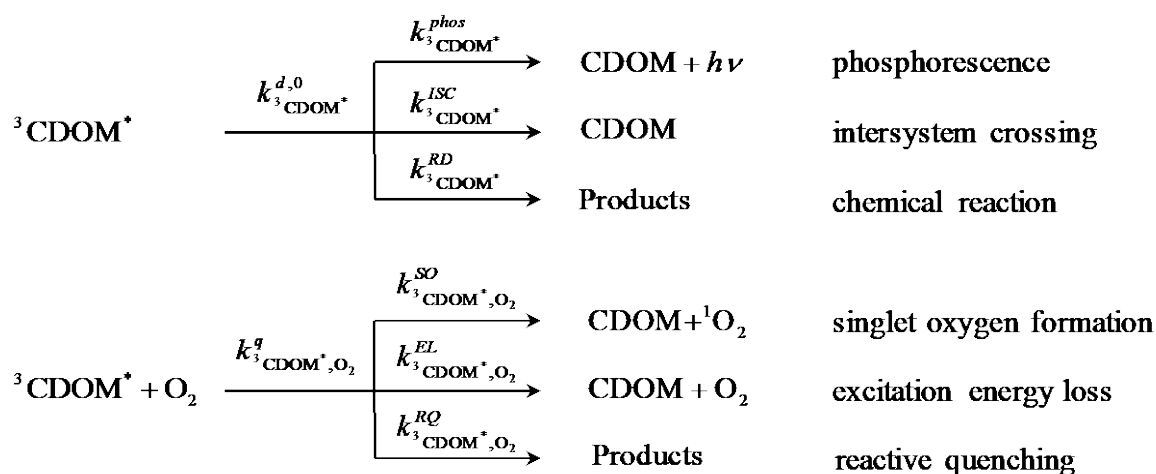
16

340 dissolved oxygen may be safely assumed to be the only relevant scavenger of $^3CDOM^*$,[55] one

341 obtains the following equation for the pseudo-first-order deactivation rate constant:

$$k^d_{^3CDOM^*} = k^{d,0}_{^3CDOM^*} + k^q_{^3CDOM^*,O_2}[O_2]$$ (13)

343   However, one has to be aware that equation 13 oversimplifies the real situation,

344 because $^3CDOM^*$ consists of a large collection of excited triplet chromophoric units, for

345 which equation 13 holds individually. The main processes and kinetic constants involved in

346 the deactivation of these chromophoric units are shown in Scheme 1. Direct measurements of

347 $k^{d,0}_{^3CDOM^*}$ and $k^q_{^3CDOM^*,O_2}$ by laser flash photolysis are not available due to the aforementioned

348 limitations. From the measurement of the oxygen concentration dependence of TMP loss

349 rates[39] and $^1O_2$ formation rates[40] under steady-state irradiation, the natural lifetimes of

350 $^3CDOM^*$ units, i.e. $\left(k^{d,0}_{^3CDOM^*}\right)^{-1}$, were estimated to fall in the range of $\approx 2-80$ μs. These

351 estimations were based on the assumption that $k^q_{^3CDOM^*,O_2} = 2\times10^9$ M^{-1}s^{-1} for all $^3CDOM^*$

352 units. The steady-state concentration of each individual $^3CDOM^*$ unit is given by:

$$\left[^3CDOM^*\right]_{ss} = \frac{r^f_{^3CDOM^*}}{k^{d,0}_{^3CDOM^*} + k^q_{^3CDOM^*,O_2}[O_2]}$$ (14)

354   From equation 14 one can conclude that an increase in oxygen concentration will lead

355 to a decrease in $\left[^3CDOM^*\right]_{ss}$ for any chromophoric unit in the CDOM. Moreover, high

356 deactivation ($k^{d,0}_{^3CDOM^*}$) and quenching rate constants ($k^q_{^3CDOM^*,O_2}$) for these units have a

357 negative effect on $\left[^3CDOM^*\right]_{ss}$.

358

$$^3CDOM^{\bullet} \xrightarrow{k_{3CDOM^{\bullet}}^{d,0}} \begin{cases} \xrightarrow{k_{3CDOM^{\bullet}}^{phos}} CDOM + h\nu & \text{phosphorescence} \\ \xrightarrow{k_{3CDOM^{\bullet}}^{ISC}} CDOM & \text{intersystem crossing} \\ \xrightarrow{k_{3CDOM^{\bullet}}^{RD}} Products & \text{chemical reaction} \end{cases}$$

$$^3CDOM^{\bullet} + O_2 \xrightarrow{k_{3CDOM^{\bullet},O_2}^{q}} \begin{cases} \xrightarrow{k_{3CDOM^{\bullet},O_2}^{SO}} CDOM + {}^1O_2 & \text{singlet oxygen formation} \\ \xrightarrow{k_{3CDOM^{\bullet},O_2}^{EL}} CDOM + O_2 & \text{excitation energy loss} \\ \xrightarrow{k_{3CDOM^{\bullet},O_2}^{RQ}} Products & \text{reactive quenching} \end{cases}$$

360

361   **Scheme 1**. Deactivation pathways for $^3CDOM^*$ in the presence and absence of

362   oxygen.

363

364   *Overview of $^3CDOM^*$ Chemistry*

365   As the excited triplet state of any excited organic chromophore, $^3CDOM^*$ can undergo

366   energy transfer to and a variety of chemical reactions with dissolved compounds, such as TCs

367   or PCs. Here we won't consider unimolecular reactions, which lead to the transformation of

368   DOM without affecting directly the transformation of other dissolved compounds. The most

369   common types of reactions that may involve $^3CDOM^*$ and a second reactant include energy

370   transfer, one-electron oxidation ($^3CDOM^*$ as an oxidant in electron transfer or hydrogen atom

371   transfer reactions) and one-electron reduction ($^3CDOM^*$ as a reductant). They are depicted in

372   Scheme 2. Further reactions such as photoinduced nucleophilic addition and cycloaddition[56]

373   are also possible, but to our knowledge no example for their occurrence in the aquatic

374   environment has been presented yet.
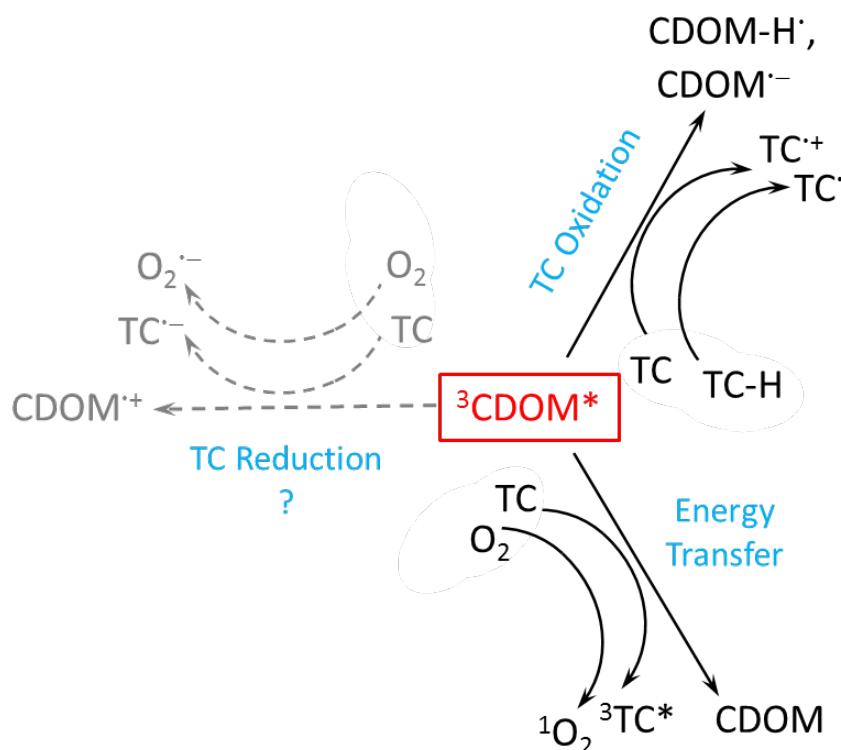
Scheme 2. Chemical fate of $^3CDOM^*$: Reactions include energy transfer resulting in the deactivation of the excited state, and reactions with a target compound (either in reduction or oxidation).

Energy Transfer

For the significance of $^1O_2$ formed through excitation energy transfer from $^3CDOM^*$ to dissolved oxygen, we refer the reader to the section below. Isomerization of 1,3-pentadiene (both *cis* and *trans* isomers) in dilute aqueous solution photosensitized by CDOM[47, 57] was attributed to energy transfer from $^3CDOM^*$ to the TC. In the resulting excited triplet state of *cis*- or *trans*-1,3-pentadiene, rotation about the double bond between the C(3) and C(4) atoms leads to isomerization. As typical for alkenes, from an excited configuration at a twisting angle of ≈90° both *cis* and *trans* isomers of the parent compound are formed after relaxation to the electronic ground state, but cases in which only one of the isomers is formed are also documented ("one-way" isomerization).[56, 58] Sorbic acid (*trans,trans*-2,4-hexadienoic acid) was also found to undergo isomerization photosensitized by CDOM and was therefore

390   proposed as a PC for ^{3}CDOM*.[41] In principle, triplet–triplet energy transfer can occur to any

391   compound exhibiting a triplet energy close to or below E^T_{CDOM}, and possible acceptors of

392   triplet energy from ^{3}CDOM* can be found in the collection of data on quenching of excited

393   triplet states of pure organic compounds.[49]

394   *Oxidations*

395   Evidence for oxidation reactions of the type depicted in Scheme 2 was found for

396   aniline in an early study by Zepp and coworkers.[57] However, ^{3}CDOM* haven't received

397   attention as photooxidants until striking similarities between DOM and some aromatic

398   ketones were demonstrated regarding their photoreactivity with phenols.[32] In a more recent

399   study, the strong decrease of TMP loss rates after borohydride reduction of CDOM suggested

400   that excited triplet ketone moieties in CDOM were involved in the phototransformation of

401   phenols.[39] In organic photochemistry, reactions involving carbonyl compounds as the

402   photoactive components and electron-rich compounds, such as amines, phenols, thiols or

403   thioethers, as the substrate undergoing oxidation, have been extensively investigated. We

404   refer to a review article for several references covering this topic.[59] The TC assessed to

405   undergo transformation initiated by ^{3}CDOM*-induced oxidation include bisphenol A,[60]

406   phenolic phytoestrogens,[61, 62] ring- or *N*-substituted anilines,[17, 63] diphenylamines,[64]

407   sulfonamide antibiotics,[17, 65-67] aminopyrimidine antibiotics,[68, 69], and phenylurea herbicides.[70]

408   Both electron transfer[71] and hydrogen transfer, in terms of a proton-coupled electron

409   transfer,[72] have been proposed as initial steps in the transformation mechanisms in aqueous

410   solution, based on results obtained using model photosensitizers. For pure electron transfer

411   reactions from a TC to an excited triplet photosensitizer ^{3}Sens*, the condition for their

412   occurrence is that the standard one-electron reduction potential of ^{3}Sens* is greater than the

413   corresponding (negative) oxidation potential of the TC.[71] The reduction potential of ^{3}CDOM*

414   was estimated to be $\approx$1.6–1.8 V vs. NHE.[59, 73]

Reductions

Chromophores may also act as one-electron donors in their excited triplet states, as

exemplified in the case of rose Bengal[74] or eosin-Y.[75] Oxygen is a readily available electron

acceptor in such a case, and the reaction product is the superoxide radical anion.[76] To our

knowledge, in the aquatic environment reductive indirect phototransformation of TCs has

been attributed to the hydrated electron in homogeneous solution[77] or in an intrahumic

environment,[78, 79] but reduction proceeding directly from $^3CDOM^*$ has not been identified

yet. We suspect that one of the reasons for this lack of evidence might be the presence of

oxygen, which, acting as a secondary electron acceptor, would efficiently re-oxidize any

formed reduced radical, $TC^{\cdot-}$, to its parent compound, thus preventing any reductive

transformation of a TC.

Measurement of $^3CDOM^*$

As noted above, flash photolysis does not allow to uniquely identify $^3CDOM^*$ or the

single chromophoric units. The consequence is that it would be extremely difficult and

uncertain to characterize and quantify the reactivity of these excited triplet states from flash

photolysis studies of CDOM, and one is forced to use an adequate PC to fulfill these tasks.

The two aforementioned conjugated dienes, namely 1,3-pentadiene[47, 57] and sorbic

acid,[41, 80] have been used so far as PCs in the low-concentration limit (see equations 4 and 5)

to quantify steady-state concentrations of $^3CDOM^*$. Moreover, sorbic acid[81] as well as

isoprene[64, 65, 82] have been employed as quenchers of $^3CDOM^*$ based on their efficient energy

transfer interaction. Oxygen can also be considered as an energy transfer quencher, and

reducing or augmenting its concentration has been exploited as a mechanistic tool to assess

the involvement of $^3CDOM^*$ in phototransformations.[32, 65, 82] However, one should be aware

of potential complications caused by the side reactions of oxygen with other RIs. These side

reactions include scavenging of the hydrated electron[77, 83] or ketyl radicals and the

440 corresponding anions,[71, 84, 85] yielding the superoxide radical anion, scavenging of carbon-

441 centered radicals to produce organic peroxyl radicals,[86] as well as enhanced production of

442 $^1O_2$.[40, 87]

443   Phenols bearing electron-donating substituents were found to be transformed by

444 CDOM-derived RIs characterized as $^3CDOM^*$.[32] Among the most reactive phenols (with

445 respect to photosensitization by CDOM), TMP and 3,4-dimethoxyphenol were proposed as

446 PCs for $^3CDOM^*$.[63] TMP has later been used in various studies,[18, 39, 60, 80, 88-92] mainly at low

447 concentrations suitable to quantify $[^3CDOM^*]_{ss}$ (see equations 4 and 5) and in some cases

448 also at higher concentrations to quench $^3CDOM^*$.[39, 60, 88] Compared to other organic

449 contaminants susceptible to transformation by $^3CDOM^*$, TMP not only has a very high

450 reactivity, but is also unaffected by the adverse inhibition side-effect ascribed to antioxidant

451 moieties of CDOM.[17, 18, 90] Moreover its direct phototransformation under (simulated)

452 sunlight is slow,[90] which makes it a very suitable PC for predicting the fate of dissolved

453 contaminants in surface waters. A drawback of electron-rich phenols as PCs for $^3CDOM^*$ is

454 constituted by their reactivity with still unidentified CDOM-derived RIs characterized as

455 long-lived photooxidants[63, 93] (*vide infra*). The effect of this side-reaction is important at sub-

456 micromolar concentration, but should be of limited relevance for $[TMP]\geq 5$ μM.[93] Despite the

457 widespread use of TMP as a PC for $^3CDOM^*$, not much is known about its transformation

458 pathways (see Scheme 3), which most probably comprise an initial electron transfer step to

459 form the corresponding phenoxyl radical.[32, 71] The good agreement between the rate constants

460 for transformation ($k^t_{^3Sens^*,TMP}$) and quenching ($k^q_{^3Sens^*,TMP}$) obtained for the reaction of TMP

461 with the excited triplet states of model aromatic ketones ($^3Sens^*$)[71] suggests a high efficiency

462 for this process. The subsequent reaction steps probably involve addition of superoxide,

463 which is widely available during CDOM photoirradiation, to yield cyclohexadienone-type

464 hydroperoxides, as suggested by pulse radiolysis studies.[94, 95] Such hydroperoxides should

465 then react to further unidentified degradation products.

466

467
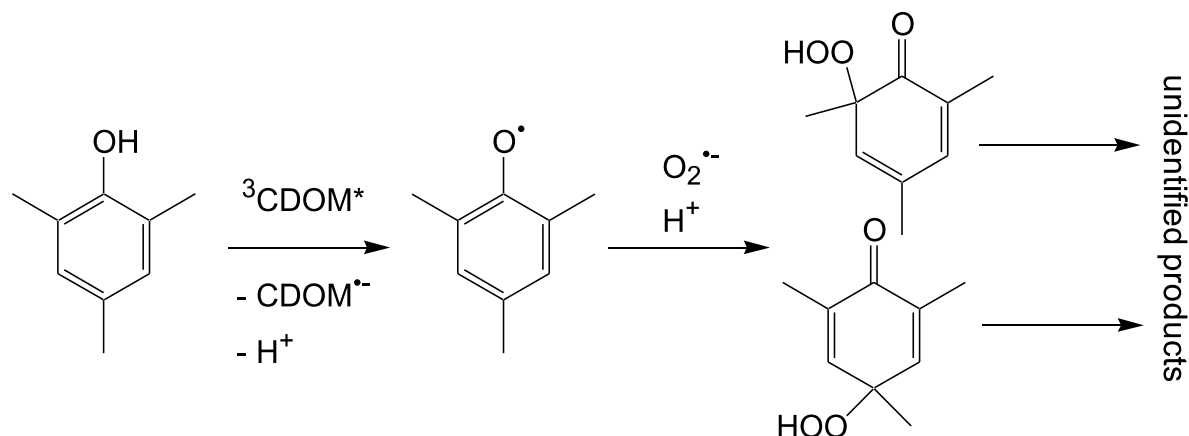
468   **Scheme 3**. Proposed reaction pathway between $^3CDOM^*$ and 2,4,6-trimethylphenol

469 (TMP).

Singlet Oxygen

471   As discussed above, dissolved oxygen is a well-known quencher of excited states and

472 will quench $^3CDOM^*$. Upon quenching of $^3CDOM^*$, $^1O_2$ is formed (see Scheme 2). $^1O_2$ refers

473 to the lowest excited singlet state of oxygen, which is present in solution in its ground-state

474 triplet configuration ($^3O_2$). There are two possible excited singlet states for oxygen (labeled

475 as $^1\Delta_g$ and $^1\Sigma_g$), located at 94 and 156 kJ mol^{-1} above the triplet ground state, however in

476 solution, only the $^1\Delta_g$ state is important as the higher energy state quickly decays in solution

477 (for a recent review on the chemistry of singlet oxygen, we refer the reader to the work by

478 Latch [96]).

479   Zepp and co-authors reported the formation of $^1O_2$ in natural waters as early as 1977.

480 [42] In this initial report, $^1O_2$ was measured by adding the compound 2,5-dimethylfuran and the

481 measured steady state concentrations were in the order of $2–22\times10^{-13}$ M. Its formation was

482 attributed to reactions associated with fulvic acids, a component of CDOM. Subsequent work

483   has established that CDOM, and more specifically $^3CDOM^*$, is the precursor for the

484   formation of $^1O_2$. Although it is selective in its reactions with organic contaminants, $^1O_2$ is

485   also important in the photochemically-driven inactivation of pathogens in surface waters. [97-

486   100]

487   In sunlit environmental waters, the steady-state concentration of $^1O_2$ ranges from 10^{-15}

488   to 10^{-12} M. [96] The quantum yields for its formation are in the range of 1.4–4.6% for surface

489   waters, DOM isolates, and samples collected from wastewater outfalls [29, 42, 43, 46, 101]. An

490   overview of $^1O_2$ photochemistry and quantification is given below. For more comprehensive

491   discussion of $^1O_2$ chemistry, we refer the reader to a recent review. [96]

492   The formation of $^1O_2$ from the quenching of $^3CDOM^*$ can be described by equations

493   15 and 16. Due to the relatively low energy needed to form $^1O_2$ (94 kJ mol^{-1}), it is expected

494   that most of the excited triplet states of CDOM will be able to produce it (see Figure 2),

495   however the efficiency of this process is unknown.

496   $$O_2 + {}^3CDOM^* \rightarrow {}^1O_2 + CDOM \tag{15}$$

497   $$r^f_{{}^1O_2} = k^{SO}_{{}^3CDOM^*,O_2}[O_2][{}^3CDOM^*] = k^q_{{}^3CDOM^*,O_2}\gamma_{{}^1O_2}[O_2][{}^3CDOM^*] \tag{16}$$

498   In equation 16, the second-order rate constants are defined as in Scheme 1 and $\gamma_{{}^1O_2}$ is

499   the yield for this reaction, defined as number of $^1O_2$ molecules formed per number of excited

500   triplet states quenched by O_2 (for a range of $\gamma_{{}^1O_2}$ values for diverse excited triplet states of

501   photosensitizers, we refer the reader to the work by Wilkinson and co-workers[87]). As

502   discussed above, the value for $k^q_{{}^3CDOM^*,O_2}$ has been estimated as 2×10^9 M^{-1} s^{-1}. [40]

503   Since $^1O_2$ is created by the deactivation of $^3CDOM^*$, it can be thought of as a TP of

504   oxygen, which is used as a PC for $^3CDOM^*$. Indeed, the quantum yield of $^1O_2$ offers an order

505   of magnitude estimate of the quantum yield for $^3CDOM^*$, or the intersystem crossing

506   quantum yield. However, under environmental conditions, there is no direct equivalency

507   between these two values. Equation 17 describes the quantum yield expression for $^1O_2$, with

508   the numerator describing its formation rate from $^3CDOM^*$ as a precursor, as shown in

509   equation 15.

510
$$\phi_{CDOM}^{^1O_2} = \frac{k_{^3CDOM*,O_2}^{q}\gamma_{^1O_2}\left[O_2\right]\left[^3CDOM*\right]}{r_{CDOM}^{abs}}$$
(17)

511   Equation 17 can be combined with equations 11 and 14 to yield equation 18.

512
$$\frac{\phi_{CDOM}^{^1O_2}}{\phi_{CDOM}^{ISC}} = \frac{k_{^3CDOM*,O_2}^{q}\gamma_{^1O_2}\left[O_2\right]}{k_{^3CDOM*}^{d,0} + k_{^3CDOM*,O_2}^{q}\left[O_2\right]}$$
(18)

513   Under environmental conditions regarding oxygen concentrations, and assuming

514   values for $k_{^3CDOM*,O_2}^{q}$ of 2×10^9 M^{-1} s^{-1}, and an estimated value for $k_{^3CDOM*}^{d,0}$ of $<5\times10^4$ s^{-1},[40]

515   equation 18 simplifies to

516
$$\frac{\phi_{CDOM}^{^1O_2}}{\phi_{CDOM}^{ISC}} \cong \frac{k_{^3CDOM*,O_2}^{q}\gamma_{^1O_2}\left[O_2\right]}{k_{^3CDOM*,O_2}^{q}\left[O_2\right]} = \gamma_{^1O_2}$$
(19)

517   This indicates that $\dfrac{\phi_{CDOM}^{^1O_2}}{\phi_{CDOM}^{ISC}}$ is equal to the weighted average value of $\gamma_{^1O_2}$ for the

518   different components of CDOM. A similar approach can be used to approximate the steady-

519   state concentration of $^1O_2$.

520   As with other RIs, $^1O_2$ is typically measured using PCs. Although a direct method to

521   measure $^1O_2$ exists via measurement of its phosphorescence at 1270 nm, most reports in the

522   environmental chemistry literature are based on the use of PCs. [15, 30, 42, 46, 101, 102] Amongst the

523   PCs, the most widely used is FFA. FFA reacts with $^1O_2$ to produce a series of compounds, as

524   shown in Scheme 4.

525

Major Product

526
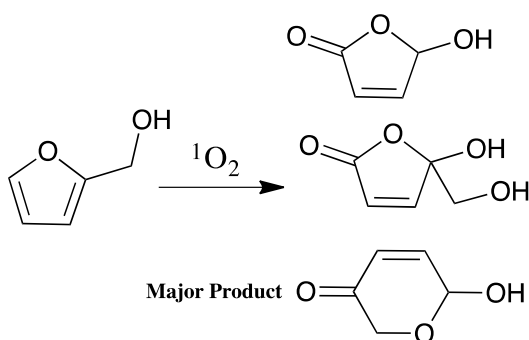
Scheme 4. Reaction between $^1O_2$ and furfuryl alcohol (FFA) showing the three

products. The major product (~85% yield) is also indicated. Scheme adapted from Haag et al.

1984. [31]

530

Originally introduced by Haag et al. [31], the use of FFA is based on the monitoring of

its disappearance (pseudo-first order decay) and then extracting the steady state concentration

of $^1O_2$ which allows the calculation of its quantum yield.

$$\frac{d[\text{FFA}]}{dt} = -k^t_{^1O_2,FFA}[^1O_2]_{ss}[\text{FFA}]$$

(20)

$$[^1O_2]_{ss} \cong \frac{r^{abs}_{CDOM}\phi^{^1O_2}_{CDOM}}{k^{d,0}_{^1O_2} + k^q_{^1O_2,FFA}[\text{FFA}]}$$

(21)

where $k^t_{^1O_2,FFA}$ is the second-order rate constant for the transformation of FFA induced by

reaction with $^1O_2$ and $k^q_{^1O_2,FFA}$ is the second-order rate constant for $^1O_2$ quenching by FFA. In

equation 21, the assumption has been made that the contribution of natural scavengers, such

as DOM,[103] to the decay of $^1O_2$ is negligible. Thus, its decay in the absence of added PCs or

quenchers is dominated by the reaction with water, with $k^{d,0}_{^1O_2} = 2.5\times10^5$ s[-1].[104, 31, 105] The use

of equation 21 to calculate the values for the steady-state concentration and quantum yield for

$^1O_2$ typically assumes that $k^{d,0}_{^1O_2} >> k^q_{^1O_2,FFA}[\text{FFA}]$. When using FFA as a probe molecule, the

concentration of added FFA must be low enough to satisfy the conditions presented in

equations 3 and 4 above. Given the value of $k^q_{^1O_2,FFA} \approx k^t_{^1O_2,FFA}$ equal to 1.2×10^8 M^{-1} s^{-1} [31, 106],

545   this indicates that the use of FFA for the assessment of $^1O_2$ should be limited to FFA

546   concentrations below 110 μM (in this case $\dfrac{k_{^1O_2}^{d,0}}{k_{^1O_2}^{d,0} + k_{^1O_2,FFA}^{q}[\text{FFA}]} = 0.95$, which introduces less

547   than 5% deviation). If this condition is not met, then equation 21 should not be simplified and

548   the quenching of $^1O_2$ by FFA needs to be considered.

549   Other species are typically used as quenchers for $^1O_2$ in order to assess the role of this

550   RI in a specific process. Quenchers include azide,[107, 96, 102, 106] β-carotene [96], and 1,4-

551   diazabicyclo[2,2,2]octane. [108]

552   An interesting observation regarding $^1O_2$ is the potential to reach higher steady-state

553   concentrations within a hypothesized hydrophobic cavity compared to the bulk solution. [102,

554   109] This work, conducted utilizing probes with different hydrophobic character, is relevant to

555   conditions where a contaminant or pathogen is closely associated with DOM, as has been

556   reported before. However, hydrophilic phase probes such as FFA are preferred when

557   investigating processes that occur in bulk, aqueous solution.

558   In a general sense, the method of quantifying $^1O_2$ in solution is well established,

559   especially using FFA. FFA is known to react with •OH, with a reaction rate constant of $1.5 \times$

560   10^{10} M^{-1} s^{-1}. [83] Therefore, in applications when the expected •OH concentrations are high, this

561   pathway needs to be considered and quenched.

562   An important consideration with the use of FFA is the potential for oxygen depletion.

563   The reaction between FFA and $^1O_2$ will consume oxygen. However, as long as the FFA

564   concentrations are low, as should be the case when using this method (see above), this should

565   not be an issue. Lastly, the formation of the major product also yields H_2O_2, although the

566   formation of this weak oxidant does not interfere with the FFA degradation. [31]

Hydroxyl Radical

$^{\bullet}$OH is an important environmental oxidant. $^{\bullet}$OH is a very reactive species, with typical second-order reaction rate constants in the order of 10^8–10^{10} M^{-1} s^{-1}. [83] Interest in $^{\bullet}$OH as a photooxidant in natural waters dates back to early reports on the photochemically-driven degradation of organic compounds in water. [110] In subsequent work, it has been established that $^{\bullet}$OH is an important oxidant in surface waters, with different implications to environmental systems. [23, 37, 108, 111-117]

The steady state concentrations of $^{\bullet}$OH in sunlit waters are in the order of 10^{-14}–10^{-17} M. In environmental waters, the main scavengers of $^{\bullet}$OH are carbonate, bicarbonate, nitrite and DOM.[83] In brackish waters, bromide will also be important.[118] Based on published values for the second order reaction rate constants for the scavenging reactions, including the reaction with DOM [119, 120], the lifetime of $^{\bullet}$OH can be estimated as in the order of 5–10 μs.

Contrary to the case of $^1O_2$, there is an incomplete understanding of the pathways that lead to the formation of $^{\bullet}$OH in surface waters, partially due to the concomitant effect of different chemical reactions. Photolysis of nitrate and nitrite is known to form $^{\bullet}$OH, with quantum yields in the order of 0.007–0.014 for nitrate and 0.024–0.078 for nitrite. [121-123] However, given the relatively low absorbance of nitrate and nitrite in environmental systems, a direct CDOM path to $^{\bullet}$OH will dominate under conditions where the concentration of these species is low. [114, 115] $^{\bullet}$OH formation through Fenton chemistry is another well-known pathway [123-128], although the efficiency of this process diminishes as the pH nears neutral. [27, 124]

Over the past 10 years, there has been an expanded interest in the direct formation of $^{\bullet}$OH from photochemical reactions within CDOM. [29, 113-115, 123, 129] When discussing the photochemical formation of $^{\bullet}$OH from CDOM, two general pathways have been suggested:

591   an H_2O_2-dependent (photo-Fenton) and H_2O_2-independent pathway.[27] These pathways could

592   also be categorized as oxygen-dependent or -independent pathways, although care must be

593   taken when assessing the role of oxygen as certain PCs necessitate oxygen for their reactions

594   (see below) and because there is no clarity regarding the actual formation pathway for this

595   species. The magnitude of both pathways seems to be CDOM-specific. For example, studies

596   of $^{\bullet}$OH production from DOM isolates using different PCs and quenchers show that the

597   H_2O_2-dependent pathway could account for up to ~ 50% of $^{\bullet}$OH production. [113, 123] In the case

598   of wastewater, the H_2O_2-dependent pathway seems to account for up to 30–40% of the $^{\bullet}$OH

599   production, based on data from Dong and Rosario-Ortiz. [115] The contribution of this pathway

600   can be eliminated via the addition of catalase (quenching of H_2O_2), or removing iron from

601   solution.

602   The mechanism for the formation of $^{\bullet}$OH through the H_2O_2-independent pathway is

603   not clear. Inherently, this pathway is ascribed to photochemical reactions within CDOM

604   components. Reported quantum yields for CDOM (including isolates, samples and

605   wastewater-derived) are in the order of 10^{-5}–10^{-4}, [115, 130, 131] indicating that this pathway is

606   quite inefficient when compared to the formation quantum yields of other RIs. Early reports

607   hypothesized that $^{\bullet}$OH was formed through the oxidation of water by excited triplet species

608   from CDOM [114, 132, 133] , although recent evidence indicates that no free $^{\bullet}$OH is formed via

609   these reactions.[134-138] Recent work has shown that hydroxyaromatic acids could be

610   photolyzed to yield $^{\bullet}$OH with quantum yields of 10^{-4}, which could potentially identify a

611   source of this species from CDOM photolysis. [129] In conclusion, there is still an incomplete

612   understanding regarding the formation of $^{\bullet}$OH from CDOM.

613   Historically, the most common PC type used for the quantification of $^{\bullet}$OH in

614   environmental photochemistry has been through the hydroxylation of arenes (e.g.,

615   terephthalic acid, benzene, nitrobenzene, benzoic acid) [114, 115, 117, 124, 129, 130, 139], however other

616 probes have also been used (e.g., butyl chloride [140]). The specific methods vary between the

617 monitoring of the degradation of a parent compound or the formation of a product.

618 Additionally, not all probes are transparent in the same wavelength range. Regarding the use

619 of quenchers for •OH, the most commonly used are the aliphatic alcohols, such as *t*-butanol

620 and isopropanol. [90, 108]

621   In the general case of the hydroxylation of an arene, the initial step is the addition of

622 •OH to the ring to yield a hydroxycyclohexadienyl radical (hydrogen abstraction is not a

623 significant pathway [141]). Scheme 5 shows the hydroxylation of benzene as an example of the

624 type of reaction mechanism common for arenes. The reaction rate constants between •OH and

625 different arenes are all in the order of 10^9 M^{-1} s^{-1}, close to the diffusion-controlled limit.[83]

626 Following the formation of this radical, the next step is an oxidation to yield the hydroxylated

627 product(s) (phenol in the case of benzene). It is expected that in most aerated solutions the

628 oxidant is oxygen, which adds to the radical to form a peroxyl radical which will decay into

629 the hydroxylated product(s). However, it has been shown that other oxidants could also

630 impact the formation of the final product, which limits the use of product ratios as an

631 assessment of •OH formation when using probes with multiple sites capable of being

632 hydroxylated. [113]

633

634
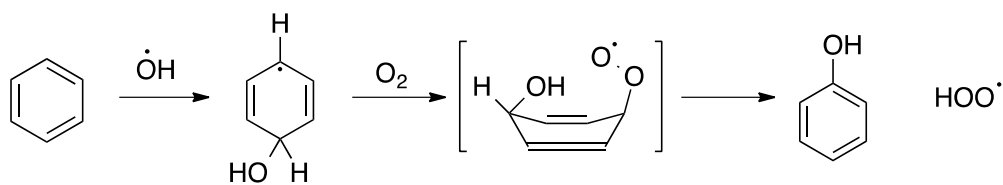
635   **Scheme 5**. Hydroxylation of benzene by •OH in aqueous solution. Note that the

636 secondary oxidant in this case is oxygen.

637

638   An alternative method, introduced by Vaughan and Blough, is based on the use of a

639 trapping agent such as DMSO, which upon reaction with •OH forms the methyl radical,

640   which upon further reactions is trapped into a fluorescent product that could be determined

641   by HPLC. [123, 134, 142] However it has been shown that this method is not immune to potential

642   interferences, mostly dealing with the capacity of other oxidants to react with the PC. [142]

643   Nevertheless, this method could be combined with the use of methane, which will selectively

644   form a methyl radical upon its reactions with $^\bullet$OH, followed by the trapping of this methyl

645   radical via the formation of a fluorescent product.[143]

646   There are several considerations that must be addressed when selecting a probe to

647   measure $^\bullet$OH. First, when using an arene, the reaction necessitates oxygen (or another

648   oxidant) to yield the final hydroxylated product. As has been established beforehand, the

649   presence of other oxidants in solution can interfere with this process. In the case where

650   oxygen is the oxidant, its addition to the hydroxycyclohexadienyl radical yields the

651   superoxide radical anion. This species will disproportionate into H_2O_2 and oxygen, and this

652   reaction is impacted by pH (the pK_a of the $HO_2^\bullet$ radical is 4.8).[144] However, under

653   environmental conditions, the formation rate of H_2O_2 from this process should be small when

654   compared to the photoproduction rate of H_2O_2 from other pathways.

655   A second aspect is the yield for the formation of the product, which is needed to

656   estimate the formation rate per equation 9. In the case of the hydroxylation of benzene, the

657   yield of phenol, which is the main stable product, has been reported in the range of 0.6 to 0.9.

658   [114, 124, 130] A recent report examined the yield as a function of temperature and obtained a

659   yield of 0.69 near circumneutral pH. [130] When comparing quantum yields between

660   publications, a note should be made of the yield assumption.

661   Lastly, and perhaps the most important consideration when measuring $^\bullet$OH, is the fact

662   that its quantification can be impacted by the formation of other oxidants. In the case of $^\bullet$OH,

663   it has been shown that the formation of what is believed to be an exciplex can lead to the

664   production of the hydroxylated product of a PC such as benzene [113, 130]. The most likely

31

665 candidate for this is the interaction between quinones and water, as quinones are known to be

666 active components of CDOM [145, 146]. The potential of this exciplex, otherwise characterized

667 as a lower-level hydroxylating species, to account for some of the observed $^{\bullet}OH$ -like

668 behavior has also been studied. The contribution of this pathway could be as high as 50%

669 based on experiments with methane [113]. In a recent study on activation energies, this pathway

670 was described as having small activation energies [130]. This indicates that a fraction of the

671 $^{\bullet}OH$-like PC reactivity reported could be assigned to such a species. It is unkown whether the

672 formation of this exciplex could have significance regarding the degradation of organic

673 contaminants.

674 Moving forward, there is a need to develop a quantitative $^{\bullet}OH$-specific method for

675 photochemical studies involving CDOM. Ideally, a chemical probe could be found that is

676 only selective to $^{\bullet}OH$ and not to other observed hydroxylating pathways. Alternatively, a

677 method based on competitive kinetics where different chemical compounds are used could be

678 implemented to assess only $^{\bullet}OH$ formation.

679 **Other Reactive Intermediates**

680 So far, we have focused our discussion on three RIs, which arguably represent the

681 three most studied species in environmental photochemistry. However, there are other

682 transient species that are formed via photochemical reactions in aqueous systems. These

683 include $O_2^{-\bullet}$, the carbonate radical ($CO_3^{\bullet-}$), as well as poorly defined DOM-derived radicals.

684 In this section, the use of PCs for the measurement of these species will be examined. Note

685 that we haven't included the hydrated electron in this paper because of its limited impact in

686 aquatic photochemistry and the detailed representation of this topic given in a recent critical

687 review.[29] Additionally, as mentioned above, we do not discuss H_2O_2 since this is not

688 considered to be a short-lived species.

Carbonate Radical ($CO_3^{\bullet-}$)

690   The formation of $CO_3^{\bullet-}$ in photoirradiated natural waters was originally attributed to

691   the reaction of $^\bullet OH$ with the bicarbonate and carbonate anions.[147] A study performed using

692   model quinone and aromatic ketone photosensitizers also showed the plausibility of $CO_3^{\bullet-}$

693   formation through the reaction of these anions with $^3CDOM^*$.[148] $CO_3^{\bullet-}$ is a weaker oxidant

694   than $^\bullet OH$ [30] and thus exhibits second-order reaction rate constants that are generally several

695   orders of magnitude lower than for $^\bullet OH$.[149] Due to poor scavenging by water matrix

696   components, steady-state concentrations of $CO_3^{\bullet-}$ are estimated to be higher than for $^\bullet OH$,[147,

697   150] which can often make up for the drawback of lower reactivity with TCs. Anilines,

698   particularly those bearing electron-donating substituents, were found to have high second-

699   order rate constants (up to $\approx 2\times10^9$ M^{-1} s^{-1}) for their reaction with $CO_3^{\bullet-}$.[147, 148, 151, 152] Thus,

700   contaminants exhibiting such functionalities are probably among the most prone to $CO_3^{\bullet-}$-

701   induced transformation in sunlit waters.[147] Based on its high reactivity, *N,N*-dimethylaniline

702   was later employed as a PC to determine $[CO_3^{\bullet-}]_{ss}$.[108, 153] Nevertheless, we have serious

703   reservations about the use of anilines for such a goal, because they were shown to be also

704   very reactive with $^3CDOM^*$ and excited triplet states of model photosensitizers,[17, 63, 72] and in

705   addition their transformation induced by one-electron oxidation is inhibited by DOM.[17, 154]

706   These side-reactions of the anilines are likely to impair the quantification of $[CO_3^{\bullet-}]_{ss}$.

707   *Superoxide Radical Anion ($O_2^{\bullet-}$)*

708     $O_2^{\bullet-}$ is formed via the one-electron reduction of oxygen in water. It is not clear what

709   the identity of this reductant is, however it is unlikely that the solvated electron is the main

710   precursor. [77, 155] Recent work has suggested that the reducing species is the result of an

711   intramolecular electron transfer, possibly involving electron donors such as phenolic species

712   and acceptors such as quinones. [93, 156, 157] In surface waters (including marine systems), there

713   are both biotic and abiotic (dark and light) sources of $O_2^{\bullet-}$.[158] Under conditions typical of

714   environmental photochemical reactions, the most likely reductants are functional groups

715   within DOM [29, 158], although the details regarding the specific mechanism are still unclear. It

716   has been reported that the yields for $O_2^{\bullet-}$ formation follow linearly the phenolic content of

717   DOM or wastewater-derived organic matter.[159]

718   Recent publications have used the compound 2-methyl-6-(4-mehoxyhenyl)-3,7-

719   dihydroimidazo[1,2-a]pyrazine-3(7H)-one (MCLA for short) as a PC for $O_2^{\bullet-}$. MCLA has

720   been used as a PC for biological systems for some time [160-162] and its application to

721   environmental systems has been recently evaluated [163, 164]. MCLA reacts with $O_2^{\bullet-}$ (Scheme

722   6) and $^1O_2$. [160] In the case of $O_2^{\bullet-}$, the reaction involves hydrogen abstraction and addition of

723    $O_2^{\bullet-}$, followed by decarboxylation and finally emission of a photon at 457 nm. In the case of

724   the reaction with $^1O_2$, the mechanism is similar. Given the interference with $^1O_2$, the use of

725   MCLA for $O_2^{\bullet-}$ involves either a delay to allow the $^1O_2$ to decay before measurement [30, 163] or

726   addition of $^1O_2$ quenchers. Care must be taken when analyzing samples with high

727   concentrations of redox active metals, as these could enhance $O_2^{\bullet-}$ formation. [163]

728
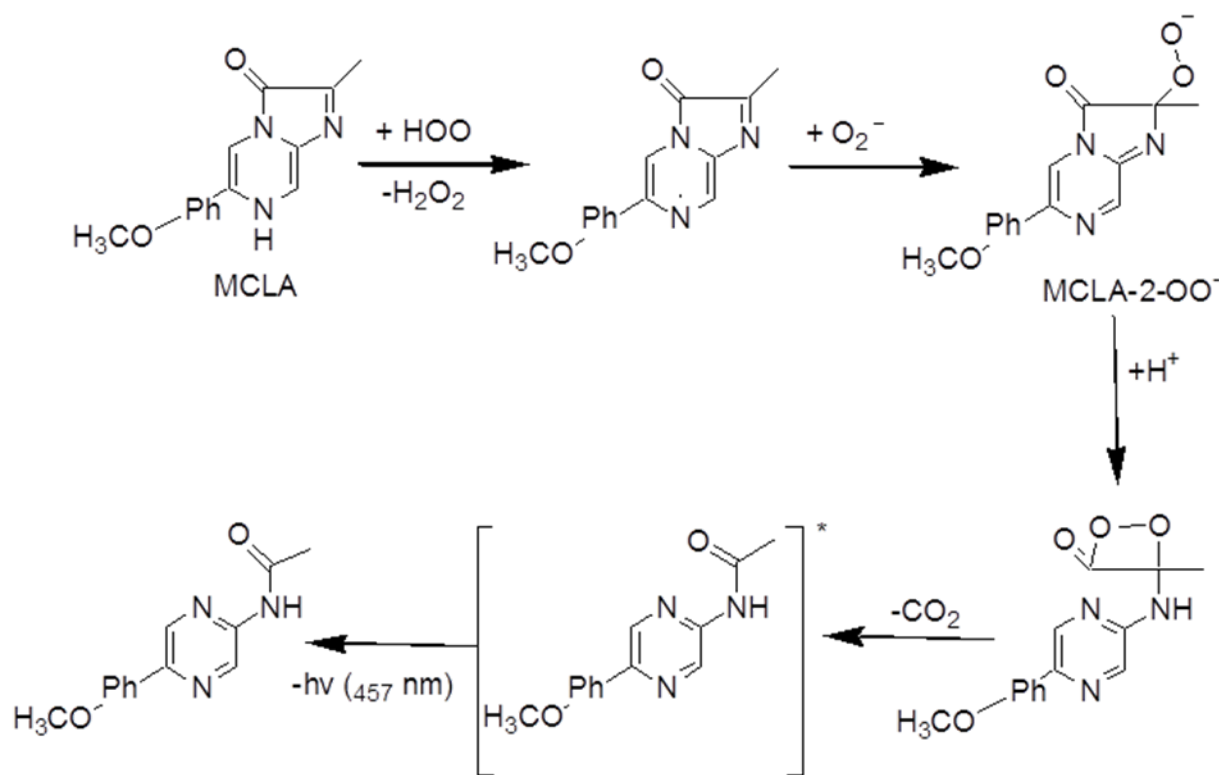
Scheme 6. Reaction between $O_2^{\bullet-}$ and MCLA resulting in the emission of a photon at 457 nm. The potential interfering reaction with $^1O_2$, leading also to the emission of light, is not shown. Adapted from Kambayashi and Ogino, 2003. [160]

Organic Peroxyl Radicals

In the 1980's, organic peroxyl radicals derived from photoirradiated CDOM were thought to be important photooxidants for the sunlight-induced transformation of organic contaminants in surface waters.[110, 165] This suggestion was never supported by convincing pieces of evidence, although the formation of such radicals must take place as a consequence of the well-established addition of molecular oxygen to C-centered alkyl-type radicals,[86] which have been measured in photoirradiated solutions of humic substances.[166, 167] Moreover, evidence for the formation of individual organic peroxyl radicals in natural waters, including estimation of their production rates, were inferred by the measurement of photoproduced alkyl nitrates.[168, 169] The quantification of their steady-state concentration and effect on the transformation of TCs appears to be difficult because of the expected high

743   variability in their chemical structure and reactivity (as a result of CDOM complexity) and

744   the current lack of research about appropriate PCs.

745   *"Long-Lived" Photooxidants*

746   The pseudo-first-order rate constants for CDOM-photosensitized transformation of

747   electron-rich phenols, including TMP, were found to increase with decreasing initial phenol

748   concentration in the range of ≈0.1−5 μM, while they only slightly depended on the initial

749   phenol concentrations above 5 μM.[93, 170] Such a kinetic behavior was interpreted as the result

750   of the action of two basic categories of photooxidants on the photosensitized transformation

751   of these phenols: (1) photooxidants for which the lifetime was determined by the water

752   matrix and which were proposed to include $^3CDOM^*$, and (2) photooxidants whose lifetime

753   was significantly controlled by the phenol itself and which were named "long-lived"

754   photooxidants (LLPOs). [93] In a later study,[63] TMP and 3,4-dimethoxyphenol (DMOP) were

755   proposed as possible probe compounds to discriminate between "short-lived" photooxidants

756   (SLPOs) and LLPOs. The method consisted in quantifying the pseudo-first-order rate

757   constant for the transformation of the used PCs at 5.0 and 0.1 μM initial concentrations,

758   respectively. The rate constant for the higher initial concentration would mainly reflect the

759   effect of SLPOs on the transformation of the PC (with the contribution of LLPOs assumed to

760   be negligible) while the rate constant for the lower concentration, would reflect the effect of

761   both types of photooxidants on the transformation of the PC. The difference of these two

762   constants would represent the minimum contribution of LLPOs to the transformation of the

763   PC. For solutions of CDOM as well as most studied surface water samples LLPOs appeared

764   to be the dominating factor in the photosensitized transformation of TMP and DMOP.[63] The

765   absence of a corresponding effect using 4-methylphenol as a PC lead to the conclusion that

766   LLPOs were weaker photooxidants than SLPOs. Moieties of the CDOM with radical cation,

767   phenoxyl, alkoxyl or peroxyl radical character are plausible candidates of LLPOs, but their

768   chemical nature remains unresolved.

Holistic Approach

770   Upon photoirradiation of CDOM a whole suite of RIs are produced that can

771   contribute to the transformation of a TC or a PC. In the low-concentration limit of the PC

772   (see derivation of equation 4) the following kinetic equation holds:

$$r_{PC}^t = \frac{d[\text{PC}]}{dt} = -[\text{PC}] \sum_m k_{RI_m,PC}^t [\text{RI}_\text{m}]_{ss} \tag{22}$$

774   where each individual RI has been indexed with the subscript m. Although a PC is chosen to

775   react exclusively with a single RI, named selected RI (RI_S) in the following, its possible

776   reaction with other RIs has to be carefully considered. This means that for the transformation

777   of a PC the contribution of any other occurring RI, i.e. $k_{RI_m,PC}^t [\text{RI}_\text{m}]_{ss}$ ($m{\neq}$S), has to be

778   scrutinized and compared with the contribution of RI_S, i.e. $k_{RI_S,PC}^t [\text{RI}_\text{S}]_{ss}$, to ascertain whether

779   the use of the PC can ensure a correct quantification of $[\text{RI}_\text{S}]_{ss}$ or not. In general, the higher

780   the reactivity of a RI, the higher its potential to interfere with the measurement of a less

781   reactive RI as a consequence of the higher second-order rate constant $k_{RI_m,PC}^t$. Here we

782   consider the most common photooxidants amongst the RIs formed in aerated waters.

783   Assuming a correlation of the reactivity of these photooxidants with their oxidative

784   "strength", expressed as their standard one-electron reduction potential (for the couples

785   RI/RI⁻, indicated in V vs. NHE in parenthesis after each photooxidant in the following list),

786   one can derive the following sequence in order of decreasing reactivity: $^{\bullet}$OH (1.90)[171] >

787   ^{3}CDOM* ($\approx$1.6–1.8)[59, 73] > $CO_3^{\bullet-}$ (1.59)[172] > $^1O_2$ (0.83). Deviations from this reactivity scale

788   can occur, for instance if the reaction of a PC with a given photooxidant is not initiated by

789   one-electron oxidation. This is the case for $^1O_2$, which is known to react with activated

790   double bonds by cycloaddition.[31] Within the considered group of photooxidants, $^{\bullet}OH$ is the

791   one that can be assumed to react at nearly diffusion-controlled rates with any PC selected to

792   detect the other photooxidants. The subsequent step in assessing the possible interference by

793   $^{\bullet}OH$ is to evaluate its steady-state concentration, accessible through independent experiments

794   using an appropriate PC for $^{\bullet}OH$. If this is not possible, due for instance to too low $[^{\bullet}OH]_{ss}$,

795   resulting in excessively long photoirradiation times, or if the contribution of $^{\bullet}OH$ exceeds a

796   recommended fraction of 5% of the transformation rate of the used PC (i.e., $k^t_{^{\bullet}OH,PC}[^{\bullet}OH]_{ss} >$

797   $0.05 \times k^t_{RI_S,PC}[RI_S]_{ss}$), then experiments to determine $[RI_S]_{ss}$ should be done in the presence of

798   an added $^{\bullet}OH$ scavenger (e.g., 2-propanol or t-butanol). The described procedure can be

799   applied by analogy to interferences caused by RIs other than $^{\bullet}OH$. When employing

800   scavengers or quenchers, analogous considerations as for the use of PCs apply. One has to be

801   aware that a scavenger/quencher can potentially affect the concentration of an RI it is not

802   intended to, or interfere in the transformation pathways of PCs or TCs. For instance, almost

803   any scavenger/quencher of the above-mentioned photooxidants can efficiently scavenge $^{\bullet}OH$.

804   Therefore, one should be extremely cautious in interpreting rate constant values obtained by

805   the application of these compounds. The use of several different scavengers/quenchers for a

806   specific RI may help to overcome the problem of such interferences.

807   The issue of adequate PC concentrations is very important and should be thoroughly

808   studied before experimental planning. For the determination of steady-state concentrations of

809   an RI (see equation 4), we propose that the depression of [RI] by the presence of a PC should

810   not exceed 5%, i.e., $k^d_{RI,PC}[PC] < 0.05 \times \left(k^{d,0}_{RI} + \sum_i k^q_{RI,Q_i}[Q_i] \right)$. Even if this condition is

811   fulfilled, one should try and use the minimal PC concentration that allows an accurate

812   measurement of k^{obs}_{PC}. If the organic carbon content of an added PC is at least of the same

813   order of magnitude as the one of DOM, there is a high risk of interference by photoproducts

38

814   formed during the transformation of PC, which could act as quenchers/scavengers or

815   additional photosensitizers for the measured RI. A significant chemical modification of DOM

816   could also take place. Of course, some of these considerations also apply in the case of high

817   PC concentration used to determine RI formation rates. For example, when using the high PC

818   concentration method, one needs to meet the conditions stated in eq. 6, where the PC is at a

819   sufficiently high concentration to dominate the overall scavenging of a particular RI.

820   A further critical issue that we noticed in several studies regards the presence of an

821   unwanted quencher in the experimental solutions, which can significantly reduce the

822   concentration of the RI under analysis. This is observed when experimentalists dissolve a PC

823   in an organic solvent to create a stock solution, which is then added to system to be studied.

824   In this case, even microliter volumes of an organic solvent could have a significant impact in

825   the determination of a particular RI. For example, when performing experiments to determine

826    $[^\bullet OH]_{ss}$, we discourage the use of organic solvents in any stock solution, which could finally

827   result to be the main scavenger of $^\bullet OH$ in the experimental solutions.

828   With RIs that comprise a whole category of homologous reactive species, as in the

829   case of $^3CDOM^*$, it is hard to measure their steady-state concentrations as well as formation

830   rates. Even if this were done, it would be unfeasible to calculate transformation rates of any

831   TC due to the ill-defined character of $^3CDOM^*$ and of the second-order rate constant for the

832   transformation of the TC induced by $^3CDOM*$, $k^t_{^3CDOM^*,TC}$. The PCs currently available to

833   measure $^3CDOM^*$ make a selection of triplet states according to their triplet energy (the

834   energy transfer-PCs, such as the conjugated dienes) or their reduction potential (the

835   oxidizable PCs, such as TMP). To date, to predict pseudo-first-order rate constants for the

836   oxidative transformation of a TC, $k^{obs}_{TC}\left(^3CDOM^*\right)$, the best available method is to determine

837    $[^3CDOM^*]_{ss}$ by applying a $k^t_{^3Sens^*,PC}$ determined using a single model photosensitizer (Sens)

838   and a single PC (e.g., TMP), and then use the second-order rate constant for the

39

839   transformation of the TC induced by ^{3}Sens*, $k^t_{^3Sens^*,TC}$. This procedure is equivalent to

840   applying the following equation:

841    $$k^{obs}_{TC}\left(^3\text{CDOM}^*\right) = k^{obs}_{PC}\left(^3\text{CDOM}^*\right)\frac{k^t_{^3Sens^*,TC}}{k^t_{^3Sens^*,PC}} \qquad (23)$$

842   The second-order rate constants for these reactions are highly dependent on the

843   difference between the reduction potential of ^{3}Sens* and the oxidation potential of the TC (or

844   PC). In view of the broad distribution expected for the reduction potential of single

845   chromophores constituting ^{3}CDOM*, it is obvious that relying on a single photosensitizer to

846   predict $k^{obs}_{TC}\left(^3\text{CDOM}^*\right)$ can be very far from reality, especially for TC that have very

847   different oxidation potential from TMP. The choice of an adequate photosensitizer to be used

848   for the application of equation 23 also remains uncertain. A way out of this dilemma would

849   be to use a suite of PCs with known oxidation potential and define, with a fixed second-order

850   rate constant (e.g. 3×10^9 M^{-1}s^{-1}), nominal concentrations for the subsets of ^{3}CDOM* that are

851   captured by each individual PC. [^{3}CDOM*]$_{ss}$ could then be expressed as a function of the

852   oxidation potential of any substrate. This would lead to the calculation of $k^{obs}_{TC}\left(^3\text{CDOM}^*\right)$

853   using the mentioned fixed second-order rate constant. An analogous method could be applied

854   to estimate the transformation rates of TCs undergoing energy transfer reactions induced by

855   ^{3}CDOM*. In this case, a suite of PCs with known excited triplet energies should be selected.

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Notes

860   The authors declare no competing financial interest.

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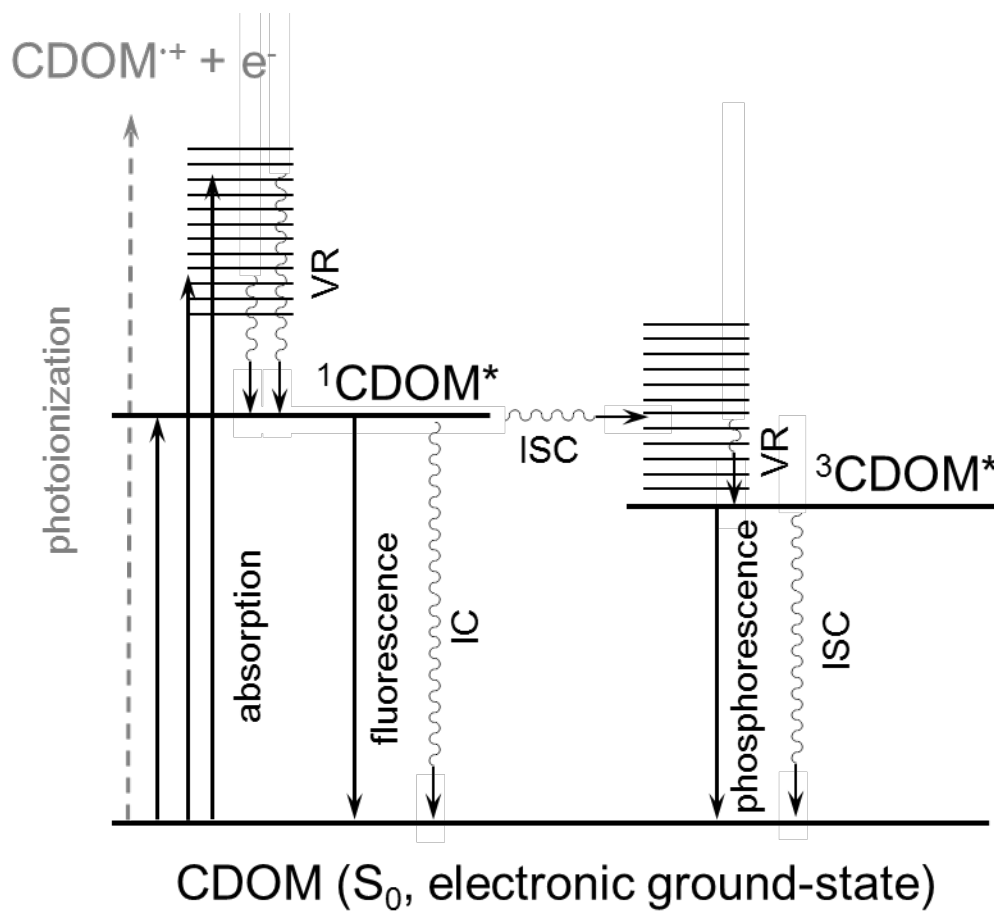1333

Figure 1. Jablonski diagram representing the processes dominating the photophysics of CDOM (Abbreviations: IC = internal conversion; ISC = intersystem crossing; VR = vibrational relaxation).
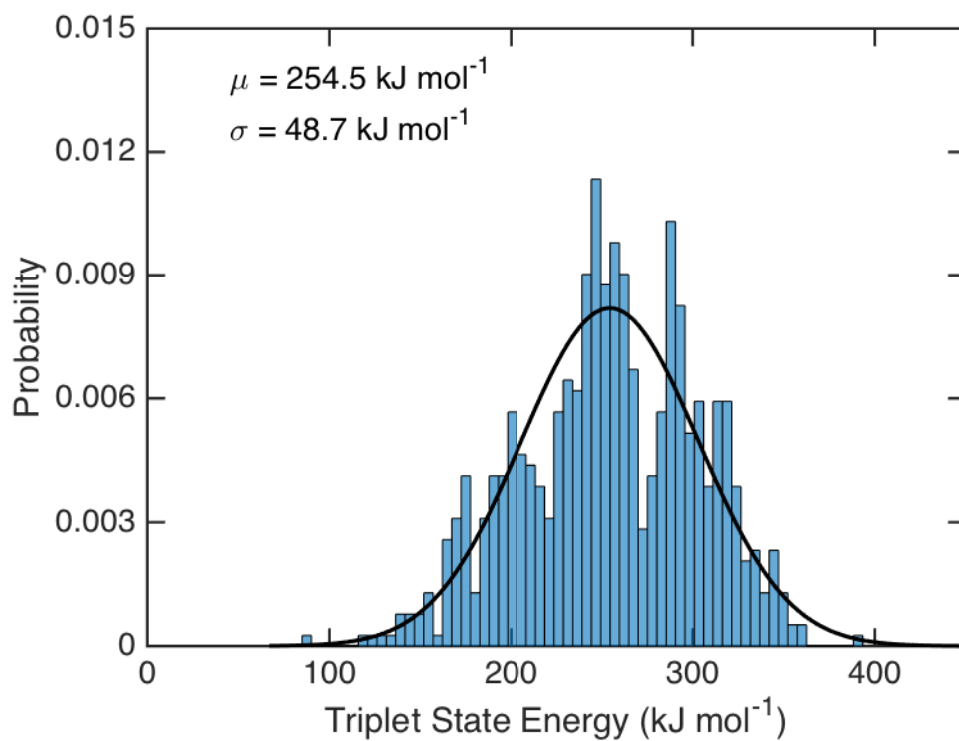
Figure 2. Triplet state energy (in polar solvent) for 755 different organic compounds. The data was taken from reference [49]. The line represents the fitted normal distribution function with average value μ and standard deviation σ.