Optical Properties and Photochemical Production of Hydroxyl Radical and Singlet Oxygen after Ozonation of Dissolved Organic Matter

Frank Leresche^{□,⊥}, Jeremy A. Torres-Ruiz[†], Tyler Kurtz[⊥], Urs von Gunten^{#,‡}, and Fernando L.
Rosario-Ortiz^{□,⊥,*}

[⊥]Environmental Engineering Program, University of Colorado Boulder, Colorado 80309, United
States

[□]Department of Civil, Environmental and Architectural Engineering, University of Colorado
Boulder, Colorado 80309, United States

[†]Department of Chemistry, University of Puerto Rico, Mayagüez, Puerto Rico 00682, United
States

[#]Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133,
CH-8600 Dübendorf, Switzerland

[‡]School of Architecture, Civil and Environmental Engineering (ENAC), Ecole Polytechnique
Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

* Corresponding author: Fernando.rosario@colorado.edu

19 **Abstract**

20 This study focuses on the effects of ozonation on the optical and photochemical properties
21 of dissolved organic matter (DOM). Upon ozonation, a decrease in light absorption properties of
22 DOM was observed concomitantly with a large increase in singlet oxygen ($^1\text{O}_2$) and hydroxyl
23 radical ($\cdot\text{OH}$) quantum yields ($\Phi_{1\text{O}_2}$ and $\Phi_{\cdot\text{OH}}$, respectively). The decrease in absorbance was
24 linked to the reaction of DOM chromophores with ozone and $\cdot\text{OH}$, formed as a secondary oxidant,
25 while the increase in $\Phi_{1\text{O}_2}$ and $\Phi_{\cdot\text{OH}}$ are linked to the formation of quinone-like moieties from the
26 reaction of ozone with phenolic DOM moieties. Investigations using benzoic acid as a $\cdot\text{OH}$ probe
27 and methanol as a $\cdot\text{OH}$ scavenger indicated that not only is $\cdot\text{OH}$ formed, but that other
28 hydroxylating species ($\cdot\text{OH}$ -like) are also produced upon DOM photo-irradiation.

29 Graphical Abstract

30

Introduction

Ozonation is a process widely applied in drinking water treatment and more recently also as a polishing step in secondary effluents of municipal wastewater treatment plants.(1, 2) In addition to its use as a disinfectant and for the oxidation of micropollutants, ozone reacts with the dissolved organic matter (DOM) present in the water, leading to changes in its physico-chemical properties.(3-5) Because wastewater is usually discharged to a receiving water body (e.g., river, lake, sea) and can represent an important fraction of the receiving water, ozone-induced transformations of DOM are of interest.(6, 7)

DOM plays an important role in the photo-transformation of contaminants in surface waters by absorbing a portion of sunlight, thereby decreasing the direct photo-transformation of contaminants (equation 1), and by producing reactive intermediates (RI). These RI include excited triplet state of the DOM ($^3\text{DOM}^*$, equation 2), singlet oxygen ($^1\text{O}_2$, equation 3) and hydroxyl radical ($^{\bullet}\text{OH}$, equation 4, where DOM' represents a transformed chromophore of DOM). The RI can react with contaminants leading to transformation products (equation 5).(8-12) Ozonation of DOM will affect this photochemistry by changing the light absorption properties of DOM and potentially affecting the yields of the RI.(3)

Studies have shown that ozonation of DOM leads to changes in its physicochemical properties, including decreases in light absorption, fluorescence intensity, chemical oxygen demand, and electron donating capacity, as well as the formation of low molecular weight compounds such as carboxylic acids and aldehydes.(1, 3, 4, 13, 14) DOM moieties have a large range of reactivity towards ozone. DOM moieties with high ozone reactivities are believed to be mostly phenolic compounds that react to quinones, ketones, catechol, and ring opening products.(15, 16) Ozone reactions with DOM were covered in recent publications and we refer the reader to those for more information.(3, 4) Reaction of ozone with DOM forms $\cdot\text{OH}$ as a side product that can further react with the DOM.(17-19) $\cdot\text{OH}$ reacts with aromatic or conjugated DOM moieties by $\cdot\text{OH}$ addition or with unsaturated moieties by $\text{H}^\bullet$ abstraction.(1) Typical second order rate constants for $\cdot\text{OH}$ addition reactions are between $1\text{-}10\times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ and are relatively faster than $\text{H}^\bullet$ abstraction reactions, with typical second order rate constants in the range of $0.1\text{-}1\times 10^9 \text{ M}^{-1} \text{ s}^{-1}$.(20)

In a previous publication, we discussed the effects of ozone on the optical properties of DOM and its effect on the photochemical generation of $^1\text{O}_2$.(3) Specifically, it was shown that during ozonation in the presence of *t*-butanol to quench $\cdot\text{OH}$ (formed via ozone decomposition), both the fluorescence and $^1\text{O}_2$ quantum yield ($\Phi_{1\text{O}_2}$) increased concomitantly. The fluorescence quantum yield increased by 92-111% for increasing specific ozone doses from 0 to 1 $\text{mmol O}_3 \text{ mmol C}^{-1}$. Similarly, the $\Phi_{1\text{O}_2}$ increased by 273-844%. In this previous publication it was hypothesized that this was due to the preferential reactions of ozone with phenolic DOM moieties and low fluorescence chromophores. This hypothesis is based on the following observations: (1) Ozone reacts towards DOM faster at high pH than at low pH.(4) This can be explained by the reaction of ozone towards phenolate being several orders of magnitude faster than towards phenols

(e.g. $k_{O_3, \text{phenolate}} = 1.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ vs $k_{O_3, \text{phenol}} = 1.3 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ (1)). (2) The aforementioned formation of quinones from ozonation of phenols. (3) The fact that phenols have low $\Phi_{^1O_2}$ (1-6%) (21) while quinones have generally higher $\Phi_{^1O_2}$ (20-98%). (22-25) (4) Low ozone doses can induce an increase in 1O_2 production rate. (3) It should be noted that ref. (3) attributes the observed increase in fluorescence quantum yield upon DOM ozonation to a preferential ozone reaction towards chromophores absorbing at long wavelengths that have low fluorescence quantum yields. Such preferential reactions of ozone with chromophores absorbing at long wavelengths may also explain part of the observed increase in $\Phi_{^1O_2}$. However, given the magnitude of the effect, it cannot be the sole explanation for the observed increase in $\Phi_{^1O_2}$.

The goal of this study is to expand on our earlier work and investigate the impact of ozone on the photochemical formation of $^{\bullet}OH$ from DOM. Specifically, this paper focuses on the effects of ozonation on (1) the optical properties of DOM, such as the absorbance spectra and of parameters that can be derived from the absorbance, and (2) the potential of DOM to generate singlet oxygen (1O_2) and hydroxyl radical ($^{\bullet}OH$) under simulated sunlight irradiation. In this study, we evaluated the impact of ozone *without* a $^{\bullet}OH$ quencher, therefore, providing a realistic depiction of the expected effects during the application of ozone in engineered systems. In addition to these effects of ozone, this paper also provides new insights on the molecular composition of DOM.

92 **Materials and Methods**

93 **Analytical instrumentation**

94 UV-Vis spectra and absorbance were measured on a Cary 100 Bio UV-Visible
95 spectrophotometer using 0.2, 1, or 5 cm pathlength quartz cuvettes. The pH was measured using a
96 calibrated Orion Star A211 pH-meter using a Thermo Scientific Orion pH electrode model
97 8157BNUMD.

98 Following the photo-irradiation experiments, concentrations of the probe compounds and of
99 the actinometer were measured in duplicate using an Agilent 1200 high-performance liquid
100 chromatography (HPLC) system equipped with a UV-Vis detector, a fluorescence detector and an
101 Agilent Eclipse Plus C-18 5 μ m particle size reverse phase column. Details of the applied isocratic
102 method are provided in Table S1 of the electronic supplementary information (ESI).

103 **Chemicals and solutions**

104 Chemicals were used as received except for *p*-nitroanisole (PNA), which was recrystallized
105 in hexane, and benzoic acid (BA), which was recrystallized in water to remove some salicylic acid
106 (SA) impurities from the commercially available product. For a complete list of chemicals, refer
107 to Text S1 (ESI). All solutions were prepared in ultrapure water (resistivity 18.2 M Ω cm) obtained
108 from a Sartorius Stedim or equivalent dispenser. A 100 mM buffer stock solution was prepared by
109 adding the appropriate amount of sodium phosphate salts. Diluted 1/10 v/v with water, it resulted
110 in a 10 mM pH 7 phosphate buffer used for the experiments.

111 Two DOM isolates were selected from the International Humic Substance Society (IHSS,
112 St-Paul, MN, USA) as representative of autochthonous (Pony Lake fulvic acid (PLFA), 1R109F)
113 and allochthonous (Suwannee River fulvic acid (SRFA), 1S101F) DOM. The carbon content of

the DOM solutions was measured spectrophotometrically using the specific ultraviolet absorbance value at the wavelength $\lambda=254$ nm ($SUVA_{254}$) of 4.2 and 2.5 L mg_C⁻¹ m⁻¹ for SRFA and PLFA, respectively.(5) Stock solutions of the two DOM types were prepared at concentrations of ≈ 50 mg_C L⁻¹ in pH 7 buffer. After stirring for 3-4 hours, the stock solutions were filtered by ultrapure water prewashed 0.45 μ m pore size polyethersulfone filters (Whatman) and the carbon content was then measured spectrophotometrically.

Ozonation experiments

An ozone/oxygen gas mixture was obtained from an Ozone Solutions ozone generator model TG-40 and bubbled in a cooled (2°C) 2 L cylindrical vessel filled with ultrapure water. An aqueous ozone stock solution with an ozone concentration of ≈ 1 mM was obtained and was measured using a 0.2 cm path length quartz cuvette on the aforementioned spectrophotometer and a molar absorption coefficient value of 3200 M⁻¹ cm⁻¹ at $\lambda=260$ nm.(1) The ozone solution was then added to buffered (pH 7, using the aforementioned phosphate buffer) DOM solutions (final concentration 5 mg_C L⁻¹) at various specific ozone doses (≤ 1 mmol_{O₃} mmol_C⁻¹) and the solutions were kept for a minimum of 2 days at 4°C to allow ozone to react to completion. The specific ozone doses were chosen to be similar to those used in drinking water or wastewater facilities.(26-29) It should be noted that for the specific ozone doses of 0.75 and 1 mmol_{O₃} mmol_C⁻¹ only the UV-vis absorbance spectra are presented. These two doses have low absorbance values (< 0.01 at $\lambda=350$ nm) that could suffer from systematic errors on the absorbance measurements and we decided not to use them for further calculations.

Irradiation experiments

The methods to determine ¹O₂ steady-state concentration ($[^1O_2]_{ss}$) and quantum yield (Φ_{1O2}) are described elsewhere.(3) Briefly, $[^1O_2]_{ss}$ during photoirradiation of the ozonated DOM solutions

was measured by spiking the solution with 22.5 μM of furfuryl alcohol (FFA) and 0.1 M of methanol (to quench $\cdot\text{OH}$). At this FFA concentration, $^1\text{O}_2$ is mostly ($\approx 99\%$) deactivated by collision with water and the presence of FFA does not affect but allows to determine the $[^1\text{O}_2]_{\text{ss}}$.(30) The solutions were then irradiated in 5 mL glass vials in a Rayonet photo-reactor model RPR-100 equipped with 16 RPR-3500A lamps that had an emission spectrum centered around 366 nm (see Figure S1 (ESI) for an emission spectrum of the lamps). 100 μL aliquots were taken at regular time intervals (total irradiation time ≈ 1 hour), diluted 1/1 (v/v) with ultrapure water and subsequently the FFA concentration was analyzed by HPLC. The abatement of FFA was fitted to a first-order kinetic model using the software Origin 2018. The steady-state concentration of $^1\text{O}_2$ ($[^1\text{O}_2]_{\text{ss}}$) during the experiments was determined using the second-order rate constant $k_{\text{FFA},^1\text{O}_2}$ of $(1.00 \pm 0.04) \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$.(31) $\Phi_{^1\text{O}_2}$ was then determined by dividing $[^1\text{O}_2]_{\text{ss}}$ by the rate of light absorption.

The experiments to determine the production rate of $\cdot\text{OH}$ and the $\Phi_{\cdot\text{OH}}$ were conducted similarly as for the determination of $^1\text{O}_2$ and are described in Text S2 (ESI). Briefly, we spiked the samples with 1 mM of BA and the $\cdot\text{OH}$ production was determined by following the zero-order production of SA from the reaction of BA with $\cdot\text{OH}$ using the yield of the reaction of $\text{BA} + \cdot\text{OH} \rightarrow \text{SA}$ of 15.5%.(32) For the experiments that used both BA and methanol as $\cdot\text{OH}$ probes, the experiment's irradiation time was relatively longer due to the overall lower rate of SA production and we observed a small ($\approx 10\%$ in control experiments containing the ozonated DOMs, 100 nM SA and 0.01 M methanol, irradiated for 225 minutes) degradation of SA during the experiments. The rate of SA production was calculated in these experiments by fitting the SA concentration to a zero-order production rate followed by a first-order degradation rate for SA. The corresponding equation is derived in Text S3 (ESI) and an example of fitting is provided in Figure S8 (ESI).

Concomitantly to the FFA or BA irradiation experiments, we ran chemical actinometry daily to determine the irradiance of the lamps in the reactor. We used the PNA + pyridine actinometer at a concentration of 10 μM of PNA and of 5 mM of pyridine.(33) At this pyridine concentration, the quantum yield for the phototransformation of PNA (which depends on the pyridine concentration) is 1.74×10^{-3} .(34) All results were normalized by using the daily measured PNA phototransformation rate to the average PNA phototransformation value of $8.68 \times 10^{-4} \text{ s}^{-1}$. The photon fluence rate in the interval $\lambda = 340\text{-}410\text{nm}$ was calculated to be of $1.40 \times 10^{-3} \text{ einstein m}^{-2} \text{ s}^{-1}$.

Calculation of optical parameters

Optical parameters calculated from the UV-Vis absorption spectra are often used because absorption spectra are easy to measure and the optical parameters correlate with DOM properties that are harder to measure such as Φ_{IO_2} and Φ_{OH} . Several optical parameters were calculated using the measured UV-Vis absorbance spectra of the solutions. The specific UV absorbance $SUVA_{254}$ (unit $\text{L mgC}^{-1} \text{ m}^{-1}$) was calculated by dividing the absorbance at the wavelength $\lambda = 254\text{nm}$ by the carbon concentration (5mgC L^{-1}). Fitting a single-parameter exponential regression to the absorbance spectra in the wavelength range 300-600nm, the spectral slope (S , unit nm^{-1}) was calculated as the coefficient of the exponential fit (according to equation 6) using $Abs_{\lambda=350\text{nm}}$ as a constant. The $E2/E3$ ratio was calculated as the ratio of the absorbance values at the wavelengths $\lambda = 250$ over $\lambda = 365\text{nm}$. Both the spectral slope and the $E2/E3$ ratio inversely correlate with the molecular weight of the DOM and positively correlate with Φ_{IO_2} and Φ_{OH} .(35-37) The wavelength averaged specific absorption coefficient ($SUVA_{\text{avg}}$, unit $\text{L mgC}^{-1} \text{ m}^{-1}$), which is a proxy for the amount of light absorbed by the solutions, was calculated in the wavelength interval 340-410 nm

according to equation 7, where $SUVA_\lambda$ is the specific absorption coefficient at a wavelength λ (unit $L\ mgC^{-1}\ m^{-1}$) and I_λ is the normalized photon irradiance of the Rayonet reactor.

$$Abs_\lambda = Abs_{\lambda=350nm} e^{-S(\lambda-350nm)} \quad (6)$$

$$SUVA_{avg} = \frac{\int_{340nm}^{410nm} SUVA_\lambda I_\lambda d\lambda}{\int_{340nm}^{410nm} I_\lambda d\lambda} \quad (7)$$

Standard deviation

The standard deviations were calculated for the first-order degradation curves of FFA and PNA and for the zero-order production lines for SA and propagated from there. The error on the rate constants from the literature used in the calculations were also incorporated in the error calculation. The standard errors calculated for FFA degradation and SA production were used in addition to the standard errors for $SUVA_{avg}$ in the calculation of Φ_{1O2} and $\Phi_{\bullet OH}$. The standard errors for $SUVA_{avg}$ were calculated as the standard deviation of triplicate UV-Vis absorbance measurements of the DOM solutions and propagated from there. Examples of calculations are shown in Text S4 (ESI).

195 **Results and discussion**

196 **Effects of ozonation on the optical properties of DOM**

197 *Absorption properties*

198 As shown previously, ozonation induces a large decrease in the UV-Vis absorption of the
199 two DOM isolates tested in this study (Figure 1). The allochthonous SRFA absorbs more light
200 because it has a higher content of aromatic moieties than PLFA. While the absolute difference
201 ($A_{O_3 \text{ dose}=0} - A_{O_3, \text{dose}}$) of the absorbance is more important at shorter wavelengths (Figures 1C and
202 D), the relative decrease ($A_{O_3}/A_{O_3=0}$) in absorbance is smaller at shorter wavelengths (Figures 1E
203 and D). Upon ozonation, the relative decrease in absorbance ($A_{O_3}/A_{O_3=0}$) differs for the two DOM
204 types (Figures 1E and D). For PLFA, the relative decrease is more significant at longer
205 wavelengths, whereas for SRFA the wavelength dependence is relatively small (see Figure S2,
206 ESI). Typically, small ozone doses induce a large decrease in absorbance (Figures 1 and S2). For
207 SRFA at $\lambda=254$ nm, the absorbance decreases by 52% for a specific ozone dose of 0.2 mmol_{O_3}
208 $\text{mmol}_{C^{-1}}$ while for PLFA the decrease is 63%.

Figure 1. Effects of the specific ozone doses on the absorbance spectra of the two DOM samples Pony Lake and Suwannee River fulvic acid (PLFA and SRFA respectively). (A) and (B): Mass absorptivity as a function of the specific ozone dose for (A) PLFA, and (B) SRFA. (C) and (D): variation of the mass absorptivity ($A_{O_3, \text{dose}=0} - A_{O_3, \text{dose}}$) as a function of the specific ozone dose for (C) PLFA and (D) SRFA. (E) and (F): Ratio of the absorptivity ($A_{O_3, \text{dose}}/A_{O_3, \text{dose}=0}$) as a function of the specific ozone dose for (E) PLFA and (F) SRFA. The samples were buffered at pH 7 using 10 mM phosphate buffer, with a DOM concentration of $5 \text{ mg}_C \text{ L}^{-1}$.

The observed effects of ozone on the absorption properties of DOM for specific ozone doses of up to $0.2 \text{ mmol}_{O_3} \text{ mmol}_C^{-1}$ are similar to a previous study conducted with the same DOM

types in presence of a $\cdot\text{OH}$ scavenger (as seen by similar reduction in $SUVA_{254}$, $SUVA_{280}$ and $SUVA_{350}$ values, see Figure S3, ESI)(3) and are similar to what was observed for three specific ozone doses for the same DOM types in the absence and presence of a $\cdot\text{OH}$ scavenger.(5) These observations can be mainly rationalized by the reaction of ozone with highly reactive activated aromatic compounds such as phenols. For specific ozone doses $> 0.2 \text{ mmolO}_3 \text{ mmolC}^{-1}$, the extent of $SUVA_{254}$ decrease was higher in the current study compared to our previous investigation.(3) This can be attributed to a depletion of the pool of highly ozone-reactive chromophores for specific ozone doses $> 0.2 \text{ mmolO}_3 \text{ mmolC}^{-1}$ and the reactions of $\cdot\text{OH}$ with these chromophores, which is possible due to the absence of a $\cdot\text{OH}$ scavenger. Such chromophores can be non-activated aromatic moieties such as benzene or methylated benzenes, which have low second order rate constants for the reactions with ozone but are highly reactive towards $\cdot\text{OH}$ ($k_{\cdot\text{OH},\text{benzene}} = 3.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$).(38) The reaction of benzene with $\cdot\text{OH}$ produces phenol, which then reacts quickly with ozone.

Optical parameters

Optical parameters such as $SUVA_{254}$, the ratio $E2/E3$, and the spectral slope are often calculated from the UV-Vis absorbance spectra for the characterization of waters.(35) In turn, these parameters are used to develop correlations with other reactivity measures of DOM, including the photochemical formation of $^1\text{O}_2$ or $\cdot\text{OH}$ as well as other properties such as aromaticity and molecular weight. (37, 39-41) Changes in these parameters are presented in Figure S4 (ESI) as a function of the specific ozone dose. A marked decrease in $SUVA_{254}$ with increasing specific ozone dose can be observed for both SRFA and PLFA (Figure S4A, ESI). In contrast, an increase in both the ratio $E2/E3$ and the spectral slope can be observed for increasing specific ozone doses (Figures S4B and C, ESI), respectively), being more pronounced for PLFA than for SRFA.

The results for ozonation in the presence and absence of an $\cdot\text{OH}$ scavenger (*t*-butanol) also show that the decrease in $SUVA_{254}$ and the increases in $E2/E3$ and the spectral slope are more important for PLFA in absence of an $\cdot\text{OH}$ scavenger (Figure S4, ESI). For SRFA, $SUVA_{254}$ behaves similarly, however, for $E2/E3$ and the spectral slope the observed increases are more pronounced in presence of the $\cdot\text{OH}$ scavenger. The spectral slope PLFA results are in contradiction with the results presented in ref. (5) where the absence of the $\cdot\text{OH}$ scavenger produced the opposite effect. The observed difference could be attributed to differences in the experimental conditions, with different DOM concentrations ($10 \text{ mg}_C \text{ L}^{-1}$ in ref. (5) vs $5 \text{ mg}_C \text{ L}^{-1}$ in the current study) and a difference in the way the ozonation experiments was conducted (in ref. (5) the residual ozone was quenched after 2 hours vs 2 days in the present experiments).

As aforementioned, the $SUVA_{254}$, the ratio $E2/E3$, and the spectral slope correlate with $\Phi_{\cdot\text{OH}}$ and Φ_{IO_2} . These correlations are presented in Figure S5 and Table S2 (ESI). The slopes of the Φ_{IO_2} regressions (Figures S5A-C) are comparable (factor 2) to our previous study with the same isolates in the presence of a $\cdot\text{OH}$ scavenger and to Everglades DOM (Table S2, ESI).^(3, 37) The slopes of the $\Phi_{\cdot\text{OH}}$ regressions in this study are significantly larger (by a factor of 2-6) than that of the Everglades DOM.⁽³⁷⁾ This observed difference in slope for the $\Phi_{\cdot\text{OH}}$ regressions makes the direct use of any of the parameters ($SUVA_{254}$, the ratio $E2/E3$, and the spectral slope) as predictors of $\Phi_{\cdot\text{OH}}$ questionable. The difference in slope may reflect a change in DOM chemical composition due to ozonation, as opposed to DOM composition of the Everglades DOM.

Effects of ozonation on the photochemical generation of $^1\text{O}_2$ from DOM

The extent of light absorbed in the wavelength interval 340-410 nm by the two DOM isolates can be characterized by $SUVA_{\text{avg}}$ as a proxy, which decreases in an exponential-like fashion upon ozonation (Figure 2A). The steady-state concentration of $^1\text{O}_2$ produced upon UV irradiation (centered at $\lambda=366$ nm, see Figure S1, ESI) increases slightly for low specific ozone doses (0 to 0.1 $\text{mmolO}_3 \text{ mmolC}^{-1}$) and then decreases sharply (by a factor of 3) for PLFA and moderately for SRFA (Figure 2B). Because $[^1\text{O}_2]_{\text{ss}}$ is decreasing relatively less compared to $SUVA_{\text{avg}}$ (Figure 2A) it can be concluded that $\Phi_{1\text{O}_2}$ (i.e., the ratio of the production of $^1\text{O}_2$ over the extent of light absorption) should increase with increasing specific ozone doses. This is illustrated in Figure 2C, where $\Phi_{1\text{O}_2}$ increases close to linearly with increasing specific ozone doses. The increase in $\Phi_{1\text{O}_2}$ is more significant for PLFA, with an increase from 1.9% (non-ozonated) to 13.1% (0.5 $\text{mmolO}_3 \text{ mmolC}^{-1}$), than for SRFA for which an increase from 1.0 to 8.5% for the same specific ozone doses was observed.

Figure 2. Effects of specific ozone doses on (A) the light absorption, (B) the generation of singlet oxygen (¹O₂) and (C) the ¹O₂ quantum yields (Φ_{¹O₂}) for Pony Lake and Suwannee River fulvic acids (PLFA and SRFA, respectively). (A) Specific UV-Vis absorption (SUVA_{avg}) in the wavelengths range 340-410 nm. (B) Measured steady-state ¹O₂ concentration ([¹O₂]_{ss}) as a function of the specific ozone doses. (C) ¹O₂ quantum yield (Φ_{¹O₂}) as a function of the specific ozone dose. Red squares/lines: PLFA; black circles/lines: SRFA, at a concentration of 5 mg_C L⁻¹. Solutions were buffered with 10 mM phosphate at pH 7, without hydroxyl radical scavenger. Error bars represents standard errors in (B) and (C) were obtained from pseudo-first order fittings (FFA experiments in duplicate). Lines are shown to guide the eye.

Increases in Φ_{1O_2} were similar in a previous study on wastewater ozonation (13) and in a study with the same DOM isolates but in the presence of a $\cdot OH$ scavenger (3) (see Figure S7, ESI for a comparison with the data from ref. (3)). The Φ_{1O_2} values were $3.2 \pm 0.4 \%$ for the non-ozonated wastewater and increased to $9.3 \pm 1.8 \%$ for a specific ozone dose of $0.25 \text{ mmol}_{O_3} \text{ mmol}_C^{-1}$, (13) which is similar to the observations for PLFA. For ozonation of the same DOM isolates in presence of an $\cdot OH$ scavenger, Φ_{1O_2} also increased close to linearly for low specific ozone doses ($< 0.35 \text{ mmol}_{O_3} \text{ mmol}_C^{-1}$) but leveled off for higher specific ozone doses. (3) This increase in Φ_{1O_2} was attributed to the formation of quinones from the reaction of ozone with phenolic DOM moieties and the observed increase of Φ_{1O_2} in Figure 2 can most likely also be attributed to a formation of quinones.

The normalized (to the non-ozonated experiment) Φ_{1O_2} in this study was compared to the data from ref. (3), where the ozonation was carried out in the presence of an $\cdot OH$ scavenger (see Figure S7, ESI). The ratio of Φ_{1O_2} from this study and the data from ref. (3) for PLFA is close to 1 for specific ozone doses up to $0.35 \text{ mmol}_{O_3} \text{ mmol}_C^{-1}$ and increases to 1.5 for a specific ozone dose of $0.5 \text{ mmol}_{O_3} \text{ mmol}_C^{-1}$ (see Figure S7, ESI). To determine if this specific ozone dose was anomalous, Φ_{1O_2} was examined in detail by compiling the corresponding extent of 1O_2 production and extent of light absorption as a function of the ozone dose (see Table S3, ESI). The increase to a value of 1.5 for a specific ozone dose of $0.5 \text{ mmol}_{O_3} \text{ mmol}_C^{-1}$ can be attributed to a decrease in the extent of 1O_2 production. (3) Ref. (3) presents additional specific ozone doses, which confirm that this observation is reproducible. Therefore, the observed value of a ratio of 1.5 observed in Figure S7 (ESI) is not an outlier. For SRFA, the ratio decreases to a value of 0.7 for specific ozone doses up to $0.15 \text{ mmol}_{O_3} \text{ mmol}_C^{-1}$ and then increases to 1.5 for specific ozone doses between 0.15 and to $0.5 \text{ mmol}_{O_3} \text{ mmol}_C^{-1}$.

309 The increase in the normalized quantum yield ratio to 1.5 for higher specific ozone doses
310 can likely be attributed to the reaction of $\cdot\text{OH}$ with non-activated aromatics, which are less reactive
311 to ozone. For the experiments in presence of a $\cdot\text{OH}$ scavenger, the pool of chromophores that are
312 non-reactive towards ozone are maintained and should overall have a lower $\Phi_{1\text{O}_2}$ than the pool of
313 ozonated chromophores. Alternatively, in the present study in absence of a $\cdot\text{OH}$ scavenger, the
314 pool of ozone-resistant chromophores should react with $\cdot\text{OH}$ to produce phenols and then quinones
315 upon reaction with ozone,(15) which have a higher $\Phi_{1\text{O}_2}$.

Effects of ozonation on the $\cdot\text{OH}$ generation potential of DOM during UV irradiation

An approach similar to the $\Phi_{1\text{O}_2}$ experiments was chosen to elucidate the factors influencing $\Phi_{\cdot\text{OH}}$ upon ozonation. The light absorbed by the two DOM types (quantified by $SUVA_{\text{avg}}$) decreases dramatically as a function of the specific ozone dose (Figure 2A). Figure 3A shows that the rate of $\cdot\text{OH}$ production upon irradiation, $r_{\cdot\text{OH}}$, is fairly constant with increasing specific ozone doses for PLFA, while for SRFA it first increases for specific ozone doses $\leq 0.2\text{mmolO}_3\text{ mmolC}^{-1}$ and then decreases for higher specific ozone doses. Because $SUVA_{\text{avg}}$ is decreasing while $r_{\cdot\text{OH}}$ remains fairly constant with increasing specific ozone doses, it can be concluded that $\Phi_{\cdot\text{OH}}$ is increasing, similarly to $\Phi_{1\text{O}_2}$. This is confirmed in Figure 3B, with a significant increase in $\Phi_{\cdot\text{OH}}$ for PLFA (by a factor of 28) and for SRFA (by a factor of 8.6) when the specific ozone dose increases from 0-0.5 $\text{mmolO}_3\text{ mmolC}^{-1}$.

Figure 3. UV irradiation of DOM: Effects of the specific ozone dose on (A) the hydroxyl radical ($\cdot\text{OH}$) production rate (r_{OH}) and (B) the $\cdot\text{OH}$ quantum yield (Φ_{OH}) for Pony Lake and Suwannee River fulvic acid (PLFA and SRFA, respectively). Red squares/lines: PLFA; black circles/lines: SRFA at a concentration of 5 mg C L^{-1} . Solutions were buffered with 10 mM phosphate at pH 7, without hydroxyl radical scavenger. Error bars represent standard errors obtained from pseudo-first order fittings (BA experiments in duplicate). Lines are shown to guide the eye.

The formation pathway of $\cdot\text{OH}$ upon DOM irradiation is not entirely elucidated and many pathways have been proposed, which are summarized in a recent review.(11) Two main mechanisms are usually considered, H_2O_2 -dependent and H_2O_2 -independent pathways. The H_2O_2 -dependent pathways involve Fenton and photo-Fenton chemistry and account for up to 50% of the

$\cdot\text{OH}$ production in some samples.(42, 43) A variety of possibilities have been proposed for the H_2O_2 -independent pathways. They include the photolysis of hydroxy substituted benzoic acids and phenols(44) and the oxidation of water or hydroxide ions by $^3\text{DOM}^*$, a mechanism that was seen to take place for a few photosensitizers such as 1-nitronaphthalene or anthraquinone-2,6-disulfonate.(45-47) If the production of $\cdot\text{OH}$ was due only to the photolysis of hydroxy substituted benzoic acids and phenols, one would expect $\Phi_{\cdot\text{OH}}$ to decrease upon ozonation due to the reaction and decomposition of the phenolic DOM moieties with ozone.(4, 15, 16, 48) The observed increase in $\Phi_{\cdot\text{OH}}$ indicates that the source of $\cdot\text{OH}$ is probably the oxidation of water or hydroxide ions by $^3\text{DOM}^*$, or the formation of H_2O_2 during ozonation, which could induce the H_2O_2 -dependent pathway.

The one-electron oxidation potentials of water and hydroxide are -2.73 V and -1.90 V respectively.(49) Quinones are known to have a high intersystem crossing yield and under photoirradiation form triplets that have a one-electron reduction potential in the range of 2.2-2.4 V (9) which is high enough to react with hydroxide and potentially with water. Therefore, it is hypothesized that the observed increase in $\Phi_{\cdot\text{OH}}$ could be attributed to the formation of quinones, which are common products from the reactions of ozone with hydroxylated aromatic compounds (e.g., phenols). (4, 15, 16, 48) It should be noted that for non-ozonated DOM, the photolysis of hydroxy substituted benzoic acids and phenols should not be excluded as a $\cdot\text{OH}$ source. A scenario for which ozonation increases the $\cdot\text{OH}$ production from DOM quinone moieties and decreases the production of $\cdot\text{OH}$ from the photolysis of substituted benzoic acid and phenols is also possible.

In studies on the oxidation of water or hydroxide ions by $^3\text{DOM}^*$, it has been shown that some other hydroxylating species ($\cdot\text{OH}$ -like) are also generated, that have similar hydroxylating properties as $\cdot\text{OH}$ but lower reactivity.(42) Experiments involving photosensitizers indicate that

some of them produce $\cdot\text{OH}$ (45, 50), but that for some photosensitizers, irradiation yielded species that were assigned to sensitizer-water exciplexes that could potentially be $\cdot\text{OH}$ -like species.(46, 51-53)

It should be noted that the probe used to quantify $\cdot\text{OH}$ in our experiments, BA, could potentially also react with $\cdot\text{OH}$ -like species (42, 52) and that part of the $\cdot\text{OH}$ measured in the experiments presented in Figure 3 could be caused by $\cdot\text{OH}$ -like species.

Qualitative assessment of the production of $\cdot\text{OH}$ and of other hydroxylating species ($\cdot\text{OH}$ -like)

To qualitatively assess the potential formation of $\cdot\text{OH}$ -like species upon DOM ozonation, we designed the following competition kinetic experiment using BA as a probe and methanol as an $\cdot\text{OH}$ scavenger (it should be noted that it is also possible to quantify the formation of $\cdot\text{OH}$ using methanol but we only used it as a scavenger here). The assumption of these experiments is that the ratio $\frac{k_{\text{BA},\cdot\text{OH}}}{k_{\text{MeOH},\cdot\text{OH}}}$ (where $k_{\text{BA},\cdot\text{OH}}=5.9\times10^9 \text{ M}^{-1} \text{ s}^{-1}$ or $k_{\text{MeOH},\cdot\text{OH}} = 9.7\times10^8 \text{ M}^{-1} \text{ s}^{-1}$ are the second-order rate constants for the reactions between BA or methanol and $\cdot\text{OH}$, respectively)(20) will be different than the ratio $\frac{k_{\text{BA},\cdot\text{OH-like}}}{k_{\text{MeOH},\cdot\text{OH-like}}}$ (where $k_{\text{BA},\cdot\text{OH-like}}$ and $k_{\text{MeOH},\cdot\text{OH-like}}$ are the second-order rate constants for the reactions between BA or methanol and $\cdot\text{OH}$ -like species, respectively). The methanol quenching experiments were modeled using equation 8, where f is the fraction of $\cdot\text{OH}$ reacting with BA, $k_{\text{DOM},\cdot\text{OH}}$ is the second-order constant between DOM and $\cdot\text{OH}$ (4.6×10^8 and $1.9\times10^8 \text{ M}^{-1} \text{ s}^{-1}$ for PLFA and SRFA respectively (54)) and $k_{\text{NO}_2^-, \cdot\text{OH}}$ is the second-order rate constant for the reaction between nitrite and $\cdot\text{OH}$ ($1\times10^{10} \text{ M}^{-1} \text{ s}^{-1}$ (20)).

$$f = \frac{k_{\text{BA}, \cdot\text{OH}}[\text{BA}]}{k_{\text{BA}, \cdot\text{OH}}[\text{BA}] + k_{\text{MeOH}, \cdot\text{OH}}[\text{MeOH}] + k_{\text{DOM}, \cdot\text{OH}}[\text{DOM}] + k_{\text{NO}_2^-, \cdot\text{OH}}[\text{NO}_2^-]} \quad (8)$$

383 Note that the reactions of $\cdot\text{OH}$ with the phosphate buffer and carbonate/bicarbonate from
 384 atmospheric CO_2 were neglected, as the fractional consumption of $\cdot\text{OH}$ by these components was
 385 small (f value <0.0001 for the phosphate buffer and <0.01 for the carbonate/bicarbonate,
 386 considering saturation conditions at 20°C for the carbonate/bicarbonate concentration).
 387 A control experiment was performed where nitrite was used as an alternative source of $\cdot\text{OH}$ to
 388 confirm the validity of equation 8 and to verify that methanol acts as an ideal quencher (a potential
 389 drawback of the use of methanol is that its reaction with $\cdot\text{OH}$ produces $\text{O}_2^{\cdot-}$ quantitatively,(1) and
 390 $\text{O}_2^{\cdot-}$ disproportionation forms H_2O_2 that is potentially an additional source of $\cdot\text{OH}$ in the system).

391
 392 **Figure 4.** Normalized salicylic acid (SA) production rate as a function of the methanol
 393 concentration. (A) Control nitrite experiment (violet, $6\mu\text{M NaNO}_2$). (B) Pony Lake Fulvic Acid
 394 (PLFA) treated with specific ozone doses of 0, 0.2 and $0.5\text{ mmolO}_3\text{ mmolC}^{-1}$. (C) Suwannee River
 395 Fulvic Acid (SRFA) treated with specific ozone doses of 0, 0.2 and $0.5\text{ mmolO}_3\text{ mmolC}^{-1}$. The

black lines represent the fraction of $\cdot\text{OH}$ reacting with BA according to Eq. 8. Benzoic acid concentration 1mM, carbon concentration 5mgC L^{-1} . Solutions were buffered with 10 mM phosphate at pH 7. Lines are shown to guide the eye.

Figure 4 presents the normalized (to $[\text{methanol}]=0$) rate of SA production. Figure 4A shows that equation 8 accurately describes the experiments performed with the photolysis of nitrite as a source of $\cdot\text{OH}$. In Figures 4B and 4C, a deviation from equation 8 is observed, indicating that BA is not only reacting with $\cdot\text{OH}$ in the system. In contrast, $\cdot\text{OH}$ -like species might also be produced and involved in BA abatement. The ratio of the calculated value from equation 8 over the experimental value is 1 for the nitrite control experiment. For PLFA the ratio is fairly constant for all specific ozone doses with a mean value of 0.47 for the non-ozonated samples and 0.46 and 0.55 for specific ozone doses of 0.2 and 0.5 $\text{mmolO}_3 \text{ mmolC}^{-1}$, respectively. For SRFA the ratio is 0.66 for the non-ozonated samples and 0.48 and 0.92 for specific ozone doses of 0.2 and 0.5 $\text{mmolO}_3 \text{ mmolC}^{-1}$, respectively.

The results for non-ozonated PLFA and SRFA can be compared to studies in which methane was used as a probe that is specific for $\cdot\text{OH}$ and non-reactive towards $\cdot\text{OH}$ -like species.(42) Methane quenching experiments indicate that for PLFA both $\cdot\text{OH}$ and $\cdot\text{OH}$ -like species are produced, while for SRFA $\cdot\text{OH}$ is produced.(42) One reason for this discrepancy might be the unknown sensitivity of terephthalic acid used in ref. (42) towards $\cdot\text{OH}$ -like species. This might not capture the full extent of $\cdot\text{OH}$ -like species produced in the SRFA experiments in ref. (42).

Comparison between the effects of ozonation on the generation potential of $^1\text{O}_2$ and $\cdot\text{OH}$ during UV irradiation.

The measured Φ_{1O_2} values were approximately two orders of magnitude higher than $\Phi_{\bullet OH}$. This reflects the fact that 1O_2 is formed by oxygen reacting with $^3DOM^*$ (eq. 3) with an efficiency that was evaluated to be approximately 30% (55), while $\bullet OH$ can be formed through several pathways that are relatively inefficient (vide supra).

A comparison of Φ_{1O_2} and $\Phi_{\bullet OH}$ (Figure 5) shows that the two quantum yields increase significantly with increasing specific ozone doses. Figure 5A shows that $\Phi_{\bullet OH}$ and Φ_{1O_2} increase

more for PLFA than for SRFA. Plotting $\frac{\Phi_{\bullet OH, O_3}}{\Phi_{\bullet OH, O_3=0}} / \frac{\Phi_{1O_2, O_3}}{\Phi_{1O_2, O_3=0}}$ (i.e., the relative ratio of increases of

the two quantum yields; Figure 5B) indicates that for PLFA, the increase in $\Phi_{\bullet OH}$ is more important than for Φ_{1O_2} , while for SRFA the ratio of increase of the two quantum yields is near unity for most specific ozone doses except for 0.2 and 0.25 $mmol_{O_3} mmol_C^{-1}$, where higher ratios were observed.

Figure 5. Comparison between the effects of ozonation on the hydroxyl radical quantum yield ($\Phi_{\bullet OH}$) and the singlet oxygen quantum yield (Φ_{1O_2}) for Pony Lake and Suwannee River fulvic acids (PLFA and SRFA, respectively). (A) $\% \Phi_{\bullet OH}$ vs $\% \Phi_{1O_2}$. (B) Ratio of the normalized (to a specific ozone dose of 0 $mmol_{O_3} mmol_C^{-1}$) $\Phi_{\bullet OH}$ over the normalized (to a specific ozone dose of 0 $mmol_{O_3} mmol_C^{-1}$) Φ_{1O_2} as a function of the specific ozone dose. Red squares/lines: PLFA; black circles/lines: SRFA. DOM at concentrations of 5 $mg_C L^{-1}$. Solutions were buffered with 10 mM phosphate at pH 7, without hydroxyl radical scavenger. Error bars represent standards errors obtained from the pseudo-first order fittings (FFA or BA experiments in duplicate). Lines are shown to guide the eye.

440

441 The observed increase in Φ_{1O_2} was attributed to the formation of quinones from the
442 ozonation of phenolic DOM moieties. Only a relatively small subset of DOM chromophores
443 produces $\cdot OH$. The relative increase in $\Phi_{\cdot OH}$ is related to either a selective destruction of DOM
444 chromophores that do not produce $\cdot OH$ or the formation of new DOM moieties that can produce
445 $\cdot OH$. An example of such new DOM moieties is quinones that are formed during ozonation and
446 that under photoradiation form triplets that have potentially high enough triplet one-electron
447 oxidation potential to form $\cdot OH$ from water or hydroxide ions (see above).

448 Based on the current understanding of DOM photochemistry, it is difficult to develop an
449 overarching model that explains the results observed in this study, as well as in other investigations
450 by the authors. It is possible that the results observed in this study are explained by the recalcitrance
451 of quinone-derivatives to transformation by ozone and $\cdot OH$. However, it is not clear if the
452 photochemistry of quinones results in direct $\cdot OH$ formation or the formation of $\cdot OH$ -like species
453 and the relative efficiency of these two processes. It is also not clear how hydroxylation of
454 quinones would affect 1O_2 formation.

455 In a previous study, we had also observed a positive correlation between the fluorescence
456 quantum yield and the yield for the formation of 1O_2 during pre-ozonation in the presence of an
457 $\cdot OH$ scavenger.(3) Although we did not measure the fluorescence quantum yield in the current
458 study, we expect that the same positive relationship would apply. Given that quinones are not
459 fluorescent, it is unclear whether these observed correlations have any causal basis. More
460 work is needed to ascertain these intercorrelations.

Conclusion

This study focused on the effects of ozonation on the optical and photochemical properties of two DOM types. Ozonation induced a significant decrease in DOM light absorbance properties and significant increases in the quantum yields of singlet oxygen (Φ_{1O_2}) and hydroxyl radical ($\Phi_{\bullet OH}$). For SRFA, the $\Phi_{\bullet OH}$ was observed to increase similarly to Φ_{1O_2} , while for PLFA the increase in $\Phi_{\bullet OH}$ was approximately a factor four larger than the observed increase in Φ_{1O_2} . The increases in Φ_{1O_2} and $\Phi_{\bullet OH}$ were linked to the reactions of phenolic DOM moieties with ozone, which lead to the formation of quinones. The simultaneous use of a $\bullet OH$ probe and a $\bullet OH$ scavenger allowed for the distinction between the formation of $\bullet OH$ and of other hydroxylating species ($\bullet OH$ -like species).

Overall, ozonation leads to an important decrease in the light absorption properties of DOM, which makes it a useful pre-treatment for UV-based treatment since the transparency of the water increases. For municipal wastewaters, where ozonation is used as a polishing step before discharge, the results of this study indicate that ozonation improves the visual aesthetics of the treated wastewater and also increases the potential for 1O_2 - and $\bullet OH$ -induced reactions.

476 **Acknowledgements**

477 Funding for this study came from the US National Science Foundation (1808126, 1833421, and
478 1549387). Funding from the University of Colorado SMART and Discovery Learning
479 Assistantships programs is also acknowledged.

480 481 **Associated Content**

482 **Electronic supplementary information**

483 Electronic supplementary information (ESI) available. See DOI:

484 **Author information**

485 Corresponding author

486 *E-mail: Fernando.rosario@colorado.edu

487 **ORCID**

488 Frank Leresche: 0000-0001-8400-3142

489 Jeremy Torres-Ruiz: 0000-0003-4331-7991

490 Tyler Kurtz: 0000-0001-6310-3240

491 Urs von Gunten: 0000-0001-6852-8977

492 Fernando Rosario-Ortiz: 0000-0002-3311-9089

493 **Note**

494 The authors declare no competing financial interest.

495 **References**

- 496 1. von Sonntag, C.; von Gunten, U.; *Chemistry of Ozone in Water and Wastewater Treatment: From Basic Principles to Applications*; IWA Publishing: London, UK, **2012**.
- 497
- 498 2. Eggen, R. I.; Hollender, J.; Joss, A.; Scharer, M.; Stamm, C., Reducing the Discharge of
- 499 Micropollutants in the Aquatic Environment: the Benefits of Upgrading Wastewater
- 500 Treatment Plants. *Environ. Sci. Technol.* **2014**, *48* (14), 7683-9.
- 501 3. Leresche, F.; McKay, G.; Kurtz, T.; von Gunten, U.; Canonica, S.; Rosario-Ortiz, F. L.,
- 502 Effects of Ozone on the Photochemical and Photophysical Properties of Dissolved Organic
- 503 Matter. *Environ. Sci. Technol.* **2019**, *53* (10), 5622-5632.
- 504 4. Önnby, L.; Salhi, E.; McKay, G.; Rosario-Ortiz, F. L.; von Gunten, U., Ozone and Chlorine
- 505 Reactions with Dissolved Organic Matter - Assessment of Oxidant-Reactive Moieties by
- 506 Optical Measurements and the Electron Donating Capacities. *Water Res.* **2018**, (144), 64-
- 507 75.
- 508 5. Wenk, J.; Aeschbacher, M.; Salhi, E.; Canonica, S.; von Gunten, U.; Sander, M., Chemical
- 509 Oxidation of Dissolved Organic Matter by Chlorine Dioxide, Chlorine, and Ozone: Effects
- 510 on its Optical and Antioxidant Properties. *Environ. Sci. Technol.* **2013**, *47* (19), 11147-56.
- 511 6. Fono, L. J.; Kolodziej, E. P.; Sedlak, D. L., Attenuation of Wastewater-Derived
- 512 Contaminants in an Effluent-Dominated River. *Environ. Sci. Technol.* **2006**, *40* (23), 7257-
- 513 7262.
- 514 7. Link, M.; von der Ohe, P. C.; Voss, K.; Schafer, R. B., Comparison of Dilution Factors for
- 515 German Wastewater Treatment Plant Effluents in Receiving Streams to the Fixed Dilution
- 516 Factor from Chemical Risk Assessment. *Sci. Total Environ.* **2017**, *598*, 805-813.
- 517 8. Fenner, K.; Canonica, S.; Wackett, L. P.; Elsner, M., Evaluating Pesticide Degradation in
- 518 the Environment: Blind Spots and Emerging Opportunities. *Science* **2013**, *341* (6147), 752-
- 519 8.
- 520 9. McNeill, K.; Canonica, S., Triplet State Dissolved Organic Matter in Aquatic
- 521 Photochemistry: Reaction Mechanisms, Substrate Scope, and Photophysical Properties.
- 522 *Environ. Sci.-Process Impacts* **2016**, *18* (11), 1381-1399.
- 523 10. Richard, C.; Canonica, S., Aquatic Phototransformation of Organic Contaminants Induced
- 524 by Coloured Dissolved Natural Organic Matter. In *Handbook of Environmental Chemistry*,
- 525 Springer-Verlag, Berlin, Germany, **2005**; Vol. 2, pp 299-323.
- 526 11. Vione, D.; Minella, M.; Maurino, V.; Minero, C., Indirect Photochemistry in Sunlit Surface
- 527 Waters: Photoinduced Production of Reactive Transient Species. *Chemistry* **2014**, *20* (34),
- 528 10590-606.
- 529 12. Hoigné, J.; Faust, B. C.; Haag, W. R.; Scully, F. E.; Zepp, R. G., Aquatic Humic Substances
- 530 as Sources and Sinks of Photochemically Produced Transient Reactants. *Acs Adv. Chem.*
- 531 *Ser.* **1989**, *219*, 363-381.
- 532 13. Mostafa, S.; Rosario-Ortiz, F. L., Singlet Oxygen Formation from Wastewater Organic
- 533 Matter. *Environ. Sci. Technol.* **2013**, *47* (15), 8179-86.
- 534 14. Wert, E. C.; Rosario-Ortiz, F. L.; Drury, D. D.; Snyder, S. A., Formation of Oxidation
- 535 Byproducts from Ozonation of Wastewater. *Water Res.* **2007**, *41* (7), 1481-1490.
- 536 15. Tentscher, P. R.; Bourgin, M.; von Gunten, U., Ozonation of *Para*-Substituted Phenolic
- 537 Compounds Yields *p*-Benzoquinones, Other Cyclic α,β -Unsaturated Ketones, and
- 538 Substituted Catechols. *Environ. Sci. Technol.* **2018**, *52* (8), 4763-4773.

16. Mvula E, von Sonntag C. Ozonolysis of phenols in aqueous solution. *Org. Biomol. Chem.* **2003**, 1(10), 1749-56.
17. Flyunt, R.; Leitzke, A.; Mark, G.; Mvula, E.; Reisz, E.; Schick, R.; von Sonntag, C., Determination of $\cdot\text{OH}$, $\text{O}_2^{\cdot-}$, and Hydroperoxyde Yield in Ozone Reaction in Aqueous Solution. *J. Phys. Chem. B* **2003**, 107 (30), 7242-53.
18. Buffle, M. O.; von Gunten, U., Phenols and Amine Induced $\text{HO}^{\cdot}$ Generation During the Initial Phase of Natural Water Ozonation. *Environ. Sci. Technol.* **2006**, 40 (9), 3057-3063.
19. Nöthe, T.; Fahlenkamp, H.; von Sonntag, C., Ozonation of Wastewater: Rate of Ozone Consumption and Hydroxyl Radical Yield. *Environ. Sci. Technol.* **2009**, 43 (15), 5990-5.
20. Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B., Critical-Review of Rate Constants for Reactions of Hydrated Electrons, Hydrogen-Atoms and Hydroxyl Radicals ($\cdot\text{OH}/\cdot\text{O}^{\cdot}$) in Aqueous-Solution. *J. Phys. Chem. Ref. Data* **1988**, 17 (2), 513-886.
21. Mártire D.O., Evans C, Bertolotti S.G., Braslavsky S.E., Garcia N.A. Singlet Molecular-Oxygen Production and Quenching by Hydroxybiphenyls. *Chemosphere.* **1993**, 26(9), 1691-701.
22. Wilkinson F, Helman W.P, Ross A.B. Quantum Yields for the Photosensitized Formation of the Lowest Electronically Excited Singlet-State of Molecular-Oxygen in Solution. *J. Phys. Chem. Ref. Data.* **1993**, 22(1), 113-262.
23. Schmidt R, Tanielian C, Dunsbach R, Wolff C. Phenalenone, a Universal Reference Compound for the Determination of Quantum Yield of Singlet Oxygen Sensitization *J. Photochem. and Photobiol. A-Chemistry.* **1994**, 79(1-2), 11-7.
24. Marti C, Jurgens O, Cuenca O, Casals M, Nonell S. Aromatic ketones as standards for singlet molecular oxygen photosensitization. Time-resolved photoacoustic and near-IR emission studies. *J. Photochem. and Photobiol. A-Chemistry.* **1996**, 97(1-2), 11-8.
25. Gutiérrez I, Bertolotti SG, Biasutti MA, Soltermann AT, Garcia NA. Quinones and hydroxyquinones as generators and quenchers of singlet molecular oxygen. *Canadian J. of Chem.-Revue Canadienne de Chimie.* **1997**, 75(4), 423-8.
26. Bourgin, M.; Beck, B.; Boehler, M.; Borowska, E.; Fleiner, J.; Salhi, E.; Teichler, R.; von Gunten, U.; Siegrist, H.; Mc Ardell, C. S., Evaluation of a Full-Scale Wastewater, Treatment Plant Upgraded with Ozonation and Biological Post-Treatments: Abatement of Micropollutants, Formation of Transformation Products and Oxidation By-Products. *Water Res.* **2018**, 129, 486-498.
27. Hollender, J.; Zimmermann, S. G.; Koepke, S.; Krauss, M.; Mc Ardell, C. S.; Ort, C.; Singer, H.; von Gunten, U.; Siegrist, H., Elimination of Organic Micropollutants in a Municipal Wastewater Treatment Plant Upgraded with a Full-Scale Post-Ozonation Followed by Sand Filtration. *Environ. Sci. Technol.* **2009**, 43 (20), 7862-7869.
28. Zimmermann, S. G.; Wittenwiler, M.; Hollender, J.; Krauss, M.; Ort, C.; Siegrist, H.; von Gunten, U., Kinetic Assessment and Modeling of an Ozonation Step for Full-Scale Municipal Wastewater Treatment: Micropollutant Oxidation, By-Product Formation and Disinfection. *Water Res.* **2011**, 45 (2), 605-617.
29. Hammes, F.; Salhi, E.; Koster, O.; Kaiser, H. P.; Egli, T.; von Gunten, U., Mechanistic and Kinetic Evaluation of Organic Disinfection By-Product and Assimilable Organic Carbon (AOC) Formation During the Ozonation of Drinking Water. *Water Res.* **2006**, 40 (12), 2275-2286.
30. Rosario-Ortiz, F. L., Canonica S., Probe Compounds to Assess the Photochemical Activity of Dissolved Organic Matter. *Environ. Sci. Technol.* **2016**, 50 (23), 12532-47.

31. Appiani, E.; Ossola, R.; Latch, D. E.; Erickson, P. R.; McNeill, K., Aqueous Singlet Oxygen Reaction Kinetics of Furfuryl Alcohol: Effect of Temperature, pH, and Salt Content. *Environ. Sci.-Process Impacts* **2017**, *19* (4), 507-516.
32. Qian, J. G.; Mopper, K.; Kieber, D. J., Photochemical Production of the Hydroxyl Radical in Antarctic Waters. *Deep-Sea Res. I.* **2001**, *48* (3), 741-759.
33. Dulin, D.; Mill, T., Development and Evaluation of Sunlight Actinometers. *Environ. Sci. Technol.* **1982**, *16* (11), 815-20.
34. Laszakovits, J. R.; Berg, S. M.; Anderson, B. G.; O'Brien, J. E.; Wammer, K. H.; Sharpless, C. M., *p*-Nitroanisole/Pyridine and *p*-Nitroacetophenone/Pyridine Actinometers Revisited: Quantum Yield in Comparison to Ferrioxalate. *Environ. Sci. Technol. Lett.* **2017**, *4* (1), 11-14.
35. Peuravuori, J.; Pihlaja, K., Molecular Size Distribution and Spectroscopic Properties of Aquatic Humic Substances. *Anal. Chim. Acta* **1997**, *337* (2), 133-149.
36. Boyle E.S., Guerriero N, Thiallet A, Del Vecchio R, Blough N.V. Optical properties of humic substances and CDOM: relation to structure. *Environ. Sci. Technol.* **2009**, *43*(7), 2262-8.
37. McKay, G.; Huang, W. X.; Romera-Castillo, C.; Crouch, J. E.; Rosario-Ortiz, F. L.; Jaffe, R., Predicting Reactive Intermediate Quantum Yields from Dissolved Organic Matter Photolysis Using Optical Properties and Antioxidant Capacity. *Environ. Sci. Technol.* **2017**, *51* (10), 5404-5413.
38. Dorfman L.M, Harter D.A., Taub I.A. Rate Constants for the Reaction of the Hydroxyl Radical with Aromatic Molecules. *J. Chem. Phys.* **1964**, *41*(9), 2954-2955.
39. Helms, J. R.; Stubbins, A.; Ritchie, J. D.; Minor, E. C.; Kieber, D. J.; Mopper, K., Absorption Spectral Slopes and Slope Ratios as Indicators of Molecular Weight, Source, and Photobleaching of Chromophoric Dissolved Organic Matter. *Limnol. Oceanogr.* **2008**, *53* (3), 955-969.
40. Dalrymple, R. M.; Carfagno, A. K.; Sharpless, C. M., Correlations Between Dissolved Organic Matter Optical Properties and Quantum Yield of Singlet Oxygen and Hydrogen Peroxide. *Environ. Sci. Technol.* **2010**, *44*, 5824-5829.
41. Peterson, B. M.; McNally, A. M.; Cory, R. M.; Thoemke, J. D.; Cotner, J. B.; McNeill, K., Spatial and Temporal Distribution of Singlet Oxygen in Lake Superior. *Environ. Sci. Technol.* **2012**, *46* (13), 7222-9.
42. Page, S. E.; Arnold, W. A.; McNeill, K., Assessing the Contribution of Free Hydroxyl Radical in Organic Matter-Sensitized Photohydroxylation Reactions. *Environ. Sci. Technol.* **2011**, *45* (7), 2818-2825.
43. Dong, M. M.; Rosario-Ortiz, F. L., Photochemical Formation of Hydroxyl Radical from Effluent Organic Matter. *Environ. Sci. Technol.* **2012**, *46* (7), 3788-3794.
44. Sun, L. N.; Qian, J. G.; Blough, N. V.; Mopper, K., Insights into the Photoproduction Sites of Hydroxyl Radicals by Dissolved Organic Matter in Natural Waters. *Environ. Sci. Technol. Lett.* **2015**, *2* (12), 352-356.
45. Sur, B.; Rolle, M.; Minero, C.; Maurino, V.; Vione, D.; Brigante, M.; Mailhot, G., Formation of Hydroxyl Radicals by Irradiated 1-Nitronaphthalene (1NN): Oxidation of Hydroxyl Ions and Water by the 1NN Triplet State. *Photochem. & Photobiol. Sc.* **2011**, *10* (11), 1817-1824.
46. Moore, J. N.; Phillips, D.; Nakashima, N.; Yoshihara, K., Photochemistry of 9,10-Anthraquinone-2,6-disulphonate. *J. Chem. Soc. Faraday Trans.* **1986**, *82*, 745-761.

47. Garg, S.; Rose, A. L.; Waite, T. D., Production of Reactive Oxygen Species on Photolysis of Dilute Aqueous Quinone Solutions. *Photochem. Photobiol.* **2007**, *83* (4), 904-913.
48. Ramseier, M. K.; von Gunten, U., Mechanisms of Phenol Ozonation-Kinetics of Formation of Primary and Secondary Reaction Products. *Ozone Sc. & Eng.* **2009**, *31* (3), 201-215.
49. Armstrong, D. A.; Huie, R. E.; Koppenol, W. H.; Lymar, S. V.; Merenyi, G.; Neta, P.; Ruscic, B.; Stanbury, D. M.; Steenken, S.; Wardman, P., Standard Electrode Potentials Involving Radicals in Aqueous Solution: Inorganic Radicals (IUPAC Technical Report). *Pure Appl. Chem.* **2015**, *87* (11-12), 1139-1150.
50. Kitamura, T.; Fudemoto, H.; Wada, Y.; Murakoshi, K.; Kusaba, M.; Nakashima, N.; Majima, T.; Yanagida, S., Visible Light Induced Photo-Oxidation of Water. Formation of Intermediary Hydroxyl Radicals through the Photoexcited Triplet State of Perfluorophenazine. *J. Chem. Soc. Faraday Trans.* **1997**, *93* (2), 221-229.
51. Gan, D.; Jia, M.; Vaughan, P. P.; Falvey, D. E.; Blough, N. V., Aqueous Photochemistry of Methyl-benzoquinone. *J. Physical Chemistry A* **2008**, *112* (13), 2803-2812.
52. Pochon, A.; Vaughan, P. P.; Gan, D. Q.; Vath, P.; Blough, N. V.; Falvey, D. E., Photochemical Oxidation of Water by 2-Methyl-1,4-benzoquinone: Evidence Against the Formation of Free Hydroxyl Radical. *J. Physical Chemistry A* **2002**, *106* (12), 2889-2894.
53. Loeff, I.; Treinin, A.; Linschitz, H., Photochemistry of 9,10-Anthraquinone-2-Sulfonate in Solution. 1. Intermediates and Mechanism. *J. Physical Chemistry* **1983**, *87* (14), 2536-2544.
54. Appiani, E.; Page, S. E.; McNeill, K., On the Use of Hydroxyl Radical Kinetics to Assess the Number-Average Molecular Weight of Dissolved Organic Matter. *Environ. Sci. Technol.* **2014**.
55. Schmitt, M.; Erickson, P. R.; McNeill, K., Triplet-State Dissolved Organic Matter Quantum Yields and Lifetimes from Direct Observation of Aromatic Amine Oxidation. *Environ. Sci. Technol.* **2017**, *51* (22), 13151-13160.