## **Supplementary Information for**

# Effects of C<sub>60</sub> on the Photochemical Formation of Reactive Oxygen Species (ROS) from Natural Organic Matter

Lijuan Yin, Huaxi Zhou, Lushi Lian, Shuwen Yan, and Weihua Song\*

Department of Environmental Science & Engineering, Fudan University, Shanghai 200433, P. R. China

\*Corresponding author: email: <u>wsong@fudan.edu.en</u> Tel: (+86)-21-6564-2040

<u>20 pages</u> <u>13 Figures (S1- S13)</u> <u>1 Table (S1)</u> <u>6 Text (S1- S6)</u>

#### Text S1. Determination the concentration of toluene in C<sub>60</sub> stock solution.

The concentration of toluene in  $C_{60}$  stock solution was measured as 0.53  $\mu$ M by headspace-GC/MS (GC7890A MS 5973, Agilent Technologies, USA), equipped with a split/splitless injector and nonpolar fused silica Rtx-5 capillary column (30 m × 0.20 mm i.d.).

In our experiments,  $C_{60}$  stock solution was diluted 11-856 times. The concentrations of toluene in the working solutions were ranged from 0.62 nM to 48 nM, which were significant lower than the concentration of NOM (5.26 mgc L<sup>-1</sup>). Therefore the remained toluene has minor impact on HO<sup>•</sup>.



Figure S1. The emission spectrum for the xenon lamp solar simulator and the natural sunlight.



**Figure S2.** The steady state concentration of (a) singlet oxygen and (b) triplet state  $C_{60}$  in  $C_{60}$  (0.8 mg<sub>C</sub> L<sup>-1</sup>) solution adding different concentrations of phosphate buffer (from 0.5 mM to 15.0 mM, pH = 7.3). Analyses were performed in triplicate, and the error bars indicate the standard deviation of the mean.



**Figure S3.** The photodegradation of FFA (a), TMP (b), and the formation rate of 2HTA (c) in distilled water and HA solutions ( $5.26 \text{ mg}_{\text{C}} \text{ L}^{-1}$ ). (pH = 7.3)

## Text S2. Steady state concentrations of HO'.

Varied concentrations of terephthalate acid (TA) were added to the solutions of NOM,  $C_{60}$  and their mixtures to trap HO<sup>•</sup> and produce 2HTA to explore the steady state concentrations of the radical. The initial formation rate of 2HTA would be expressed as eq 1:

$$R_{2HTA} = \frac{d[2HTA]}{dt} = \eta k_{HO\bullet,TA}[TA][HO^{\bullet}]$$
(1)

 $\eta$  is the reaction yield, which is estimated as 0.28 based on the reference.<sup>[1]</sup>  $k_{\text{HO,TA}}$  is the second-order reaction rate constant between HO' and TA, as 3.3 x 10<sup>9</sup> M<sup>-1</sup> s<sup>-1</sup>.<sup>[2]</sup> Meanwhile, the [HO'] can also be expressed as:

$$[HO^{\bullet}] = \frac{R_{HO\bullet}}{S + k_{HO\bullet,TA}[TA]}$$
(2)

Where S represents the scavenging rate constant of HO<sup>•</sup> by the matrix,  $R_{HO^•}$  is the formation rate of HO<sup>•</sup>. Combine both equations 1 & 2, then

$$R_{2HTA} = \eta k_{HO\bullet,TA} [TA] \frac{R_{HO\bullet}}{S + k_{HO\bullet,TA} [TA]}$$
(3)

In the absence of TA, it would be  $[HO^{\bullet}]_0 = \frac{R_{HO^{\bullet}}}{S}$ .

Therefore 
$$R_{2HTA} = \lim_{TA \to 0} [(\eta k_{HO,TA}[TA]) \frac{R_{HO}}{S}]$$
 (4)

And 
$$[HO^{\bullet}]_{ss} = \lim_{TA \to 0} \left[ \left( \eta k_{\bullet OH, TA} \right)^{-1} \frac{\kappa_{2HTA}}{[TA]} \right]$$
 (5)

Figure S4 presents the way how  $R_{2HTA}$  could vary as a function of [TA]. Therefore [HO<sup>•</sup>] could be calculated.



**Figure S4.** The formation rate of 2HTA by photodegradation of varied concentrations of TA (from 1.0  $\mu$ M to 0.2 mM) added in NOM (2S101H 5.26 mg<sub>C</sub> L<sup>-1</sup>) solution and the mixtures with C<sub>60</sub> (from 0.01 mg<sub>C</sub> L<sup>-1</sup> to 0.80 mg<sub>C</sub> L<sup>-1</sup>) ([phosphate] = 5 mM; pH = 7.3).

## Text S3. The effects of FFA concentrations on the $[{}^{1}O_{2}]_{ss}$

Varied concentrations of FFA (from 20  $\mu$ M to 0.2 mM) were added in the mixture of HA (5.2 mgC L<sup>-1</sup>) and C<sub>60</sub> (0.1 mg C L<sup>-1</sup>) and were irradiated in simulated sunlight to study the steady-state concentration of <sup>1</sup>O<sub>2</sub>. The loss of FFA was detected by HPLC, the isocratic mobile phase was 70% H<sub>2</sub>O containing 0.05% TFA and 30% methanol containing 0.05% TFA. The UV/Vis detection wavelength was 219 nm.

The pseudo first order rate constants of FFA ranged from  $2.75 \times 10^{-5}$  s<sup>-1</sup> to  $3.34 \times 10^{-5}$  s<sup>-1</sup>. The difference is not obvious. Therefore 20  $\mu$ M of FFA have been applied as  ${}^{1}O_{2}$  chemical probe.



**Figure S5.** Pseudo first order rate constant of varied concentrations of FFA (from 20  $\mu$ M to 0.3 mM) with addition of 5.26 mgC L<sup>-1</sup> SRHA and 0.1 mgC L<sup>-1</sup> C<sub>60</sub>. The experiment was conducted in the solar simulator and temperature was 25 ± 1 °C at pH 7.3. Analyses were performed in triplicate, and the error bars indicate the standard deviation of the mean.



**Figure S6.** The degradation of FFA (20 $\mu$ M) upon irradiation in C<sub>60</sub> solutions (from 0.01 mg<sub>C</sub> L<sup>-1</sup> to 0.80 mg<sub>C</sub> L<sup>-1</sup>) and NOM solutions adding different concentrations of C<sub>60</sub> ([phosphate] = 5 mM; pH = 7.3). Note that "NOM C<sub>60</sub>" indicates that aqueous solutions containing NOM and C<sub>60</sub> were individually prepared and mixed in the dark for 24 h before the simulated solar irradiation.



**Figure S7.** The degradation of TMP (35  $\mu$ M) upon irradiation in C<sub>60</sub> solutions (from 0.01 mg<sub>C</sub> L<sup>-1</sup> to 0.80 mg<sub>C</sub> L<sup>-1</sup>) and NOM solutions adding different concentrations of C<sub>60</sub> ([phosphate] = 5 mM; pH = 7.3).

## Text S4. Second-order rate constant of TMP and <sup>3</sup>C<sub>60</sub><sup>\*</sup>.

The transformation of 2,4,6-Trimethylphenol (TMP) was used to acquire the steady-state concentration of  ${}^{3}C_{60}^{*}$ . TMP, a selective probe, would be degraded upon irradiation in the solutions containing triplet photosensitizers.<sup>[3, 4]</sup> Figure S7 presents the transformation rate of TMP at different initial concentrations upon irradiation in the C<sub>60</sub> solution. The transformation rate r<sub>TMP</sub> can be written as following.

$$-\frac{d[TMP]}{dt} = \frac{r_{3C60*}k_{TMP}[TMP]}{k_{TMP}[TMP] + k_6[O_2] + k^*} + \frac{r_{1O2}k_2[TMP]}{k_2[TMP] + k_d}$$
(6)

The reaction rate constant of TMP with hydroxyl radicals was neglected, based on previous findings.<sup>[3]</sup> The fit of the experimental data was carried out with eq (6), with  $k_{TMP}$  is the second-order rate constant of TMP and  ${}^{3}C_{60}^{*}$ ,  $k_{d}$  is the first-order rate constant for the deactivation of  ${}^{1}O_{2}$  in water  $(2.4 \times 10^{5} \text{ s}^{-1})$ ,<sup>[5]</sup>  $k_{2}$  are the second-order rate constant of TMP and singlet oxygen  $(6.3 \times 10^{7} \text{ M}^{-1} \text{ s}^{-1})$ ,<sup>[6]</sup>  $r_{3C60*}$  and  $k_{6}[O_{2}]+k^{*}$  are the formation rate of  ${}^{3}C_{60}^{*}$  and the deactivation rate constant of  ${}^{3}C_{60}^{*}$ , respectively.  $k_{6}[O_{2}] + k^{*} = 5 \times 10^{5} \text{ s}^{-1}$ ,<sup>[5]</sup>  $r_{1O2}$  represent the formation rate of  ${}^{1}O_{2}$  under irradiation.  $r_{1O2} = (7.70 \pm 0.52) \times 10^{-9} \text{ M s}^{-1}$  for  $C_{60} (0.2 \text{ mgc L}^{-1})$  solution, and  $r_{3C60*}$  and  $k_{TMP}$  as fit variables. The fit yielded  $r_{3C60*} = (3.7 \pm 0.6) \times 10^{-9} \text{ M s}^{-1}$  and  $k_{TMP} = (2.8 \pm 0.7) \times 10^{9} \text{ M}^{-1} \text{ s}^{-1}$  for  $C_{60}$  solutions.



**Figure S8.** Transformation rate of TMP at different initial concentrations in the C<sub>60</sub> (0.2 mg<sub>C</sub> L<sup>-1</sup>) solution ([phosphate] = 5 mM; pH = 7.3).

## Text S5. Standard curve of O<sub>2</sub><sup>-</sup>.

The mixture composed of 12 M absolute ethanol, 15  $\mu$ M DTPA, 41 mM acetone and buffered to pH 12 using 1 mM borate was illuminated to form the standard O<sub>2</sub><sup>--</sup> solutions.<sup>[7]</sup> This solution was irradiated with 254 nm light to produce standard O<sub>2</sub><sup>--</sup> which was detected at once by a spectrometer (USB-4000, Ocean Optics Inc.) with the absorbance at 240 nm. The O<sub>2</sub><sup>--</sup> concentration would decrease when it was in the flow line before entering into the detector. To obtain the initial O<sub>2</sub><sup>--</sup> signal, the intensity of each specified concentration was extrapolated from its best fit to a log function, as previously described. The details can be found in prior researches.<sup>[8, 9]</sup>



Figure S9. Standard curve of intensity of photomultiplier tube signal to the concentration of O<sub>2</sub><sup>-</sup>.



**Figure S10.** The generation of  $H_2O_2$  upon irradiation in NOM (2S101H 5.26 mg<sub>C</sub> L<sup>-1</sup>) solution and the mixtures with  $C_{60}$  (from 0.01 mg<sub>C</sub> L<sup>-1</sup> to 0.80 mg<sub>C</sub> L<sup>-1</sup>) ([phosphate] = 5 mM; pH = 7.3).

## Text S6. Calculation of quantum yields and triplet quantum yield coefficient.

**Calculation of Quantum Yields.** The quantum yield ( $\Phi_i$ ) of reactive intermediate was controlled by the rate of species formation ( $R_i$ , mol L<sup>-1</sup> s<sup>-1</sup>) and the rate of light absorption ( $R_a$ , Es L<sup>-1</sup> s<sup>-1</sup>) as shown in equation 7.<sup>[10]</sup>

$$\Phi_{i} = \frac{R_{i}}{R_{a}} \tag{7}$$

The number of photons absorbed and the quantum yield ( $\Phi_{102}$ ) determined the formation rate of  ${}^{1}O_{2}$ . Steady state concentration of this species ([ ${}^{1}O_{2}$ ]<sub>ss</sub>) would be expressed as equation 8.

$$[{}^{1}O_{2}]_{ss} = \frac{k_{\alpha} \Phi_{1O2}[S]}{k_{X}[X] + k_{d}}$$
(8)

where S represents the photosensitizing molecule with the singlet oxygen quantum yield of  $\Phi_{1O2}$ ,  $k_{\alpha}$  is the specific light absorbance of the solution,  $k_X$  represents the reaction rate between singlet oxygen and any species present in the water matrix which can be ignored,<sup>[11]</sup> and  $k_d$  ( $k_{d,H2O} = 2.4 \times 10^5 \text{ s}^{-1}$ ) is the decay rate of  ${}^{1}O_2$  by physical quenching by water.<sup>[5]</sup> Therefore, the equation 8 can be written as:

$$\Phi_{102} = \frac{\left[{}^{1}O_{2}\right]_{SS}k_{d}}{[S]k_{a}} \tag{9}$$

As shown in equation 9, the numerator represents the  ${}^{1}O_{2}$  formation rate (in M s<sup>-1</sup>) which can be calculated as  $R_{i} = [{}^{1}O_{2}]_{SS} \times k_{d}$ . The denominator means the rate of light absorption ( $R_{a}$ , Es L<sup>-1</sup> s<sup>-1</sup>), a parameter which is also used to calculate the apparent quantum yield of H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub><sup>--</sup> and HO<sup>-</sup>. The specific light absorbance is calculated as equation 10:

$$R_{\alpha} = \sum_{290}^{400} \frac{E_{p}^{0} \varepsilon_{S}(\lambda) (1 - 10^{-(\alpha(\lambda) + \varepsilon_{S}(\lambda)[S])z})}{(\alpha(\lambda) + \varepsilon_{S}(\lambda)[S])z}$$
(10)

With  $E_p^0$  is the spectral photon irradiance (Einstein cm<sup>-2</sup> s<sup>-1</sup>),  $\epsilon_s$  is the apparent molar absorptivity (L cm mol<sup>-1</sup>),  $\alpha(\lambda)$  is the unit absorbance of the background matrix which can be ignored, and z is the depth (cm) of the sample.

The formula used to calculate the apparent quantum yield of  $H_2O_2$  ( $\Phi_{H2O2}$ ) can be expressed as:

$$\Phi_{\rm H202} = \frac{R_{\rm H202}}{R_{\rm a}} \tag{11}$$

The numerator of the formula, formation rate of  $H_2O_2$  ( $R_{H2O2}$ ), is acquired from Figure S10. Almost in the same way, quantum yield of HO<sup>•</sup> and  $O_2^{•-}$  were derived from the formation rate of HO<sup>•</sup> and  $O_2^{•-}$  respectively.<sup>[8, 12]</sup>

**Triplet quantum yield coefficient.** The pseudo-first order rate constants ( $k'_{TMP}$ ) of the reaction of TMP with <sup>3</sup>NOM\* and <sup>3</sup>C<sub>60</sub><sup>\*</sup> were obtained to determine the quantum yield coefficient of <sup>3</sup>NOM\* and <sup>3</sup>C<sub>60</sub><sup>\*</sup>,  $f_{TMP}$  (M<sup>-1</sup>).<sup>[13]</sup> The quantum yield coefficient is expressed as:

$$f_{\rm TMP} = \frac{k_{\rm TMP}}{R_{\rm a}} \tag{12}$$

photosensitizer	R <sub>a</sub>	$\Phi_{1O2}$	Error bar of $\Phi_{102}$	$f_{\mathrm{TMP}}$	Error bar of $f_{\text{TPM}}$
	10 <sup>-7</sup> Es L <sup>-1</sup> s <sup>-1</sup>	%	%	L Es <sup>-1</sup>	L Es <sup>-1</sup>
C <sub>60</sub> 0.01	0.65	3.24	0.16	389.60	6.92
C <sub>60</sub> 0.05	1.36	2.17	0.11	149.93	1.28
C <sub>60</sub> 0.1	2.48	1.82	0.06	153.59	1.30
C <sub>60</sub> 0.2	4.80	1.54	0.10	111.20	1.93
C <sub>60</sub> 0.4	7.67	1.11	0.15	105.41	2.15
C <sub>60</sub> 0.8	13.08	1.07	0.09	123.86	2.54
NOM	18.66	3.82	0.08	86.46	1.59

**Table S1.** Quantum Yield (coefficient) of  ${}^{1}O_{2}$  and triplet excited states in NOM or  $C_{60}$  (from 0.01 mg<sub>C</sub> L<sup>-1</sup> to 0.80 mg<sub>C</sub> L<sup>-1</sup>) samples.



**Figure S11.** Intensity/mass-averaged particle size distributions (PSDs) of different concentrations of  $C_{60}$  with or without NOM.



**Figure S12.** The molecular concentrations of  $nC_{60}$  vs the initial concentrations of  $C_{60}$ . The slope is  $27 \pm 1$ , indicating that  $C_{60}$  act as clusters, and the average of n=27.

**Table S2.** Fitting parameters for the decay rates of  $O_2^{\bullet}$  at varied concentrations of  $C_{60}$  using the equation  $([O_2^{\bullet-}] = \frac{c_0}{1+k_{obs}c_0t}exp^{-\alpha t})$ .

Conc. of C <sub>60</sub> (nM)	$C_0$ of $O_2$ (nM)	$k_{obs}(M^{-1}s^{-1})$	α
0	16.2	0.0038	0
170	10.2	0.0038	0.00235
200	9.00	0.0038	0.00476
250	6.48	0.0038	0.00654
300	5.00	0.0038	0.00974
350	3.38	0.0038	0.0125



**Figure S13.** The fitting parameter of  $\alpha$  ( $k_2[C_{60}^{\bullet-}][K_a][H^+]$ ) vs the concentrations of  $C_{60}^{\bullet-}$  in the decay of  $O_2^{\bullet-}$ .



**Figure S14**. The formation rates of  $C_{60}$  relating to the concentrations of  $C_{60}$  (concentrations range from 0.01 mg<sub>C</sub> L<sup>-1</sup> to 0.80 mg<sub>C</sub> L<sup>-1</sup>). Analyses were performed in triplicate, and the error bars indicate the standard deviation of the mean.



**Figure S15.** The normalized attenuation of  $\Delta \Phi_{102}$ ,  $\Delta \Phi_{02}$ ,  $\Delta \Phi_{1202}$ ,  $\Delta \Phi_{H0}$ , and  $f_{TMP}$  vs. the concentrations of C<sub>60</sub> (mg<sub>C</sub> L<sup>-1</sup>). Analyses were performed in triplicate, and the error bars indicate the standard deviation of the mean.

#### Reference

[1]Charbouillot, T.; Brigante, M.; Mailhot, G.; Maddigapu, P. R.; Minero, C.; Vione, D., Performance and selectivity of the terephthalic acid probe for OH as a function of temperature, pH and composition of atmospherically relevant aqueous media. *J. Photoch. Photobio. A.* **2011**, *222* (1), 70-76.

[2]Mark, G.; Tauber, A.; Rudiger, L. A.; Schuchmann, H. P.; Schulz, D.; Mues, A.; von Sonntag, C., OH-radical formation by ultrasound in aqueous solution - Part II: Terephthalate and Fricke dosimetry and the influence of various conditions on the sonolytic yield. *Ultrason. Sonochem.* **1998**, *5* (2), 41-52.

[3]Canonica, S.; Freiburghaus, M., Electron-rich phenols for probing the photochemical reactivity of freshwaters. *Environ. Sci. Technol.* **2001**, *35* (4), 690-695.

[4]Faust, B. C.; Hoigne, J., Sensitized photooxidation of phenols by fulvic acid and in natural waters. *Environ. Sci. Technol.* **1987**, *21* (10), 957-964.

[5]Rodgers, M. A. J.; Snowden, P. T., Lifetime of  $O_2({}^1D_g)$  in liquid water as determined by time-resolved infrared luminescence measurements. *J. Am. Chem. Soc.* **1982**, *104* (20), 5541-5543.

[6]Tratnyek, P. G.; Hoigne, J., Photo-oxidation of 2,4,6-trimethylphenol in aqueous laboratory solutions and natural waters: kinetics of reaction with singlet oxygen. *Journal of Photochemistry and Photobiology a-Chemistry* **1994**, *84* (2), 153-160.

[7]Fujii, M.; Rose, A. L.; Waite, T. D.; Omura, T., Superoxide-mediated dissolution of amorphous ferric oxyhydroxide in

seawater. Environ. Sci. Technol. 2006, 40 (3), 880-887.

[8]Zhang, D.; Yan, S.; Song, W., Photochemically induced formation of reactive oxygen species (ROS) from effluent organic matter. *Environ. Sci. Technol.* **2014**, *48* (21), 12645-12653.

[9]Rose, A. L.; Moffett, J. W.; Waite, T. D., Determination of superoxide in seawater using 2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo 1,2-a pyrazin-3(7H)-one chemiluminescence. *Anal. Chem.* **2008**, *80* (4), 1215-1227.

[10]Bodhipaksha, L. C.; Sharpless, C. M.; Chin, Y.-P.; Sander, M.; Langston, W. K.; Mackay, A. A., Triplet photochemistry of effluent and natural organic matter in whole water and isolates from effluent-receiving rivers. *Environ. Sci. Technol.* **2015**, *49* (6), 3453-3463.

[11]Cory, R. M.; Cotner, J. B.; McNeill, K., Quantifying interactions between singlet oxygen and aquatic fulvic acids. *Environ. Sci. Technol.* **2009**, *43* (3), 718-723.

[12]Dong, M. M.; Rosario-Ortiz, F. L., Photochemical formation of hydroxyl radical from effluent organic matter. *Environ. Sci. Technol.* 2012, *46* (7), 3788-3794.

[13]Canonica, S.; Jans, U.; Stemmler, K.; Hoigne, J., Transformation kinetics of phenols in water: photosensitization by dissolved natural organic material and aromatic ketones. *Environ. Sci. Technol.* **1995**, *29* (7), 1822-1831.