Supporting Information

Sunlight-mediated inactivation of MS2 coliphage via exogenous singlet oxygen produced by sensitizers in natural waters

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Chemicals and Reagents

Experiments were conducted in deionized (DI) water, D_2O (99.8%, Acros), or samples obtained from a WSP (fourth in series) at the Soscol Water Recycling Facility, Napa, CA, a facility treating municipal waste water. Solutions were buffered with either phosphate (NaH_2PO_4, Fisher) or bicarbonate ($NaHCO_3$, Fisher). Ionic strength was adjusted with NaCl (Fisher). Fluka humic acid (FHA; Fluka), Suwannee river humic acid (SRHA; International Humic Substance Society) and Rose Bengal sodium salt (Sigma) were used as sensitizers. Quenchers included sodium formate (99%, Fisher), L-histidine (98%, Acros), catalase (Sigma), and superoxide dismutase from bovine erythrocytes (SOD; Sigma). Desferioxamine mesylate (EMD Biosciences) was used to inhibit the Fenton reaction. Furfuryl alcohol (FFA; 99%, Aldrich) and phenol (99%, Fisher) were used to quantify $^1O_2$ and $OH^{\cdot}$, respectively. Reagents for the analysis of H_2O_2 (30%, Fisher) were ethylenediaminetetraacetic acid disoidum salt (Acros), 2,2-dipyridyl (99%, Aldrich), horseradish peroxidase (Sigma) N,N-diethyl-1,4-phenylenediamine sulfate (97%, Aldrich), perchloric acid (Fisher) and sulfuric acid (Fisher). Liquid chromatography eluents were acetonitrile (Fisher) and formic acid (88%, Fisher). Sample aliquots were diluted with dilution water (DW; Hach). Reagents for nutrient broth and agar plates contained Bacto tryptone (BD), yeast extract (EM Science), NaCl (Fisher), streptomycin sulfate (Fisher), ampicillin sodium salt (Fisher), $CaCl_2$ (EMD) and dextrose (EMD); granulated agar (Fisher) was also added the nutrient agar. Air and N_2 were purchased from Praxair Inc. (Danbury, CT).

MS2 Propagation and Enumeration.

MS2 (ATCC 15597-B1) was propagated as described in ref. *1*, using *E.coli* (ATCC 700891) as the host organism. The concentration of the MS2 stock solution was 2×10^{11} Plaque Forming Units (PFU)/mL. Enumeration was performed using the double-layer agar method (*1*). Triplicate analysis of selected samples yielded reproducible results (95% confidence intervals of 0.08 log units).

Derivation of κ_{obs}^{abs}

The procedure for calculating the average number of photons absorbed throughout the sample per unit time, $\dot{n}_{abs\ average}$ (photons/(m^2s)), was based on the approach of Morowitz (*2*) and was as modified as follows. The irradiance at any wavelength at any depth in the water sample can be calculated according to the Beer-Lambert Law (*3*):

$$\frac{\Delta I(\lambda,z)}{\Delta\lambda} = \frac{\Delta I_0(\lambda)}{\Delta\lambda} \cdot 10^{-\alpha(\lambda)z}, \qquad \text{(eq. S1)}$$

where I(λ, z) is the irradiance at wavelength λ (W/m^2) at depth z (cm), $I_0(\lambda)$ is the irradiance by wavelength λ at the sample surface (W/m^2), and α(λ) is the absorption coefficient at wavelength λ of the water sample (cm^{-1}). The typical Beer-Lambert Law has been modified in eq. S1 such that the irradiance is represented as a differential, $\frac{\Delta I(\lambda,z)}{\Delta\lambda}$, to account for the discrete measurements recorded over intervals of Δλ (= 1 nm, in the case of our spectroradiometers).

The light absorbed at any wavelength by the water column above depth z, $I_{abs}(\lambda,z)$, is equal to the difference between $I_0(\lambda)$ and I(λ,z). To calculate the average light absorbed throughout the sample, $I_{abs}(\lambda,z)$ was integrated over the reactor depth (L = 5 to 6.5 cm), and then divided by the reactor depth:

$$I_{abs,\,average}(\lambda) = \int_0^L \frac{1}{L}\left(\frac{\Delta I_0(\lambda)}{\Delta\lambda} - \frac{\Delta I(\lambda,z)}{\Delta\lambda}\right)dz = \frac{\Delta I_0(\lambda)}{\Delta\lambda}\left(1 - \frac{1}{L\cdot\alpha(\lambda)\cdot\ln(10)}\left(1-10^{-\alpha(\lambda)L}\right)\right) \qquad \text{(eq. S2)}$$

$I_{abs,\,average}(\lambda)$ can then be converted to the average number of photons absorbed at each wavelength per unit time, $\dot{n}_{abs\ average}(\lambda)$, using the relationship

$$I_{abs,\,average}(\lambda) = \dot{n}_{abs,\,average}(\lambda)\cdot\frac{hc}{\lambda} \qquad \text{(eq. S3)}$$

where h is Planck's constant (6.626 x 10^{-34} Js), and c is the speed of light (2.998*10^{8} ms^{-1}). $\dot{n}_{abs\ average}$ for all λ can be determined by summing over all wavelengths considered (320 – 700 nm):

$$\dot{n}_{abs,\ average} = \sum_{\lambda=320nm}^{700nm} \left(\dot{n}_{abs,\ average}(\lambda)\right)\Delta\lambda \qquad \text{(eq. S4)}$$

The number of photons absorbed at any point during the experiment, $n_{abs,\ average}$, is calculated by multiplying $\dot{n}_{abs\ average}$ by the exposure time of the sample to simulated sunlight. κ_{obs}^{abs} can thus be calculated from the slope of a plot of ln(PFU/mL) vs. average number of photons absorbed.

Literature cited

(1) APHA; AWWA; WEF *Standard Methods for the Examination of Water & Wastewater*; 21 ed.; American Public Health Association, American Water Works Association, and Water Environment Federation: Washington, DC, 2005.

(2) Morowitz, H. J. Absorption effects in volume irradiation of microorganisms. *Science* **1950**, *111*, 229-230.

(3) Stumm, W.; Morgan, J. M. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*; 3rd ed.; John Wiley & Sons, Inc.: New York, 1996.