Environmental Science & Technology

Subscriber access provided by The Library | University of Bath

Article

Photoinactivation of Eight Health-Relevant Bacterial Species: Determining the Importance of the Exogenous Indirect Mechanism

Peter Maraccini, Jannis Wenk, and Alexandria B. Boehm

Environ. Sci. Technol., **Just Accepted Manuscript** • DOI: 10.1021/acs.est.6b00074 • Publication Date (Web): 27 Apr 2016

Photoinactivation of Eight Health-Relevant Bacterial Species: Determining the Importance of the Exogenous Indirect Mechanism

Peter A. Maraccini[†§], Jannis Wenk[**‡§], Alexandria B. Boehm[*†§]

[†]Department of Civil and Environmental Engineering, Stanford University, Stanford, California 94305, United States

[‡]Department of Civil & Environmental Engineering, University of California, Berkeley, California 94720-1710, United States

[§]Engineering Research Center (ERC) for Re-inventing the Nation's Urban Water Infrastructure (ReNUWIt)

[*]**Corresponding author:** Tel: 650 724-9128, Fax: 650 723-7058, E-mail: aboehm@stanford.edu

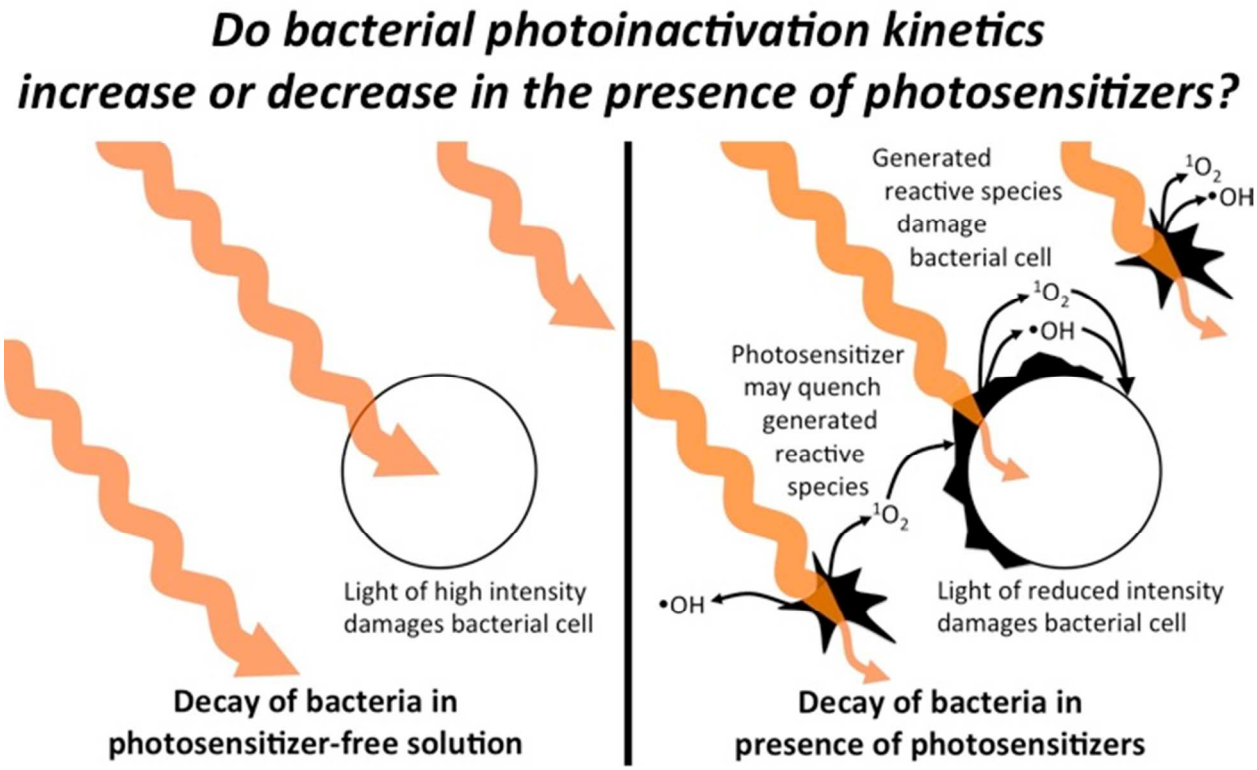
[**]**Present Address:** Department of Chemical Engineering and Water Innovation & Research Centre (WIRC), University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom.

Keywords: bacteria, sunlight, disinfection, photoinactivation, exogenous, sensitizers, reactive oxygen species, enterococci, E. coli

ABSTRACT

It is presently unknown to what extent the endogenous direct, endogenous indirect, and exogenous indirect mechanisms contribute to bacterial photoinactivation in natural surface waters. In this study, we investigated the importance of the exogenous indirect mechanism by conducting photoinactivation experiments with eight health-relevant bacterial species (*Bacteroides thetaiotaomicron, Campylobacter jejuni*, *Enterococcus faecalis*, *Escherichia coli* K12, *E. coli* O157:H7, *Salmonella enterica* serovar Typhimurium LT2, *Staphylococcus aureus*, and *Streptococcus bovis*). We used three synthetic photosensitizers (methylene blue, rose bengal, and nitrite) and two model natural photosensitizers (Suwannee River natural organic matter and dissolved organic matter isolated from a wastewater treatment wetland) that generated singlet oxygen and hydroxyl radical. *B. thetaiotaomicron* had larger first order rate constants than all other organisms under all conditions tested. The presence of the synthetic photosensitizers generally enhanced photoinactivation of Gram-positive facultative anaerobes (*Ent. faecalis, Staph. aureus, Strep. bovis*). Among Gram-negative bacteria, only methylene blue with *E. coli* K12 and rose bengal with *C. jejuni* showed an enhancing effect. The presence of model natural photosensitizers either reduced or did not affect photoinactivation rate constants. Our findings highlight the importance of the cellular membrane and photosensitizer properties in modulating the contribution of the exogenous indirect mechanism to the overall bacterial photoinactivation.

38 ***TOC ART***


Do bacterial photoinactivation kinetics increase or decrease in the presence of photosensitizers?
Light of high intensity damages bacterial cell
Decay of bacteria in photosensitizer-free solution
Generated reactive species damage bacterial cell
$^1O_2$
•OH
Photosensitizer may quench generated reactive species
Light of reduced intensity damages bacterial cell
Decay of bacteria in presence of photosensitizers

39

INTRODUCTION

Forty-four percent of the world's population resides within 150 km of the coastline[1], and eight of the world's ten largest urban centers are coastal. The shoreline and coastal waters are valuable natural resources, providing society with food, recreation, revenue, and numerous ecosystem services (such as nutrient cycling, runoff detoxification, and biodiversity). Despite the importance of clean coastal waters to our economy and well-being, declining water quality along the world's coastlines threatens ecosystem and human health[2,3].

It is estimated that globally, exposure to coastal waters polluted with pathogens from wastewater causes an excess 120 million gastrointestinal (GI) and 50 million severe respiratory illnesses per year[4]. In an effort to reduce recreational waterborne illnesses, US states are required through provisions outlined in the BEACH Act to implement beach monitoring programs that use densities of fecal indicator bacteria (FIB) to assess risk. Similar monitoring programs are in place around the globe, guided by recommendations from the World Health Organization[5,6]. Although they are not usually etiologic agents of recreational waterborne illness, FIB are used to evaluate beach water quality because their densities in coastal waters contaminated with wastewater and urban runoff have been linked quantitatively to swimmer illness in epidemiology studies[7]. When FIB densities exceed threshold values, beach advisories or closures are issued warning swimmers that exposure may lead to illness.

The number of beach advisories and closures has grown in the US over the last 20 years, in part due to the increasing number of beaches monitored by local and state agencies[4]. Mitigation of microbial pollution in coastal waters has proven challenging because there are many potential point and non-point sources (including runoff, sewage, bird and wild animal feces, oceanic outfalls, decaying plants, and sediments)[8], and there is imperfect knowledge about

the factors that modulate the abundance and distribution of different indicators and pathogens once they are introduced into the environment[9,10]. Science that yields insight into the physical, chemical, and biological controls of the abundance and distribution of microbial pollution in coastal waters is needed and of both national and international importance. Such knowledge would guide remediation efforts, and assist in the identification of safe and dangerous conditions for swimming.

Field observations in both marine and fresh surface waters suggest that sunlight is one of the most important factors modulating FIB concentrations[11–13]. Photoinactivation of bacteria, or inactivation by means of sunlight or radiant energy, may occur via three different mechanisms: endogenous direct, endogenous indirect, and exogenous indirect[14,15]. Endogenous direct photoinactivation results from the damage of cellular chromophores by photons, such as UVB damage to DNA[16,17], while indirect photoinactivation involves energy or electron transfer to form reactive species either within (endogenous) or external (exogenous) to the cell that can then cause cell death. In practice, it is difficult to tease apart the endogenous mechanisms so they can be considered together[18]. In coastal waters, colored dissolved organic matter[19,20] can act as the photosensitizers that absorb and transfer the light energy outside of the bacteria cell (exogenous indirect), while chromophores within the cell can inadvertently do the same (endogenous indirect).

It is presently unknown to what extent the three different mechanisms contribute to bacterial inactivation in natural waters. The inactivation of *Escherichia coli* and to a lesser extent enterococci has been measured in a wide range of sunlit waters and the observed inactivation rate constants vary over several orders of magnitude[15,21–26]. There are limited studies on sunlight inactivation of waterborne bacterial pathogens such as *Campylobacter*, *Vibrio cholerae*, *Shigella*,

and *Salmonella*[27–34]. These studies primarily have investigated how inactivation rate constants vary between the pathogens and indicator organisms; the results are equivocal with some studies reporting similar inactivation among organisms and others reporting widely differing inactivation rate constants. Furthermore, a number of studies have attempted to elucidate the mechanisms of bacterial inactivation in sunlit, natural waters by using FIB[14,15,18,21,22,24,35–38]. The extent to which each of the three photoinactivation mechanisms (endogenous direct, endogenous indirect, and exogenous indirect) contributed to the overall photoinactivation varied between studies. Further, it is unclear whether those results can be extended to bacterial pathogens.

The present study tests whether the exogenous indirect mechanism of bacterial photoinactivation is important relative to the endogenous mechanisms in natural waters. We conducted photoinactivation experiments in a solar simulator with eight health-relevant bacterial species (*Bacteroides thetaiotaomicron, Campylobacter jejuni*, *Enterococcus faecalis*, *E. coli* K12, *E. coli* O157:H7, *Salmonella enterica* serovar Typhimurium LT2, *Staphylococcus aureus*, and *Streptococcus bovis*). Experiments were carried out in the presence and absence of model synthetic and natural photosensitizers under full spectrum simulated sunlight. While studies have previously investigated the exogenous indirect photoinactivation pathway for viruses[19–21,39–43], *E. coli*[14,15,18,21,22,36,37], and enterococci[14,15,18,21,37,44] this is the first study to do so for a wide range of bacterial species and sensitizers. The results of this study will be used in future efforts to accurately model and predict bacterial photoinactivation rate constants in natural waters as they inform whether the exogenous indirect mechanism is critical to include.

EXPERIMENTAL MATERIALS AND METHODS

Bacterial cultivation. The following bacteria were obtained from the American Type Culture Collection (ATCC): *Bacteroides thetaiotaomicron* (ATCC 29741), *Campylobacter jejuni* (ATCC 29428), *Enterococcus faecalis* (ATCC 19433), *Escherichia coli* K12 (ATCC 10798), *Escherichia coli* O157:H7 (ATCC 43895), *Staphylococcus aureus* (ATCC 25923), and *Streptococcus bovis* (ATCC 33317). The attenuated strain *Salmonella enterica* serovar Typhimurium LT2 was obtained from the Falkow Lab of Stanford University. These organisms were chosen to represent both Gram negative and positive bacteria. Each strain is a bacterial pathogen or bacterial indicator organism. Individual properties and justifications for choice of each bacterial species are provided in Table S1.

Tryptic soy broth (TSB, BD Bacto, Sparks, MD; ATCC medium 18) was used as the growth media for the facultative anaerobic microorganisms (*Ent. faecalis, E. coli* K12, *E. coli* O157:H7, *S. enterica*, *Staph. aureus*, and *Strep. bovis*) and made following manufacturer's direction using deionized water. When TSB was used as the chemostat medium, it was diluted to 25% strength. Brucella broth (BD BBL, Sparks, MD; ATCC medium 1116) was used as the growth media for the microaerophilic microorganism *Campylobacter jejuni*, and made following manufacturer's direction using deionized water. The obligate anaerobe *Bacteroides thetaiotaomicron* was grown in modified TYG medium (see SI). All media were autoclaved at 121°C for 15 minutes prior to use. Facultative anaerobes were grown in an aerobic environment (ambient air conditions) while *C. jejuni* and *B. thetaiotaomicron* were grown in a microaerophilic and anaerobic environment, respectively (see SI).

A single stock of each bacterial species was generated for storage at -20°C. This single -20°C stock was used to generate the biomass to seed the photoinactivation experiments in order to minimize variance associated with starting bacterial population[45].

For facultative anaerobes, experiment seed was generated using chemostats (see SI) to minimize the variability among replicates[45]. Chemostats could not be used for microaerophiles and obligate anaerobes due to the difficulty of controlling the atmosphere. *B. thetaiotaomicron* and *C. jejuni* seed were generated from batch cultures (see SI). Cells were harvested by pelleting and re-suspending the cells in 1 mM carbonate buffer saline (CBS, pH 7.64) three times.

Experimental solutions and procedures. Stock solutions of Suwannee River natural organic matter (SRNOM, International Humic Substance Society, St. Paul, MN), isolated DOM (iDOM), methylene blue (MB), sodium nitrite, and rose bengal (RB) were filter sterilized before use (for further details, including iDOM isolation method, see SI). The panel of photosensitizers was chosen to include both natural (SRNOM and iDOM) and synthetic sensitizers (MB, RB, and nitrite), a range of surface charges (anionic for SRNOM, iDOM, RB, and nitrite; cationic for MB), and a range of reactive oxygen species generated (Table S2). Stock sensitizer solutions were added to individual 100 mL experimental beakers to reach final concentrations of 0.5 μM RB, 200 mg/L as NO_2^-, 0.5 μM MB, 20 mg C SRNOM/L, and 20 mg C iDOM/L each at a total volume of 50 mL. All solutions were buffered using 1 mM CBS at pH 7.64. A single bacterial species was then added to each reactor so that the final starting concentrations were 5.2×10^3 – 5.6×10^6 colony forming units (CFU)/ml for *B. thetaiotaomicron,* 9.2×10^5 – 1.2×10^7 CFU/ml for *C. jejuni*, 2.9×10^6 – 3.0×10^7 CFU/ml for *E. coli* K12, 1.9×10^6 – 1.6×10^7 CFU/ml for *E. coli* O157:H7, 6×10^5 – 1.1×10^7 CFU/ml for *Ent. faecalis*, 1.4×10^7 – 2.9×10^7 CFU/ml for *S. enterica*, 2.0×10^7 – 4.2×10^7 CFU/ml for *Staph. aureus,* and 2.4×10^4 – 1.9×10^6 CFU/ml for *Strep. bovis* (Table S3).

Due to the time and cost of acquiring the iDOM, experiments with iDOM were limited to representative Gram negative and Gram positive organisms: *E. coli* K12 and *Ent. faecalis.* We also limited experiments with methylene blue to *E. coli* K12 and *Ent. faecalis*.

Beakers containing a single sensitizer and a single bacterial species were placed in a recirculating water bath, set at a temperature of 15°C, in a solar simulator (Altas Suntest CPS+; Linsengericht-Altenhaßlau, Germany). Beakers were stirred continuously. An additional beaker containing 1 mM CBS and the bacterial species was covered with foil and placed in the same recirculating water bath to serve as dark control for each experiment; however, the dark control was not continuously stirred and was only mixed upon sampling. The temperature of experimental solutions was followed during initial experiments to confirm isothermal conditions (15°C); thereafter temperature measurements were discontinued. The solar simulator was set at an irradiance of 400 W/m^2 and was equipped with a coated quartz filter and a UV special glass filter to block the transmission of wavelengths below 290 nm to simulate natural sunlight (passing wavelength, 290 nm < λ < 800 nm)[46]. The resulting light spectrum as measured using a spectroradiometer (ILT950; International Light, Peabody, MA) compared favorably to the light intensity at the ground level (17 m above sea level) in Palo Alto, California, USA (37.4292° N, 122.1381° W) at midday in the summer (Figure S1).

All experiments were performed in triplicate. A single chemostat (for facultative anaerobes) or batch culture (for microaerophiles and obligate anaerobes) generated the bacterial seed for an entire set of experiments (i.e., a CBS experiment, a SRNOM experiment, a rose bengal experiment, and a nitrite experiment), but separate chemostats or batch cultures generated the bacterial seeds for each experimental biological replicate (i.e, CBS replicate 1, CBS replicate 2, CBS replicate 3).

Upon exposure to the solar simulator light source, 0.5 ml samples were aseptically withdrawn from the beakers over a time course (anywhere from every 2 to 60 minutes, depending on how quickly the organism lost culturability during pilot experimental runs). Samples were serially diluted with CBS and bacterial colonies were enumerated by spread plating appropriate dilutions in duplicate on appropriate agars (see SI). Concentrations were calculated using counts from all plates with between 10 and 400 colonies after accounting for the dilution and volume applied to the agar.

Chemical Probes. The steady-state bulk concentrations of singlet oxygen ($^1O_2$) and hydroxyl radical (·OH) were indirectly measured in experimental solutions identical to those used in the inactivation experiments by monitoring the decay of probe compounds furfuryl alcohol (FFA) and phenol[47–49] (see SI). The probe compounds did not decay due to direct photolysis and only decayed in the presence of the photosensitizers (Figure S2).

Data Analysis. The inactivation data obtained from each experiment were fit using a shoulder-log linear model[50]:

$$\frac{C(t)}{C_0} = e^{-kt}\left(\frac{e^{kS}}{1+(e^{kS}-1)e^{-kt}}\right) \quad (1)$$

where t is time (min), $C(t)$ (CFU/ml) is the measured concentration at time t, C_0 is the measured concentration at time 0, S (min) is the shoulder or lag time over which there is minimal inactivation of the bacteria, and k (min^{-1}) is the rate constant for the log linear portion of the inactivation curve after completion of the lag time. All fitted values are non-negative. If the 95% confidence interval of the shoulder length, S, crossed zero, then the inactivation data were refit using a simpler first order decay model:

$$\frac{C(t)}{C_0} = e^{-kt} \quad (2)$$

In some cases, biological replicates were fit with different models (either Equation 1 or Equation 2) if one replicate had a shoulder and the other did not. It should be noted that for the case when the shoulder is not different from 0, then Equation 1 simplifies to Equation 2. Fit parameters, and their standard deviations and 95% confidence intervals were obtained using IGOR PRO (WaveMetrics Inc., Lake Oswego, OR). A χ^2 value was generated for each model (Eq. 1 and 2) and the Pearson's χ^2 test for goodness of fit was used to determine the P value, with the assumed null hypothesis that the model predicted the experimental data. Model parameters were corrected for light screening of UVB photons as described in the SI, and the corrected parameters are hereon referred to as $\hat{k}$ and $\hat{S}$ and reported with units m^2/MJ and MJ/m^2, respectively.

$\hat{k}$ values were compared between bacterial species and experimental solutions using analysis of variance (ANOVA) and Tukey post hoc tests. $\hat{k}$ values were $\log_{10}$ transformed for the analysis to achieve normality. The presence or absence of a shoulder was compared between experiments using Pearson's chi-square test. Statistics were completed using IBM SPSS statistics (version 22).

$\hat{k}$ values from treatment and controls conducted with organisms from the same chemostat or batch culture (Table S4) were compared pairwise, with the null hypothesis that the $\hat{k}$ values were the same[18]. This approach was taken rather than pooling biological replicates as variability among replicate experiments conducted with different bacterial seed has been well documented[45]. If the paired t-test indicated the control and treatment were significantly different ($p<0.05$) in two or more of the three biological replicate experiments and the effect indicated the same trend (i.e. enhanced or suppressed inactivation in treatment relative to the control), then the treatment was deemed to have a potential effect on photoinactivation, and the $\hat{k}$ values in

treatment and controls were considered different. We chose this approach to be inclusive in considering treatments with potential effects.

The inactivation rate constants $\hat{k}$ (corrected for light screening) were pooled and averaged across experimental treatments and CBS controls for which the $\hat{k}$ values were different and higher in the treatments than in the controls. $\hat{k}_{endogenous}$, defined as the inactivation rate constant in the absence of exogenous sensitizers, was estimated as the average $\hat{k}$ from the CBS controls for each particular bacterial species. $\hat{k}_{exogenous}$, defined as the increase in inactivation rate constant due to the presence of exogenous sensitizers, was estimated as the difference between the average $\hat{k}$ from the experiments with a particular photosensitizer and the average $\hat{k}$ of the CBS controls ($\hat{k}_{endogenous}$). $k_{\text{exogenous}}$ was then expressed in units of per time by multiplying by the depth-average UVB intensity (averaged across control and treatment replicates) (Table S5). Pearson's r assessed whether $k_{\text{exogenous}}$ was correlated with steady state singlet oxygen or hydroxyl radical concentrations.

RESULTS

Curve fits. Inactivation data (Figure 1) were fit using either a shoulder-log linear model or first-order decay model. Model fit parameters and their 95% confidence intervals can be found in Table S3. For 114 (99%) of the data series of the 115 individual experiments, the Pearson's χ^2 test values for fitted curves were above 0.05, indicating that the applied model well fitted the data (Table S4).

A shoulder was observed for 79 of the 115 inactivation curves ranging from 0.000055 to 0.0051 MJ/m^2 (Table S4). The remaining 36 data series did not require a shoulder and were fit

with the simplified model. Pearson χ^2 tests showed that the proportion of experiments with a shoulder varied between bacterial species and sensitizer ($p<0.05$ for both). For example, experiments conducted with iDOM (n=6) all had a shoulder and nearly 90% of experiments conducted in CBS (n=31) exhibited a shoulder; the remaining sensitizer experiments showed similar proportions of models with and without a shoulder. All experiments conducted with *Ent. faecalis* (n= 21) and *Strep. bovis* (n=12) had shoulders and 85% of the experiments conducted with *Staph. aureus* (n=13) had a shoulder, while 17% of experiments done with *S. enterica* had a shoulder. For experiments with the other organisms, close to 50% of models included a shoulder. A coarser analysis indicates that of the 47 experiments conducted with Gram-positive bacteria, 96% had a shoulder while 50% of the experiments conducted with Gram-negative bacteria (n=68) had a shoulder.

Inactivation data of the dark controls were fit with a first-order decay model; however, the slopes did not differ from zero ($p < 0.05$, data not shown).

Photoinactivation in the absence of photosensitizers. In CBS, $\hat{k}$ values ranged from 1045 to 16907 m^2/MJ with a median of 1925 m^2/MJ (Table S4). $\hat{k}$ was significantly different between organisms (ANOVA, $p<0.05$). Tukey post hoc testing indicated *B. thetaiotaomicron* $\hat{k}$ was greater than $\hat{k}$ of all other organisms (average $\hat{k}$ = 12678 m^2/MJ). *C. jejuni*, *Strep. bovis*, and *Staph. aureus* decayed similarly ($\hat{k}$ = 2100-3950 m^2/MJ) with lower $\hat{k}$ than *B. thetaiotaomicron*, but higher $\hat{k}$ than the remaining organisms (1470-1944 m^2/MJ).

Effect of photosensitizers on photoinactivation. The measured bulk phase steady state singlet oxygen and hydroxyl radical concentrations for all photosensitizers at the chosen concentration are shown in Table S2. For RB the singlet oxygen concentration was 1.0×10^{-12} M (Table S2). MB, SRNOM, and iDOM all generated similar steady state concentrations of singlet

oxygen, ranging from 8.6 x 10^{-14} to 1.2 x 10^{-13} M (Table S2). The singlet oxygen concentration for the nitrate solution is not reported due to potential side reactions and contribution of hydroxyl radical to FFA decay[51–53]. MB, RB, and nitrite generated similar steady state concentrations of hydroxyl radicals, from 4.5 x 10^{-15} to 8.3 x 10^{-15} M, while SRNOM and iDOM generated steady state concentrations approximately 10 times lower (~1.4 x 10^{-16} to 2.1 x 10^{-16} M (Table S2)). Note that phenol is a rather unspecific probe compound for hydroxyl radical as it may considerably react with other types of reactive species[47]. Therefore, the presented values represent upper limits for bulk phase steady state hydroxyl radical concentrations. The contribution of carbonate radical to phenol depletion under the chosen experimental conditions was calculated and can be assumed negligible (see SI).

$\hat{k}$ measured in the presence of photosensitizers ranged from 367 to 20510 m^2/MJ with a median of 2424 m^2/MJ (Table S4). $\hat{k}$ varied between organisms, photosensitizer present in solution, and their interaction (ANOVA, $p<0.05$ for all three terms). Post hoc testing indicated *B. thetaiotaomicron* generally had the highest $\hat{k}$, followed by *C. jejuni*, then the Gram-positive facultative anaerobes followed by the Gram-negative facultative anaerobes. Among the experimental treatments containing sensitizers, treatments with MB had among the highest $\hat{k}$. Treatments containing RB and nitrite had smaller $\hat{k}$ than those containing MB but higher $\hat{k}$ than those containing iDOM and SRNOM which tended to have the lowest $\hat{k}$.

Based on the criteria described in the methods section, we identified which sensitizers had a potential effect on the photoinactivation of each bacterial species. For *Ent. faecalis*, the presence of MB, RB, and nitrite increased $\hat{k}$ compared to the CBS control while iDOM decreased $\hat{k}$. For *Staph. aureus*, RB and nitrite increased $\hat{k}$ and SRNOM decreased $\hat{k}$. For

Strep. bovis, RB increased $\hat{k}$. For *E. coli* K12, SRNOM and RB decreased $\hat{k}$ while MB increased $\hat{k}$ relative to CBS. For *E. coli* O157:H7, RB, nitrite, and SRNOM decreased $\hat{k}$. For *S. enterica*, SRNOM decreased $\hat{k}$. SRNOM and nitrite decreased $\hat{k}$ of *B. thetaiotaomicron*. RB increased $\hat{k}$ of *C. jejuni*. No other differences, identified based on our criteria, were observed. The average $\hat{k}$ from pooled biological replicate experiments for the CBS control and photosensitizer treatments are shown in Figure 2.

$\hat{k}_{exogenous}$ was between 900 and 11681 m^2/MJ for bacteria-photosensitizer treatments where the sensitizer increased $\hat{k}$ relative to the control (Table 1). These values correspond to $k_{exogenous}$ between 0.07 and 1.07 min^{-1} (Table 1). $\hat{k}_{exogenous}$ and $k_{exogenous}$ were not positively significantly correlated with the steady state concentrations of singlet oxygen or hydroxyl radical in the experimental solutions (ranged from -0.5 to 0.4, $p>0.05$).

The starting concentrations of bacteria varied among biological replicates. Even though we aimed to have the same starting concentration, the differences were unavoidable. Multiple linear regression modeling of k or $\hat{k}$ of each bacterial species as a function of photosensitizer and $\log_{10}$-transformed starting concentration indicated that the starting concentration no significant impact on the rate constants ($p > 0.05$).

DISCUSSION

In sensitizer-free solution, the obligate anaerobe *B. thetaiotaomicron* has a rate constant approximately three times greater than other bacteria. *B. thetaiotaomicron* is able to generate proteins to alleviate oxidative stress, but only if the stress is applied while the bacteria have access to the nutrients necessary to sustain repair mechanisms [54,55]. *B. thetaiotaomicron* was

grown in anaerobic (<1% O_2) environments, where oxygen is scarce. As such, it may not have developed the cellular machinery to deal with oxidative stress. However, simply being exposed to an oxic environment did not lead to inactivation, as the dark controls for *B. thetaiotaomicron* decayed minimally over the length of the experiment (data not shown).

A comparison between photoinactivation rate constants reported herein with those reported in previous studies is difficult because prior studies generally do not report the spectrum of the light source used in their experiments, the geometry of their reactors, and/or the absorbance of their experimental solutions. This makes it difficult to correct their reported rate constants (e.g., in units of per time) for UVB light screening for comparison with our results. Photoinactivation rate constants (uncorrected for light screening) reported in previous studies span several orders of magnitude (Table S6): *C. jejuni* ($k = 0.12 – 0.61\ min^{-1}$)[28], *E. faecalis* ($k = 0.003 – 0.64\ min^{-1}$)[21,45,56,57], *E. coli* ($k = 0.002 – 0.12\ min^{-1}$)[22,32,58,59], *Salmonella enterica* ($k = 0.001 – 0.80\ min^{-1}$)[30,32–34,60,61], *Staphylococcus spp*. ($k = 0.19\ min^{-1}$)[62], *Bacteroides ovatus* (measured by QPCR, $k = 0.06 – 0.15\ min^{-1}$)[63].

The rate constants reported herein for bacteria in sensitizer-free solutions are attributed solely to endogenous mechanisms and may be representative of those in clear natural waters (like open ocean waters). This assumes that carbonate radicals were not present at significant concentrations in the CBS solution. Indeed, the phenol probe, which reacts with both hydroxyl and carbonate radicals[64], did not decay in 1 mM CBS (Figure S2), indicating minimal steady state concentrations of both radicals. It should be noted that direct application of the rate constants to clear natural waters may be hindered due to our use of laboratory-grown bacteria instead of those sourced, uncultured, from the natural environment. A recent study suggests the latter have differential susceptibility to photoinactivation[56]. Using uncultivated organisms from

329 the natural environment (including sewage) for decay experiments represents a great
330 methodological challenge; the vast majority, if not all, pathogen inactivation experiments have
331 been conducted using laboratory-cultured organisms. Future work will need to overcome this
332 challenge to determine if results from laboratory-cultivated organisms can be extended to natural
333 systems.

334 The addition of the photosensitizers enhanced photoinactivation in some instances, and
335 diminished it in others. The panel of photosensitizers were chosen to include both natural and
336 synthetic sensitizers, a range of surface charges, and varying proportions of reactive oxygen
337 species generated, and were added at concentrations that would generate those reactive oxygen
338 species at the high end or just above what is found in the environment. In sunlit surface waters,
339 such as rivers and lakes, including municipal wastewater effluent impacted water bodies, the
340 steady-state concentration of singlet oxygen has been reported to be 5.9×10^{-14} – 15×10^{-14}
341 M[48,65], while hydroxyl radical steady state concentrations are several orders of magnitude lower
342 ranging from 10^{-19} to 2.7×10^{-16} M[66,67], although the hydroxyl radical steady state concentration
343 may be as high as 10^{-14} M in the presence of nitrate[68]. The anionic dye rose bengal was the only
344 sensitizer to generate bulk phase steady state singlet oxygen concentrations above that reported
345 in the natural environment; however, rose bengal, the cationic dye methylene blue, and
346 photoactive nitrite all generated bulk-phase hydroxyl radicals concentration approximately ten
347 times higher than would appear in the natural environment. Suwannee River NOM and isolated
348 DOM each generated environmentally relevant bulk phase concentrations of reactive oxygen
349 species.

350 The addition of the synthetic photosensitizers (rose bengal, nitrite, methylene blue)
351 tended to have an enhancing effect on the photoinactivation of Gram-positive facultative

anaerobes (*Ent. faecalis, Staph. aureus, Strep. bovis*). Among the Gram-negative bacteria, only methylene blue with *E. coli* K12 and rose bengal with *C. jejuni* had an enhancing effect, with the remaining synthetic photosensitizers having either a negligible or diminishing effect on photoinactivation rate constants. The Gram-staining procedure characterizes the cell wall structure, designating the microorganisms with thinner peptidoglycan layers and an additional outer membrane containing two lipid bilayers as Gram-negative, and those with thicker peptidoglycan layers and without the additional outer membrane as Gram-positive[69]. The Gram-negative property of an additional outer membrane may confer some protection against the exogenous indirect mechanism of photoinactivation[44,70] that the Gram-positive bacteria do not have, possibly explaining the trends found in this and past studies [71,72]. Methylene blue, a cationic photosensitizer, enhanced photoinactivation of both the Gram-positive *Ent. faecalis* and the Gram-negative *E. coli* K12. The cationic photosensitizers have a greater affinity for the negatively charged phospholipids of Gram-negative bacteria and enter more easily via self-promoted uptake in comparison with the anionic photosensitizers, such as rose bengal, and neutral photosensitizers[73,74], and are therefore potentially better able to adsorb to the cellular membrane and generate reactive oxygen species close to vital cellular components within the bacteria[37].

The natural photosensitizers either diminished or did not affect photoinactivation rate constants of all bacteria tested. Considering that most natural photosensitizers such as Suwannee River natural organic matter are anionic at circumneutral pH[75], the charge repulsion and decreased associated sorption properties may prevent the natural sensitizers from adsorbing to the cellular membrane which is also negatively charged, to damage it with reactive oxygen species generated at locally high concentrations[20,76]. The photosensitizers may also interact with

375 the bacteria to shield the susceptible targets of photoinactivation[77], thereby reducing
376 photoinactivation to a greater degree than that for which can be corrected by light screening.
377 Lastly, photosensitizers may react with transient reactive oxygen species[78–82], thereby acting as a
378 sink for reactive oxygen species generated within or outside the cell. We used relatively high, yet
379 still environmentally relevant, concentrations of NOM in our experiments; future work could
380 explore if lower or higher NOM concentrations give rise to different results regarding exogenous
381 indirect photoinactivation. The natural photosensitizers we used in this study may differ from the
382 multitude of photosensitizers found in the natural environment in terms of sizes, charges, ROS-
383 generating potentials, and potential for associating with cellular membranes. It is possible that
384 other NOM could induce exogenous indirect photoinactivation.
385 When the presence of a photosensitizer did enhance photoinactivation, the resulting
386 exogenous indirect photoinactivation rate constant did not correlate with bulk phase steady state
387 concentrations of singlet oxygen or hydroxyl radical. The lack of a correlation suggests that the
388 exogenous indirect mechanism depends on more than the bulk phase reactive oxygen species
389 concentrations alone. A strong possibility is that the exogenous indirect mechanism is mediated
390 by interactions between the cell and the sensitizer[20,76]. When the sensitizer is associated with the
391 bacterial surface, localized concentrations of reactive species may be much higher than in the
392 bulk phase[19,20,76]. There are other reactive species that we did not consider which may have
393 contributed to and correlate with exogenous indirect photoinactivation rate constants. For
394 example, it cannot be excluded that, in the 1 mM CBS buffer solution used as matrix in the
395 experiments, no secondary carbonate radicals formed in the presence of photosensitizers.
396 Carbonate radicals are formed when carbonate or bicarbonate scavenge hydroxyl-radical or
397 through scavenging reactions with excited triplet state NOM [64,83]. If carbonate radicals did form,

398 they could potentially enhance bacterial photoinactivation. Additionally, triplet state NOM may
399 also contribute to photoinactivation. However so far, no study has provided evidence that under
400 environmentally relevant conditions carbonate radical or triplet state NOM contributes
401 significantly to bacterial inactivation. One study concluded that carbonate radicals do not
402 contribute to the inactivation of rotavirus[39], while another predicted that carbonate radicals
403 contributed ~1% and ~0.2% to the overall photoinactivations of the model viruses MS2 and
404 phiX174 in pond water, respectively[84]. For viruses, the triplet state has been associated with the
405 exogenous indirect photoinactivation mechanism for rotavirus[39,43]. Future work could examine
406 how sensitive different bacterial species are to different reactive species following what was
407 done by Mattle et al. for three model viruses[84]. While this approach would allow us to determine
408 second order rate constants for each reactive species relative to its bulk phase concentration, it
409 may be problematic since we suspect that adsorption of the photosensitizer to the cell and
410 localized high concentrations of reactive species near the photosensitizer play an important role
411 in mediating the exogenous indirect mechanism.
412 The amount of UVB light relative to other wavelengths likely affects the contribution of
413 the exogenous indirect mechanism to the overall photoinactivation of bacteria. Removal of UVB
414 entirely from the described experiments enhances the relative contribution of exogenous
415 photoinactivation to total photoinactivation significantly[85]. Thus, differences in the amount of
416 UVB present in experimental systems may affect conclusions about the importance of the
417 exogenous indirect mechanism (see supporting information of Fisher et al.[56]).
418 Our photoinactivation rate constants were correct for UVB light screening (280-320 nm)
419 solely while others have corrected rate constants using UVA+UVB light (280-400 nm).
420 Including the fluence from UVA light in the calculations of photoinactivation rate contants does

not change the results reported herein (see Tables S7 and S8 and Figure S3). Future work to uncover the precise wavelengths of light responsible for endogenous bacterial photoinactivation (through determining organism-specific action spectra[41,86,87]) will aid in the choice of wavelengths to use for light screening calculations.

Some decay profiles exhibited a shoulder, most predominantly among the Gram-positive bacteria. A shoulder may arise due to a threshold effect, for which a cell can withstand a certain level of stress before death, or because multiple cellular targets independently require inactivation to reach cell death[88–91]. Field modeling efforts typically have not incorporated a shoulder component[18] and it is unclear if the shoulder is an artifact of the experimental set up or is experienced by organisms in natural surface waters. Future work will need to address this.

Environmental relevance. Colored dissolved organic matter and other natural photosensitizers in water have the potential to both enhance photoinactivation via the exogenous indirect mechanism and diminish photoinactivation by blocking the light that would contribute to the direct and indirect endogenous mechanisms. This research suggests that the bacterial membrane properties (Gram-positive versus Gram-negative), and interactions between bacteria and photosensitizer, determine whether or not the exogenous indirect pathway is important relative to other inactivation pathways for bacteria. Furthermore, this research demonstrates the inability of Suwannee River NOM and analogous NOM to significantly accelerate bacterial photoinactivation under the conditions tested.

SUPPORTING INFORMATION

Includes additional experimental details, calculations for light screening, calculations of carbonate radical contribution, table of ROS concentrations in solution, tables of raw data from

444 photoinactivation experiments, results of model fittings and statistical analysis, and the natural
445 and simulated light spectra. This material is available free of charge.
446
447 ***ACKNOWLEDGMENTS***
448 This work was supported by the National Science Foundation (NSF) Engineering
449 Research Center for Reinventing the Nation's Urban Water Infrastructure (ReNUWIt) (EEC-
450 1028968), NSF grants CBET- 1334359 and OCE- 1129270, and an NSF Graduate Research
451 Fellowships for P.A.M., and a scholarship of the Swiss National Science Foundation (PBEZP2-
452 142887) for J.W. We acknowledge Emmanuel Assa, Sneha Ayyagari, Andrea Cheung, Lauren
453 Donovan, Robert Firme, Monique Grimaldi, Austin Hay, Brad Huang, Laura Kwong, Aloysius
454 Makalinao, Jill McClary, Carolyn Morrice, Soyun Park, Matt Pierno, Shanna Rucker, and Micah
455 Silberstein for laboratory assistance. We thank Kara Nelson for her comments on the paper.
456
457 ***REFERENCES***

458 (1) Cohen, J. E.; Small, C.; Mellinger, A.; Gallup, J.; Sachs, J.; Vitousek, P. M.; Mooney, H.
459 A. Estimates of Coastal Population. *Science (80-.).* **2008**, *278*, 1211–1212.
460 (2) Halpern, B. S.; Walbridge, S.; Selkoe, K. A.; Kappel, C. V.; Micheli, F.; D'Angrosa, C.;
461 Bruno, J. F.; Casey, K. S.; Ebert, C.; Fox, H. E.; et al. A Global Map of Human Impact on
462 Marine Ecosystems. *Science (80-.).* **2008**, *319*, 948–953.
463 (3) Halpern, B. S.; Longo, C.; Hardy, D.; McLeod, K. L.; Samhouri, J. F.; Katona, S. K.;
464 Kleisner, K.; Lester, S. E.; O'Leary, J.; Ranelletti, M.; et al. An index to assess the health
465 and benefits of the global ocean. *Nature* **2012**, *488*, 615–620.
466 (4) Dorfman, M.; Rosselot, K. S. *Testing the Water: A Guide to Water Quality at Vacation*
467 *Beaches*; 2008.
468 (5) World Health Organization. *Monitoring bathing waters – A practical guide to the design*
469 *and implementation of assessments and monitoring programmes*; Bartram, J.; Rees, G.,
470 Eds.; 2000.
471 (6) World Health Organization. *Guidelines for safe recreational water environments, Volume*
472 *1, Coastal and Fresh Waters*; 2003; Vol. 1.

(7) Boehm, A. B.; Soller, J. A. Risks Associated with Recreational Waters: Pathogens and Fecal Indicators. In *Encyclopedia of Sustainability Science and Technology*; Laws, E. A., Ed.; 2013.

(8) Boehm, A. B.; Ashbolt, N. J.; Colford, J. M.; Dunbar, L. E.; Fleming, L. E.; Gold, M. A.; Hansel, J. A.; Hunter, P. R.; Ichida, A. M.; McGee, C. D.; et al. A sea change ahead for recreational water quality criteria. *J. Water Health* **2009**, *7*, 9–20.

(9) Boehm, A. B.; Grant, S. B.; Kim, J. H.; Mowbray, S. L.; McGee, C. D.; Clark, C. D.; Foley, D. M.; Wellman, D. E. Decadal and Shorter Period Variability of Surf Zone Water Quality at Huntington Beach, California. *Environ. Sci. Technol.* **2002**, *36*, 3885–3892.

(10) Erin K. Lipp, Nancy Schmidt, Mark E. Luther, and J. B. R. Determining the Effects of El Nifio-Southern Oscillation Events on Coastal Water Quality. *Estuaries* **2001**, *24*, 491–497.

(11) Whitman, R. L.; Nevers, M. B.; Korinek, G. C.; Byappanahalli, M. N. Solar and Temporal Effects on Escherichia coli Concentration at a Lake Michigan Swimming Beach Solar and Temporal Effects on Escherichia coli Concentration at a Lake Michigan Swimming Beach. *Appl. Environ. Microbiol.* **2004**, *70*, 4276.

(12) Enns, A. A.; Vogel, L. J.; Abdelzaher, A. M.; Solo-Gabriele, H. M.; Plano, L. R. W.; Gidley, M. L.; Phillips, M. C.; Klaus, J. S.; Piggot, A. M.; Feng, Z.; et al. Spatial and temporal variation in indicator microbe sampling is influential in beach management decisions. *Water Res.* **2012**, *46*, 2237–2246.

(13) Yamahara, K. M.; Layton, B. A.; Santoro, A. E.; Boehm, A. B. Beach Sands along the California Coast are Diffuse Sources of Fecal Bacteria to Coastal Waters. *Environ. Sci. Technol.* **2007**, *41*, 4515–4521.

(14) Davies-Colley, R. J.; Donnison, A. M.; Speed, D. J.; Ross, C. M.; Nagels, J. W. Inactivation of faecal indicator micro-organisms in waste stabilisation ponds: interactions of environmental factors with sunlight. *Water Res.* **1999**, *33*, 1220–1230.

(15) Davies-Colley, R. J.; Donnison, A. M.; Speed, D. J. Towards a mechanistic understanding of pond disinfection. *Water Sci. Technol.* **2000**, *42*, 149–158.

(16) Malloy, K. D.; Holman, M. A.; Mitchell, D.; Detrich, H. W. Solar UVB-induced DNA damage and photoenzymatic DNA repair in antarctic zooplankton. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 1258–1263.

(17) Schuch, A. P.; Menck, C. F. M. The genotoxic effects of DNA lesions induced by artificial UV-radiation and sunlight. *J. Photochem. Photobiol. B, Biol.* **2010**, *99*, 111–116.

(18) Nguyen, M. T.; Jasper, J. T.; Boehm, A. B.; Nelson, K. L. Sunlight inactivation of fecal indicator bacteria in open-water unit process treatment wetlands: Modeling endogenous and exogenous inactivation rates. *Water Res.* **2015**, *83*, 282–290.

(19) Kohn, T.; Nelson, K. L. Sunlight-mediated inactivation of MS2 coliphage via exogenous singlet oxygen produced by sensitizers in natural waters. *Environ. Sci. Technol.* **2007**, *41*, 192–197.

(20) Kohn, T.; Grandbois, M.; McNeill, K.; Nelson, K. L. Association with natural organic

matter enhances the sunlight-mediated inactivation of MS2 coliphage by singlet oxygen. *Environ. Sci. Technol.* **2007**, *41*, 4626–4632.

(21) Sinton, L. W.; Hall, C. H.; Lynch, P. A.; Davies-colley, R. J. Sunlight Inactivation of Fecal Indicator Bacteria and Bacteriophages from Waste Stabilization Pond Effluent in Fresh and Saline Waters. *Appl. Environ. Microbiol.* **2002**, *68*, 1122–1131.

(22) Fisher, M. B.; Keenan, C. R.; Nelson, K. L.; Voelker, B. M. Speeding up solar disinfection (SODIS): effects of hydrogen peroxide, temperature, pH, and copper plus ascorbate on the photoinactivation of E. coli. *J. Water Health* **2008**, *6*, 35–51.

(23) Fujioka, R. S.; Hashimoto, H. H.; Siwak, E. B.; Young, R. H. F. Effect of Sunlight on Survival of Indicator Bacteria in Seawater. *Appl. Environ. Microbiol.* **1981**, *41*, 690–696.

(24) Sassoubre, L. M.; Nelson, K. L.; Boehm, A. B. Mechanisms for Photoinactivation of Enterococcus faecalis in Seawater. *Appl. Environ. Microbiol.* **2012**, *78*, 7776–7785.

(25) Sassoubre, L. M.; Walters, S. P.; Russell, T. L.; Boehm, A. B. Sources and fate of Salmonella and fecal indicator bacteria in an urban creek. *J. Environ. Monit.* **2011**, *13*, 2206–2212.

(26) Noble, R. T.; Lee, I. M.; Schiff, K. C. Inactivation of indicator micro-organisms from various sources of faecal contamination in seawater and freshwater. *J. Appl. Microbiol.* **2004**, *96*, 464–472.

(27) Sciacca, F.; Rengifo-Herrera, J. A.; Wéthé, J.; Pulgarin, C. Dramatic enhancement of solar disinfection (SODIS) of wild Salmonella sp. in PET bottles by H2O2 addition on natural water of Burkina Faso containing dissolved iron. *Chemosphere* **2010**, *78*, 1186–1191.

(28) Obiri-Danso, K.; Paul, N.; Jones, K. The effects of UVB and temperature on the survival of natural populations and pure cultures of Campylobacter jejuni, Camp. coli, Camp. lari and urease-positive thermophilic campylobacters (UPTC) in surface waters. *J. Appl. Microbiol.* **2001**, *90*, 256–267.

(29) Kehoe, S. C.; Barer, M. R.; Devlin, L. O.; McGuigan, K. G. Batch process solar disinfection is an efficient means of disinfecting drinking water contaminated with Shigella dysenteriae type I. *Lett. Appl. Microbiol.* **2004**, *38*, 410–414.

(30) Berney, M.; Weilenmann, H. U.; Simonetti, A.; Egli, T. Efficacy of solar disinfection of Escherichia coli, Shigella flexneri, Salmonella Typhimurium and Vibrio cholerae. *J. Appl. Microbiol.* **2006**, *101*, 828–836.

(31) Kramer, G. F.; Ames, B. N. Oxidative mechanisms of toxicity of low-intensity near-UV light in Salmonella typhimurium. *J. Bacteriol.* **1987**, *169*, 2259–2266.

(32) Sinton, L.; Hall, C.; Braithwaite, R. Sunlight inactivation of Campylobacter jejuni and Salmonella enterica, compared with Escherichia coli, in seawater and river water. *J. Water Health* **2007**, *5*, 357–365.

(33) Boehm, A. B.; Soetjipto, C.; Wang, D. Solar inactivation of four Salmonella serovars in fresh and marine waters. *J. Water Health* **2012**, *10*, 504–510.

(34) Bosshard, F.; Berney, M.; Scheifele, M.; Weilenmann, H. U.; Egli, T. Solar disinfection

(SODIS) and subsequent dark storage of Salmonella typhimurium and Shigella flexneri monitored by flow cytometry. *Microbiology* **2009**, *155*, 1310–1317.

(35) Sassoubre, L. M.; Ramsey, M. M.; Gilmore, M. S.; Boehm, A. B. Transcriptional response of Enterococcus faecalis to sunlight. *J. Photochem. Photobiol. B Biol.* **2014**, *130*, 349–356.

(36) Kadir, K.; Nelson, K. L. Sunlight mediated inactivation mechanisms of Enterococcus faecalis and Escherichia coli in clear water versus waste stabilization pond water. *Water Res.* **2014**, *50*, 307–317.

(37) Alves, E.; Costa, L.; Carvalho, C. M. B.; Tomé, J. P. C.; Faustino, M. A.; Neves, M. G. P. M. S.; Tomé, A. C.; Cavaleiro, J. A. S.; Cunha, A.; Almeida, A. Charge effect on the photoinactivation of Gram-negative and Gram-positive bacteria by cationic meso-substituted porphyrins. *BMC Microbiol.* **2009**, *9*, 70.

(38) Dahl, T. A.; Midden, W. R.; Hartman, P. E. Comparison of Killing of Gram-Negative and Gram-Positive Bacteria by Pure Singlet Oxygent. *J. Bacteriol.* **1989**, *171*, 2188–2194.

(39) Romero-Maraccini, O. C.; Sadik, N. J.; Rosado-Lausell, S. L.; Pugh, C. R.; Niu, X.-Z.; Croue, J.-P.; Nguyen, T. H. Sunlight induced Inactivation of Human Wa and Porcine OSU Rotaviruses in the Presence of Exogenous Photosensitizers. *Environ. Sci. Technol.* **2013**, *47*, 11004–11012.

(40) Romero, O. C.; Straub, A. P.; Kohn, T.; Nguyen, T. H. Role of temperature and Suwannee River natural organic matter on inactivation kinetics of rotavirus and bacteriophage MS2 by solar irradiation. *Environ. Sci. Technol.* **2011**, *45*, 10385–10393.

(41) Silverman, A. I.; Nguyen, M. T.; Schilling, I. E.; Wenk, J.; Nelson, K. L. Sunlight Inactivation of Viruses in Open-Water Unit Process Treatment Wetlands: Modeling Endogenous and Exogenous Inactivation Rates. *Environ. Sci. Technol.* **2015**, *49*, 2757–2766.

(42) Silverman, A. I.; Peterson, B. M.; Boehm, A. B.; McNeill, K.; Nelson, K. L. Sunlight inactivation of human viruses and bacteriophages in coastal waters containing natural photosensitizers. *Environ. Sci. Technol.* **2013**, *47*, 1870–1878.

(43) Rosado-Lausell, S. L.; Wang, H.; Gutierrez, L.; Romero-Maraccini, O. C.; Niu, X.-Z.; Gin, K. Y. H.; Croue, J.-P.; Nguyen, T. H. Roles of Singlet Oxygen and Triplet Excited State of Dissolved Organic Matter Formed by Different Organic Matters in Bacteriophage MS2 Inactivation. *Water Res.* **2013**, *47*, 4869–4879.

(44) Dahl, T.; Midden, W. R.; Neckers, D. C. Comparison of Photodynamic Action By Rose Bengal in Gram-Positive and Gram-Negative Bacteria. *Photochem. Photobiol.* **1988**, *48*, 607–612.

(45) Maraccini, P. A.; Wang, D.; McClary, J. S.; Boehm, A. B. Growth-dependent photoinactivation kinetics of Enterococcus faecalis. *J. Appl. Microbiol.* **2015**, *118*, 1226–1237.

(46) Plumlee, M. H.; Reinhard, M. Photochemical attenuation of N-nitrosodimethylamine (NDMA) and other nitrosamines in surface water. *Environ. Sci. Technol.* **2007**, *41*, 6170–

6176.

(47) Canonica, S.; Freiburghaus, M. Electron-Rich Phenols for Probing the Photochemical Reactivity of Freshwaters. *Environ. Sci. Technol.* **2001**, *35*, 690–695.

(48) Haag, W. R.; Hoigne, J. Singlet oxygen in surface waters. 3. Photochemical formation and steady-state concentrations in various types of waters. *Environ. Sci. Technol.* **1986**, *20*, 341–348.

(49) Kochanyt, J.; Bolton, J. R. Mechanisms of Photodegradation of Aqueous Organic Pollutants. 1. EPR Spin-Trapping Technique for the Determination of OH Radical Rate Constants in the Photooxidation of Chlorophenols following the Photolysis of H2O2. *J. Phys. Chem.* **1991**, *95*, 5116–5120.

(50) van Boekel, M. A. J. S. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *Int. J. Food Microbiol.* **2002**, *74*, 139–159.

(51) Calza, P.; Vione, D.; Novelli, A.; Pelizzetti, E.; Minero, C. The role of nitrite and nitrate ions as photosensitizers in the phototransformation of phenolic compounds in seawater. *Sci. Total Environ.* **2012**, *439*, 67–75.

(52) 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 (·OH/·O-) in aqueous solution. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513–886.

(53) Richard, C.; Lemaire, J. Analytical and kinetic study of the phototransformation of furfuryl alcohol in aqueous ZnO suspensions. *J. Photochem. Photobiol. A Chem.* **1990**, *55*, 127–134.

(54) Rocha, E. R.; Selby, T.; Coleman, J. P.; Smith, C. J. Oxidative stress response in an anaerobe, Bacteroides fragilis: a role for catalase in protection against hydrogen peroxide. *J. Bacteriol.* **1996**, *178*, 6895–6903.

(55) Imlay, J. A. *How oxygen damages microbes: Oxygen tolerance and obligate anaerobiosis*; 2002; Vol. 46.

(56) Fisher, M. B.; Iriarte, M.; Nelson, K. L. Solar water disinfection (SODIS) of Escherichia coli, Enterococcus spp., and MS2 coliphage: effects of additives and alternative container materials. *Water Res.* **2012**, *46*, 1745–1754.

(57) Maraccini, P. A.; Ferguson, D. M.; Boehm, A. B. Diurnal variation in Enterococcus species composition in polluted ocean water and a potential role for the enterococcal carotenoid in protection against photoinactivation. *Appl. Environ. Microbiol.* **2012**, *78*, 305–310.

(58) Berney, M.; Weilenmann, H.; Ihssen, J.; Bassin, C.; Egli, T. Specific Growth Rate Determines the Sensitivity of Escherichia coli to Thermal , UVA , and Solar Disinfection. *Appl. Environ. Microbiol.* **2006**, *72*, 2586–2593.

(59) Berney, M.; Weilenmann, H.-U.; Egli, T. Flow-cytometric study of vital cellular functions in Escherichia coli during solar disinfection (SODIS). *Microbiology* **2006**, *152*, 1719–1729.

(60) Dejung, S.; Fuentes, I.; Almanza, G.; Jarro, R.; Navarro, L.; Arias, G.; Urquieta, E.; Torrico, A.; Fenandez, W.; Iriarte, M.; et al. Effect of solar water disinfection (SODIS) on model microorganisms under improved and field SODIS conditions. *J. Water Supply Res. Technol. - AQUA* **2007**, *56*, 245–256.

(61) Smith, R. J.; Kehoe, S. C.; McGuigan, K. G.; Barer, M. R. Effects of simulated solar disinfection of water on infectivity of Salmonella typhimurium. *Lett. Appl. Microbiol.* **2000**, *31*, 284–288.

(62) Boyle, M.; Sichel, C.; Fernández-Ibáñez, P.; Arias-Quiroz, G. B.; Iriarte-Puña, M.; Mercado, A.; Ubomba-Jaswa, E.; McGuigan, K. G. Bactericidal effect of solar water disinfection under real sunlight conditions. *Appl. Environ. Microbiol.* **2008**, *74*, 2997–3001.

(63) Dong, S.; Hong, P.; Nguyen, T. H. Persistence of Bacteroides ovatus under simulated sunlight irradiation Persistence of Bacteroides ovatus under simulated sunlight irradiation. **2014**.

(64) Busset, C.; Mazellier, P.; Sarakha, M.; De Laat, J. Photochemical generation of carbonate radicals and their reactivity with phenol. *J. Photochem. Photobiol. A Chem.* **2007**, *185*, 127–132.

(65) Mostafa, S.; Rosario-Ortiz, F. L. Singlet oxygen formation from wastewater organic matter. *Environ. Sci. Technol.* **2013**, *47*, 8179–8186.

(66) Vione, D.; Falletti, G.; Maurino, V.; Minero, C.; Pelizzetti, E.; Malandrino, M.; Ajassa, R.; Olariu, R.-I.; Arsene, C. Sources and sinks of hydroxyl radicals upon irradiation of natural water samples. *Environ. Sci. Technol.* **2006**, *40*, 3775–3781.

(67) Tai, C.; Xiao, C.; Zhao, T.; Wu, L.; Han, D. Determination of hydroxyl radicals photochemically generated in surface waters under sunlight by high performance liquid chromatography with fluorescence detection. *Anal. Methods* **2014**, *6*, 8193–8199.

(68) Brezonik, P. L.; Fulkerson-Brekken, J. Nitrate-Inducted Photolysis in Natural waters: Control on Concentrations of Hydroxyl Photo-Intermediates by Natural Scavenging Agents. *Environ. Sci. Technol.* **1998**, *32*, 3004–3010.

(69) Madigan, M. T.; Martinko, J. M.; Dunlap, P. V.; Clark, D. P. *Biology of Microorganisms*; 12th ed.; Pearson Benjamin Cummings: San Francisco, CA, 2009.

(70) Mamone, L.; Venosa, G. Di; Gándara, L.; Sáenz, D.; Vallecorsa, P.; Schickinger, S.; Rossetti, M. V; Batlle, A.; Buzzola, F.; Casas, A. Journal of Photochemistry and Photobiology B : Biology Photodynamic inactivation of Gram-positive bacteria employing natural resources. **2014**, *133*, 80–89.

(71) Malik, Z.; Ladan, H.; Nitzan, Y. Photodynamic inactivation of Gram-negative problems and possible solutions bacteria. *J. Photochem. Photobiol. B Biol.* **1992**, *14*, 262–266.

(72) Demidova, T. N.; Gad, F.; Zahra, T.; Francis, K. P.; Hamblin, M. R. Monitoring photodynamic therapy of localized infections by bioluminescence imaging of genetically engineered bacteria. *J. Photochem. Photobiol. B Biol.* **2005**, *81*, 15–25.

(73) Jiang, Z.; Vasil, A. I.; Hale, J. D.; Hancock, R. E. W.; Vasil, M. L.; Hodges, R. S. Effects

of net charge and the number of positively charged residues on the biological activity of amphipathic ??-helical cationic antimicrobial peptides. *Biopolym. - Pept. Sci. Sect.* **2008**, *90*, 369–383.

(74) Taheri-Araghi, S.; Ha, B. Y. Physical basis for membrane-charge selectivity of cationic antimicrobial peptides. *Phys. Rev. Lett.* **2007**, *98*, 5–8.

(75) Au, K. K.; Alpert, S. M.; Pernitsky, D. J. Particle and Natural Organic Matter Removal in Drinking Water. In *Operational Control of Coagulation and Filtration Processes - Manual of Water Supply Practices, M37 (3rd Edition)*; 2011; pp. 1–16.

(76) Latch, D. E.; Mcneill, K. Microheterogeneity of Singlet Oxygen Distribution in Irradiated Humic Acid Solutions. *Science (80-.).* **2006**, *311*, 1743–1747.

(77) Cantwell, R. E.; Hofmann, R.; Templeton, M. R. Interactions between humic matter and bacteria when disinfecting water with UV light. *J. Appl. Microbiol.* **2008**, *105*, 25–35.

(78) Goldstone, J. V.; Voelker, B. M. Chemistry of Superoxide Radical in Seawater: CDOM Associated Sink of Superoxide in Coastal waters. *Environ. Sci. Technol.* **2000**, *34*, 1043–1048.

(79) Canonica, S.; Laubscher, H.-U. Inhibitory effect of dissolved organic matter on triplet-induced oxidation of aquatic contaminants. *Photochem. Photobiol. Sci.* **2008**, *7*, 547–551.

(80) Janssen, E. M. L.; Erickson, P. R.; McNeill, K. Dual roles of dissolved organic matter as sensitizer and quencher in the photooxidation of tryptophan. *Environ. Sci. Technol.* **2014**, *48*, 4916–4924.

(81) Wenk, J.; von Gunten, U.; Canonica, S. Effect of Dissolved Organic Matter on the Transformation of Contaminants Induced by Excited Triplet States and the Hydroxyl Radical. *Environ. Sci. Technol.* **2011**, *45*, 1334–1340.

(82) Wenk, J.; Canonica, S. Phenolic antioxidants inhibit the triplet-induced transformation of anilines and sulfonamide antibiotics in aqueous solution. *Environ. Sci. Technol.* **2012**, *46*, 5455–5462.

(83) Burns, J. M.; Cooper, W. J.; Ferry, J. L.; King, D. W.; DiMento, B. P.; McNeill, K.; Miller, C. J.; Miller, W. L.; Peake, B. M.; Rusak, S. A.; et al. Methods for reactive oxygen species (ROS) detection in aqueous environments. *Aquat. Sci.* **2012**, *74*, 683–734.

(84) Mattle, M.; Vione, D.; Kohn, T. Conceptual model and experimental framework to determine the contributions of direct and indirect photoreactions to the solar disinfection of MS2, phiX174 and adenovirus. *Environ. Sci. Technol.* **2015**, 334–342.

(85) Maraccini, P. Photoinactivation of Bacteria: Effects of Physiology, Water Conditions, and Sunlight Intensity, 2016.

(86) Nguyen, M. T.; Silverman, A. I.; Nelson, K. L. Sunlight inactivation of ms2 coliphage in the absence of photosensitizers: Modeling the endogenous inactivation rate using a photoaction spectrum. *Environ. Sci. Technol.* **2014**, *48*, 3891–3898.

(87) Fisher, M. B.; Love, D. C.; Schuech, R.; Nelson, K. L. Simulated sunlight action spectra for inactivation of MS2 and PRD1 bacteriophages in clear water. *Environ. Sci. Technol.*

2011, *45*, 9249–9255.

(88) Sinton, L. W.; Davies-Colley, R. J.; Bell, R. G. Inactivation of enterococci and fecal coliforms from sewage and meatworks effluents in seawater chambers. *Appl. Environ. Microbiol.* **1994**, *60*, 2040–2048.

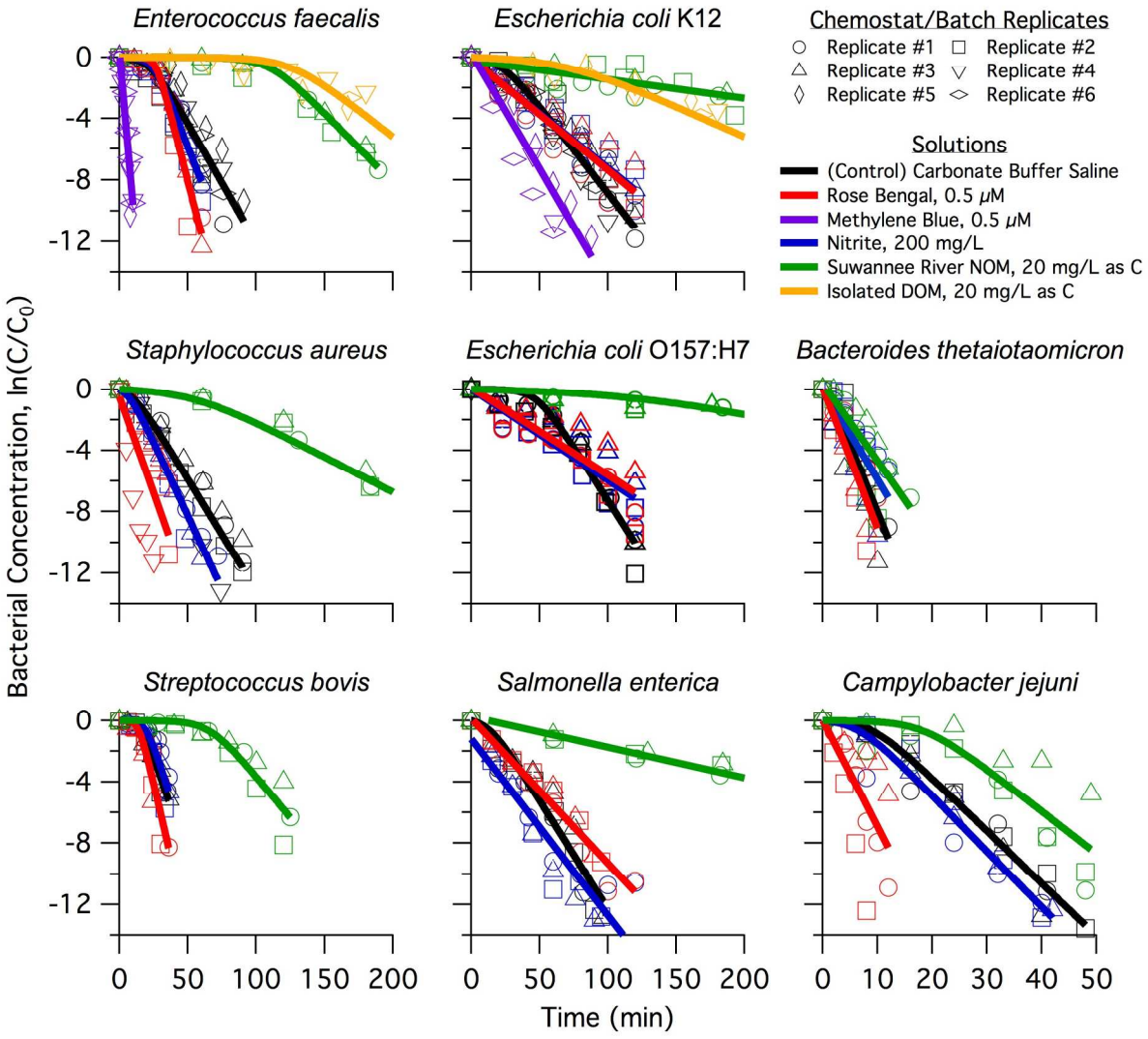
(89) Sinton, L. W.; Finlay, R. K.; Lynch, P. A. Sunlight Inactivation of Fecal Bacteriophages and Bacteria in Sewage-Polluted Seawater. *Appl. Environ. Microbiol.* **1999**, *65*, 3605–3613.

(90) Thurston-Enriquez, J. A.; Haas, C. N.; Jacangelo, J.; Riley, K.; Gerba, C. P. Inactivation of Feline Calicivirus and Adenovirus Type 40 by UV Radiation. *Appl. Environ. Microbiol.* **2003**, *69*, 577–582.

(91) Cebrián, G.; Michiels, C. W.; Mañas, P.; Condón, S. Biological approach to modeling of Staphylococcus aureus high-hydrostatic-pressure inactivation kinetics. *Appl. Environ. Microbiol.* **2010**, *76*, 6982–6990.

Bacteria: Photosensitizer	$\hat{k}_{exogenous}$ [m^2MJ^{-1}]	$k_{exogenous}$ [min^{-1}]
Ent. faecalis:		
0.5 μM Methylene Blue	11681 ± 1958	1.07 ± 0.18
0.5 μM Rose Bengal	2788 ± 367	0.24 ± 0.03
200 mg/L Nitrite	900 ± 262	0.07 ± 0.02
Staph. aureus:		
0.5 μM Rose Bengal	1992 ± 904	0.16 ± 0.07
200 mg/L Nitrite	905 ± 327	0.07 ± 0.03
Strep. bovis:		
0.5 μM Rose Bengal	1709 ± 898	0.16 ± 0.08
E. coli K12:		
0.5 μM Methylene Blue	1160 ± 548	0.09 ± 0.04
C. jejuni:		
0.5 μM Rose Bengal	8972 ± 4786	0.79 ± 0.42

Table 1. Exogenous photoinactivation rate constants for bacteria in a photosensitizer treatment where the observed $\hat{k}$ was higher than that in the control solution for at least two of the three replicates as determined by pairwise comparison. $\hat{k}_{exogenous}$ ± standard error was estimated as the difference between the average $\hat{k}$ from the pooled experiments with a photosensitizer and the average $\hat{k}$ of the pooled control experiments, then the $k_{exogenous}$ ± standard error was calculated from the depth-average UVB intensity (averaged across control and treatment replicates). *k* values, their standard deviations, and their 95% confidence intervals for individual experiments may be found in Table S3, while $\hat{k}$ values, their standard deviations, and their 95% confidence intervals for individual experiments may be found in Table S4.

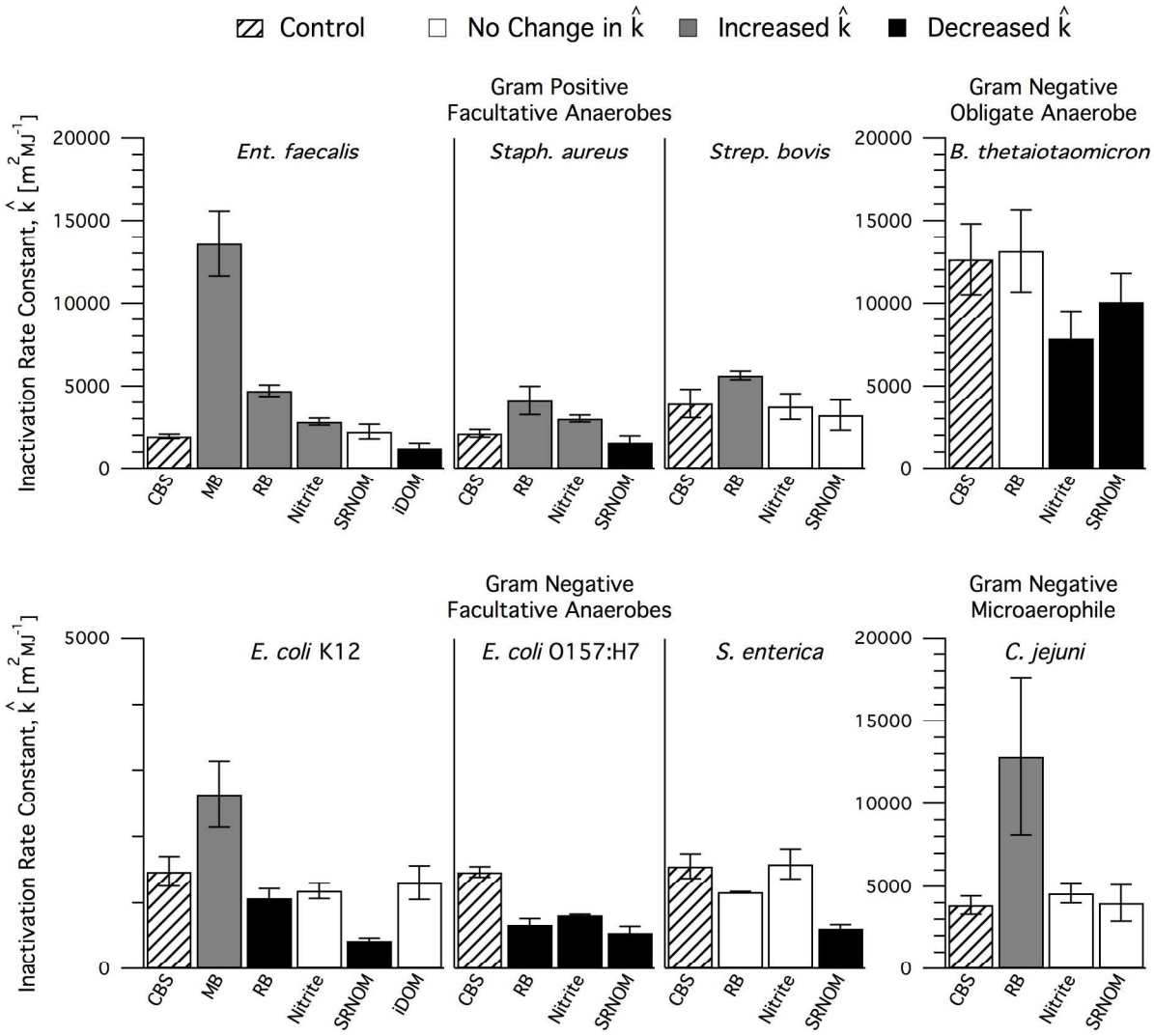


734

735 **Figure 1.** Photoinactivation curves of Gram-positive facultative anaerobes (*Ent. faecalis*, *Staph.*
736 *aureus*, *Strep. bovis*), Gram-negative facultative anaerobes (*E. coli* K12, *E. coli* O157:H7, *S.*
737 *enterica*), an obligate anaerobe (*B. thetaiotaomicron*), and a microaerophile (*C. jejuni*) in 1 mM
738 carbonate buffer saline with various photosensitizers. The data from the replicate experiments
739 (shown as different symbols, minimum three for each bacteria and solution) were aggregated
740 together and fitted with either shoulder-log linear or first order kinetics model (solid lines on
741 graphs) for ease of viewing; however the decay curves obtained from the pooled replicates were
742 not used for hypothesis testing. As described in the methods, three biological paired replicates

743 were completed for each treatment and control. The number of replicates as indicated in the
744 legend is 6 because in some cases, not all treatments could be completed at the same time with a
745 single control, so the treatments and their paired controls were split between 2 separate
746 experiments (see Table S3).

747



748

749 **Figure 2.** Inactivation rate constants of Gram-positive facultative anaerobes, Gram-negative
750 facultative anaerobes, an obligate anaerobe, and a microaerophile in 1 mM carbonate buffer
751 saline (CBS) with various photosensitizers (CBS = no photosensitizer, MB = 0.5 μM methylene
752 blue, RB = 0.5 μM rose bengal, Nitrite = 200 mg/L NO_2^-, SRNOM = 20 mg/L as C Suwannee
753 River natural organic matter, iDOM = 20 mg/L as C dissolved organic matter isolated from a
754 treatment wetland) when irradiated with simulated sunlight. Values are averages and standard
755 errors from pooled biological replicates; note that pooled values were not used in hypothesis
756 testing and are shown here for visualization. The stripped bars are the control solutions (CBS),

757 the grey bars indicate the photosensitizer increased $\hat{k}$, the black bars indicate the photosensitizer
758 decreased $\hat{k}$, the white bars indicate there was no change in $\hat{k}$ compared to the control, as
759 determined by paired t-tests between each biological replicates matched treatment and control
760 (see methods). The $\hat{k}$ values, their standard deviations, and their 95% confidence intervals for
761 individual experiments may be found in Table S4.