Electronic Supplementary Information for

Photoinactivation of uncultured, indigenous enterococci

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Materials & Methods

Wastewater and surface water physicochemical measurements. At the time of sample collection, temperature, salinity, and DO were measured using YSI-30 and ProODO probes (YSI Incorporated, Yellow Springs, OH). Upon arrival at the laboratory, we also measured dissolved nonpurgeable organic carbon (NPOC), turbidity, and absorbance of all samples. NPOC was measured on a Shimadzu TOC-L analyzer (Shimadzu Corporation, Kyoto, Japan). Turbidity was measured on a DRT-15CE turbidimeter (HF Scientific, Fort Myers, FL). Absorbance was measured on a Uvikon XL spectrophotometer (BioTek Instruments, Inc., Winooski, VT) (Figure S3). A summary of physicochemical measurements for all samples is provided in Table S2.

Tangential filtration of surface waters. Tangential filtration was used to concentrate surface water samples using an OMEGA suspended screen cassette (Pall Corporation, Port Washington, NY). The membrane was sanitized with 0.1 N sodium hydroxide and flushed with at least 5 L of sterile DI water before use for each sample. The surface water samples were passed through the membrane until ~5 L remained in the retentate.

Quantification of enterococci on mEI agar. Enterococci were quantified from raw water samples and photoinactivation experiments by membrane filtration on mEI agar following EPA Method 1600.[1] Enterococci were identified as pink colonies with blue halos growing on mEI

agar after incubation at 41 °C for 24 ± 1 hours. Each batch of mEI agar was tested with *Enterococcus faecalis* (ATCC 19433) and *Escherichia coli* K12 (ATCC 10798) as positive and negative controls, respectively. On every day that filtrations were performed, a filtration blank was made with sterile PBS to verify a lack of contamination.

Enterococci pigmentation tests. The proportion of enterococci in raw water samples that were pigmented was quantified using a previously described method, with slight variation. Individual enterococci colonies from stored mEI agar plates were inoculated into 800 µL tryptic soy broth (TSB; Sigma-Aldrich, St. Louis, MO) and incubated at 37 °C for ~24 hours. A maximum of 50 colonies was selected from each plate; the total number of colonies examined for each water sample is provided in Table 1 in the main text. After incubation, one loop of liquid culture from each isolate was streaked onto tryptic soy agar (TSA; BD Difco, Sparks, MD) and incubated at 37 °C for 24-48 hours. After incubation, each streak was identified as pigmented or nonpigmented by picking a small amount of biomass on a sterile cotton swab and visually examining the swab for pigmentation. *E. casseliflavus* (ATCC 25788) and *E. faecalis* were used as positive (pigmented) and negative (nonpigmented) controls, respectively.

Calculations for light scattering and depth-averaged light intensity. We determined the relative importance of absorbance versus light scattering in our reactor systems by comparing the light scattering coefficient and the absorbance coefficient as has been described previously [2,3]. In brief, the light scattering coefficient ($b(\lambda)$) and absorbance coefficient ($a(\lambda)$) are calculated following previously defined relationships[4,5] using Equations S1 and S2, respectively:

$$b(\lambda) = \frac{T_n}{2.03 \pm 0.26} \qquad \textbf{Equation S1}$$

$$a(\lambda) = \frac{2.303\alpha(\lambda)}{l}$$ **Equation S2**

Where T_n is the turbidity of the reactor solution (NTU), $\alpha(\lambda)$ is the absorbance at wavelength λ, and l is the pathlength (m). The scattering and absorbance coefficients are then used to calculate the relative contribution of absorbance to the light attenuation through the water column:

$$\frac{K_d(\lambda)}{a(\lambda)} = \sqrt{1 + 0.256\frac{b(\lambda)}{a(\lambda)}}$$ **Equation S3**

Where $K_d(\lambda)$ is the vertical attenuation coefficient at wavelength λ. The contribution of scattering to light attenuation was considered negligible when $K_d(\lambda)/a(\lambda) < 1.1$. For all but one sample (beach2), light scattering was determined to be unimportant in the UVB range. Because the only available methods for incorporating light scattering in fluence calculations are based on simulations as opposed to analytical solutions,[6] we opted to exclude sample beach2 from certain analyses, as described in the main text. For all other samples, we calculated the depth-averaged light intensity ($\langle I \rangle_z$) for each experiment based on the emitted irradiance of the solar simulator (Figure S1) and the absorbance of the UVB wavelengths through the reactor solutions (Figure S2) (Equations S4 and S5):

$$\langle I \rangle_z = \sum_\lambda \langle I_\lambda \rangle_z$$ **Equation S4**

$$\langle I_\lambda \rangle_z = I_{\lambda,0}\frac{(1-10^{-\alpha_\lambda z})}{2.303\alpha_\lambda z}$$ **Equation S5**

Where $\langle I_\lambda \rangle_z$ is the depth-averaged light intensity at a single wavelength λ, $I_{\lambda,0}$ is the light intensity at wavelength λ, α_λ is the absorbance of the reactor solution at wavelength λ, and z is the reactor solution depth in cm. The emitted irradiance of the solar simulator was measured using a SpectriLight spectroradiometer (International Light Technologies; Peabody, MA), and

absorbance was measured on a Uvikon XL spectrophotometer (BioTek Instruments, Inc.). We noted a discontinuity in the absorbance spectra of reactor solutions seeded with WWTP and pond bacteria at ~340 nm (Figure S4). To investigate whether the discontinuity potentially affected the study conclusions, we modeled absorbance of reactors seeded with WWTP and pond bacteria in the UVB range (280-320 nm) using a monoexponential decay function fit to the absorbance of those solutions at wavelengths between 340 and 400 nm; the mono-exponential decay in absorbance with wavelength is typically observed in natural waters.[7] Photoinactivation rate constants corrected for light screening in the UVB range increased slightly using modeled UVB absorbance in fluence calculations, but did not substantively influence the relationships observed in the study. We therefore used the originally measured absorbance spectra for all fluence calculations.

If possible, less than 10% of the reactor volume was removed during the course of photoinactivation experiments. When ≤ 10% of the total reactor volume was removed over the course of the experiments, z was assumed to remain constant and was based on the starting reactor volume. Therefore, a single value of $\langle I \rangle_z$ was calculated for each experiment and was used to determine fluence for each sample using Equation S6.

$$F = t \times \langle I \rangle_z \qquad \textbf{Equation S6}$$

Where F is fluence in kJ/m^2 and t is time in minutes. During experiments when starting concentrations were low, it was necessary to remove larger sample volumes in order to quantify enterococci concentrations. To account for this, depth-averaged light intensity calculations for these experiments were corrected for the changing depth in the reactors over the course of the experiments. In cases where > 10% of the total reactor volume was removed during the

experiments, we accounted for the change in volume by calculating a new value for $\langle I \rangle_z$ from Equations S4 and S5 based on an updated depth z after each sample n was removed. Therefore, the fluence at a particular sample time t_n is calculated from Equation S7.

$$F_n = F_{n-1} + (t_n - t_{n-1}) \times \langle I \rangle_{z,n-1} \qquad \textbf{Equation S7}$$

Where F_n is the fluence associated with sample n, F_{n-1} is the fluence associated with sample $n-1$, $(t_n - t_{n-1})$ is equivalent to the time between samples $n-1$ and n, and $\langle I \rangle_{z,n-1}$ is the depth-averaged light intensity associated with the depth of the reactor (z) after removing sample $n-1$.

Comparison to previously published literature. In order to compare the photoinactivation rate constants generated in this study to those from previous literature, we searched the literature for studies measuring sunlight inactivation of enterococci. We used Web of Science to search the literature using the following terms: enterococc* AND (sunlight OR solar) AND (decay OR inactivat*) AND water AND (fluence OR irradia*). The search retrieved 30 articles. After title and abstract review, the full text was reviewed for 12 articles that measured sunlight inactivation of enterococci in water without additional treatment methods (such as photocatalysis and/or titanium dioxide addition). After full text review of the 12 articles, only 2 (Nguyen et al. and Silverman et al.) were determined to include sufficient information for comparison to our study.[8,9] The majority of remaining articles were excluded because their inactivation was reported in terms of time (as opposed to fluence), and they did not include absorbance or irradiance spectra sufficient to convert time-based inactivation to fluence-based. In addition to articles identified by the literature search, we included three additional studies from our lab for comparison.[2,10,11] Because our work herein presents all rate constants in units of $m^2 kJ_{UVB}^{-1}$, it was necessary to perform unit conversions and estimations of light screening on values from

previously published studies. For one study, photoinactivation rate constants were reported in the same units as here, so no unit conversion was necessary.[10]

In Maraccini et al. [11], rate constants for photoinactivation of *E. faecalis* and *E. casseliflavus* are presented in units of min^{-1}. To account for light screening and convert these units to $m^2kJ_{UVB}^{-1}$, irradiance and absorbance spectra were obtained from the original authors. These spectra were not measured during the specific experiments published in Maraccini et al., but they are from unpublished experiments performed with the same solar simulator and nearly identical experimental conditions. Using these spectra and z = 2.6 cm, $<I>_z$ was calculated using Equations S4 and S5 as described above, and rate constants in terms of time were converted to units of fluence by dividing by $<I>_z$.

In Maraccini et al. [2], laboratory experiments were conducted to measure a photoinactivation rate constant for lab-cultured *E. faecalis* in various water matrices, reported in the original paper in units of m^2MJ^{-1}.[2] The authors included a correction for light screening of UVB wavelengths in this value. Therefore, simple dimensional analysis was used to determine k in units of $m^2kJ_{UVB}^{-1}$ for direct comparison to our study.

In Nguyen et al.,[8] photoinactivation rate constants of lab-cultured *E. faecalis* and *E. casseliflavus* and of wastewater-sourced enterococci are reported in units of hr^{-1}. To convert these rate constants to units of $m^2kJ_{UVB}^{-1}$, irradiance data (in Wm^{-2}) at each wavelength in the UVB spectrum was pulled from the solar simulator outputs in the Supplementary Information of their paper using PlotDigitizer. The absorbance spectrum of all reactor solutions was not included in that paper, but we approximated this using absorbance spectra from our laboratory for experiments performed under nearly identical conditions. Nguyen et al.[8] specify the reactor depth

z = 5 cm. This data was used to calculate $\langle I \rangle_z$ using Equations S4 and S5 as described above, and rate constants reported in units of per time were converted to units of per UVB fluence using Equation S8:

$$k = k_t / \langle I \rangle_z \qquad \textbf{Equation S8}$$

In Silverman et al.[9], photoinactivation rate constants of lab-cultured *E. faecalis* and wastewater-sourced enterococci are provided in units of hr^{-1} (2.63 hr^{-1} and 0.46 hr^{-1}, respectively). Depth-averaged irradiance spectra (in units of Wm^{-2}) are also provided in the Supporting Information of their paper. PlotDigitizer was used to extract depth-averaged light intensity at each UVB wavelength for both lab-cultured and wastewater-sourced enterococci experiments, and these values were summed to determine $\langle I \rangle_z$. Rate constants were then converted to units of per fluence using Equation S8. Silverman et al. also published a biological weighting function model for predicting photoinactivation rate constants for wastewater-sourced enterococci.[9] We used this model to determine photoinactivation rate constants for wastewater-sourced enterococci from our experiments. The output of the model provides k in units of time (hr^{-1}), and we converted these units back to fluence in $m^2 kJ_{UVB}^{-1}$ in order to compare against the photoinactivation rate constants measured in our experiments.

Next, we wanted to validate our pigmentation-based photoinactivation model to other studies. Two previous studies from our lab included enterococci pigmentation and photoinactivation data from the same beach,[11,12] and so we combined data from these two studies to compare to our model. We also searched the literature using Web of Science and the following terms: enterococc* AND (sunlight OR solar) AND (decay OR inactivat*) AND water AND

pigment*. Two additional articles were identified from this search, but neither included sufficient information to compare to our model.

In Boehm et al. [12], photoinactivation rate constants of enterococci at a marine beach were derived using a mass balance model and reported in units of $d^{-1}W_{UVB}m^{-2}$ without accounting for light screening in the water column. To calculate photoinactivation rate constants that accounted for light screening, the original UVB irradiance spectra incident on the water surface and the solar zenith angle at every hour during the study were obtained from the authors. Absorbance spectra were not measured in these experiments, so we instead approximated the absorbance of the marine water using the average absorbance spectra of all beach samples collected in this study. The depth *z* across which light screening is considered was set to 2 m, , the volume averaged depth of the water column in Boehm et al.[12] To account for the changing solar zenith angle over the course of a day, Equation S5 was modified to include a pathlength correction factor using the following equations:

$$\langle I_\lambda \rangle_z = I_{\lambda,0} \frac{(1-10^{-\psi \alpha_\lambda z})}{2.303 \psi \alpha_\lambda z} \qquad \textbf{Equation S9}$$

$$\psi = \left(\sqrt{1-(n^{-1}\sin\gamma)^2}\right)^{-1} \qquad \textbf{Equation S10}$$

where ψ is the pathlength correction factor, *n* is the index of refraction for water (~ 1.34), γ is the solar zenith angle in radians, and all other variables are as previously defined. Equation S4 was then used to determine the overall depth-averaged light intensity at all UVB wavelengths during each hour, and this information was used in the original mass balance, in lieu of the UVB incident on the surface of the water, to model the photoinactivation rate constant of enterococci

with light screening.[12] The same model and approach described in Boehm et al. [12] were used to find the best fit *k* value.

References

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Tables

Table S1 Description of wastewater and surface water samples and time of collection.

Sample Name	Sample Source	Collection Date	Collection Time
WWTP1	City of Sunnyvale Water Pollution Control Plant, Sunnyvale, CA	13-Sep-17	15:00
WWTP2	Palo Alto Regional Water Quality Control Plant, Palo Alto, CA	14-Sep-17	7:30
WWTP3	San Jose-Santa Clara Regional Wastewater Facility, San Jose, CA	28-Sep-17	10:00
WWTP4	Daly City Water & Wastewater, Daly City, CA	2-Oct-17	9:00
WWTP5	Oceanside Water Pollution Control Plant, San Francisco, CA	10-Oct-17	9:00
WWTP6	San Francisco Southeast Treatment, San Francisco, CA	23-Oct-17	9:00
pond1	Palo Alto Duck Pond, Palo Alto, CA	4-Dec-17	7:30
beach1	Cowell Beach, Santa Cruz, CA	9-Dec-17	6:30
beach2	Main Beach, Santa Cruz, CA	11-Jan-18	7:30
beach3	Capistrano Beach, Half Moon Bay, CA	27-Jan-18	7:00
stream1	Serra Street drainage basin, Stanford, CA	1-Feb-18	7:30
stream2	San Lorenzo River, Santa Cruz, CA	8-Feb-18	7:30
stream3	San Pedro Creek, Pacifica, CA	17-Feb-18	7:00

Table S2 Summary of physicochemical and microbial measurements from collected wastewater and surface water samples, along with the initial concentration (C_0) of enterococci in experimental photoinactivation reactors. DO = dissolved oxygen; NPOC = nonpurgeable organic carbon. Error is ± standard deviation.

Sample Name	Temp (°C)	Salinity (ppt)	DO (%)	Turbidity (NTU)	NPOC (mg/L)	Enterococci in raw water (CFU/mL)	Enterococci in experimental reactors (i.e. C_0) (CFU/mL)
WWTP1	26.4 ± 0.1	0.6	4.4	101.9 ± 0.8	57.32 ± 24.65	2.29×10^4	1.84×10^4
WWTP2	25.8 ± 0.9	0.8	19	76.4 ± 5.4	34.12 ± 20.85	3.25×10^3	6.09×10^3
WWTP3	25.5	0.8	9.7	89.5	53.51 ± 1.99	3.36×10^3	3.60×10^3
WWTP4	10.2 ± 1.3	0.5	44.4	110.1 ± 5.4	90.36 ± 0.62	2.87×10^4	1.44×10^4
WWTP5	21.5 ± 0.1	0.4	46.9	232.5 ± 23.3	35.38 ± 0.70	8.98×10^3	5.95×10^3
WWTP6	21.9 ± 0.4	1.4	28.1	112.3 ± 0.8	75.38 ± 1.26	2.24×10^3	1.35×10^3
pond1	13.2 ± 0.1	17.8	138.5	12.14 ± 0.04	9.54 ± 0.99	1.28	3.33×10^1
beach1	11.3 ± 0.1	32.5	103.7	3.57 ± 0.16	2.55 ± 0.12	0.46	4.77
beach2	13.6 ± 0.5	32.7	100.6	6.53 ± 0.06	4.36 ± 0.41	0.67	2.19
beach3	11.9 ± 0.1	31.8	97	5.66 ± 0.03	4.59 ± 0.03	1.51	1.04×10^1
stream1	14.1 ± 0.1	0.1	10.2	3.72 ± 0.03	8.00 ± 0.06	1.21	3.01×10^1
stream2	10.4 ± 0.1	2.2	92.3	1.96 ± 0.23	5.19 ± 0.05	1.05	1.39×10^1
stream3	8.4 ± 0.1	0.2	90.7	1.37 ± 0.07	5.16 ± 0.21	8.80	1.35×10^2

Table S3 Photoinactivation rate constants with respect to fluence in the UVA & UVB wavelengths (280-400 nm).

Sample Name	k ($m^2 kJ_{UVA+UVB}^{-1}$)	S ($kJ_{UVA+UVB}/m^2$)
WWTP1	0.1 (0.01)	37.0 (4.3)
WWTP2	0.07 (0.008)	27.7 (3.5)
WWTP3	0.06 (0.01)	39.0 (2.7)
WWTP4	0.06 (0.006)	39.2 (7.3)
WWTP5	0.07 (0.01)	39.2 (7.2)
WWTP6	0.04 (0.006)	45.5 (4.8)
pond1	0.1 (0.03)	12.4 (5.5)
beach1	0.01 (0.003)	-
beach2	0.08 (0.0002)	73.7 (0.09)
beach3	0.02 (0.003)	-
stream1	0.04 (0.006)	17.8 (7.0)
stream2	0.03 (0.007)	39.1 (9.4)
stream3	0.04 (0.005)	30.9 (8.4)

Figures

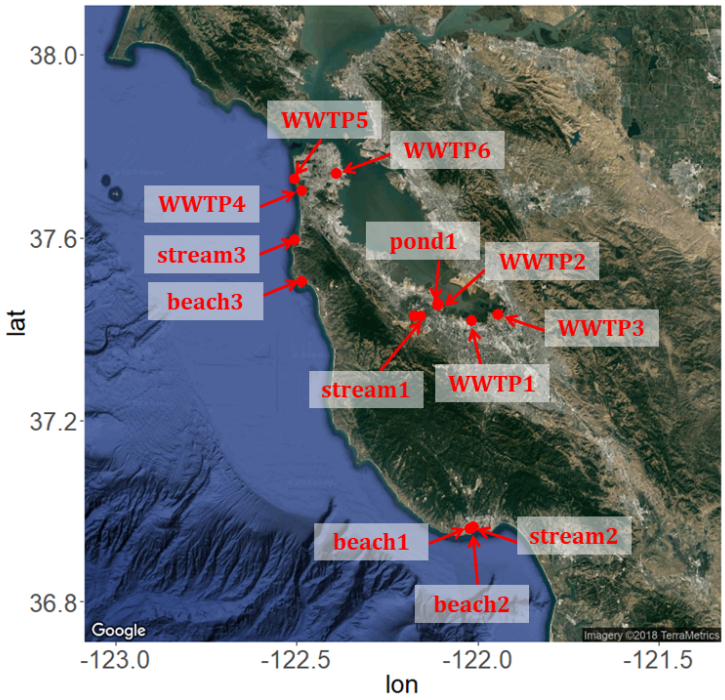


Figure S1 Map of wastewater and surface water sample locations. Lat = latitude; lon = longitude.

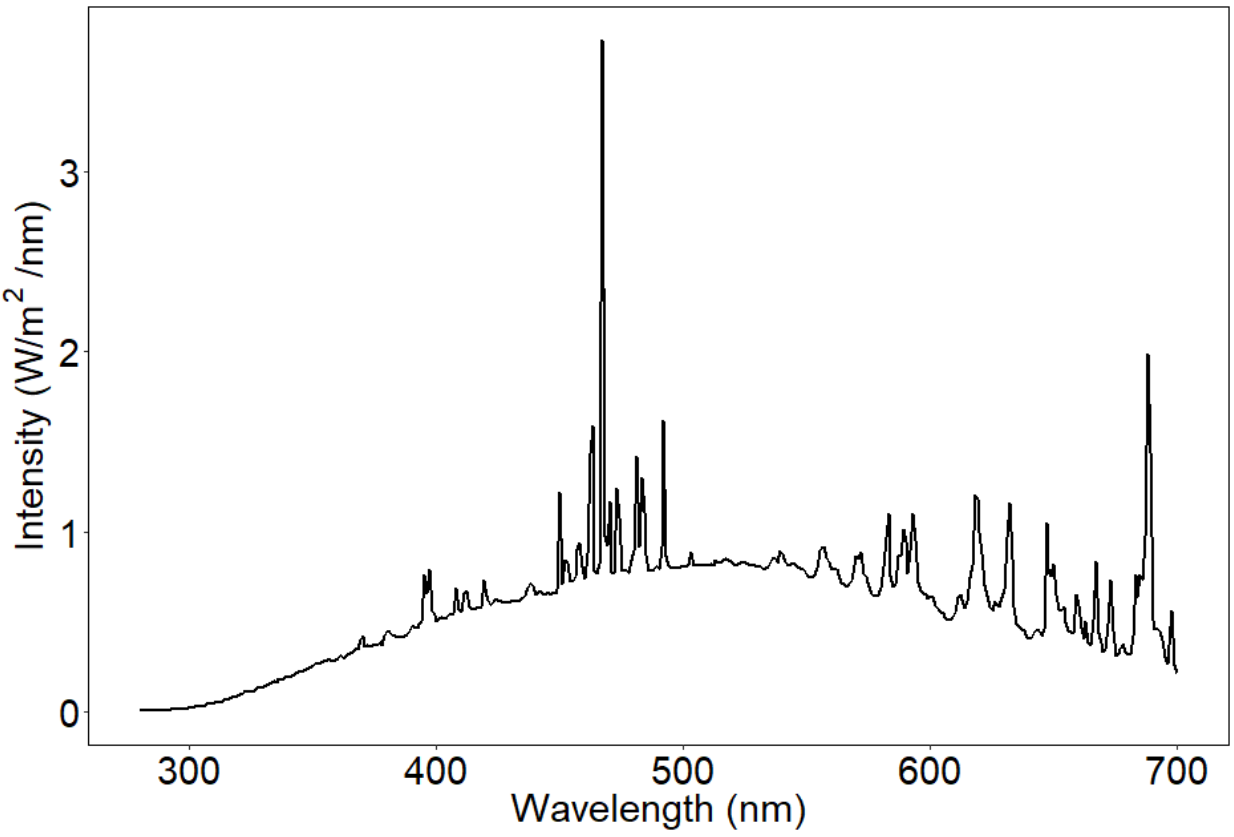


Figure S2 Irradiation emission spectrum from solar simulator.

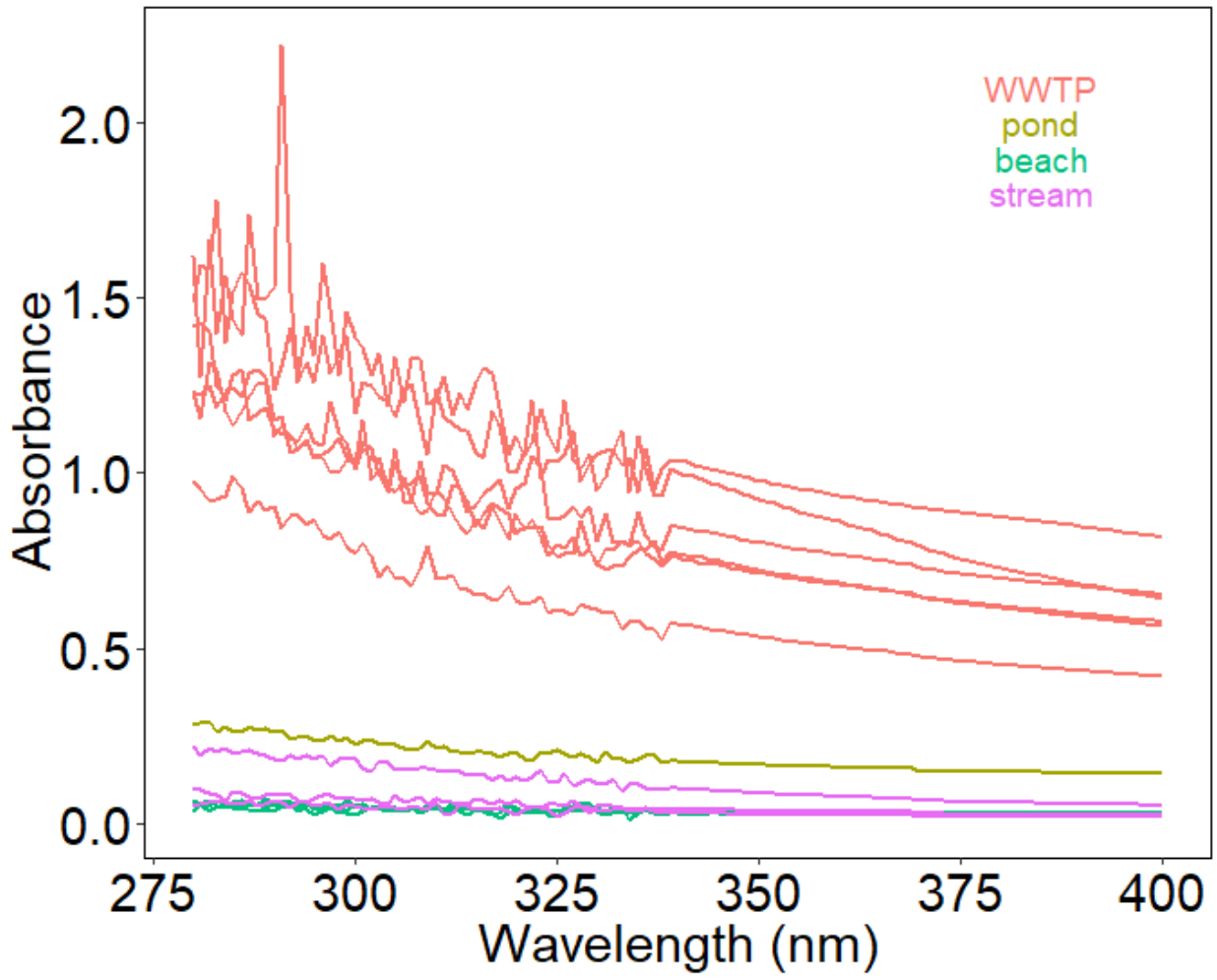


Figure S3. Absorbance of raw water samples in the UVA & UVB range (280-400 nm). Colors correspond to sample type.

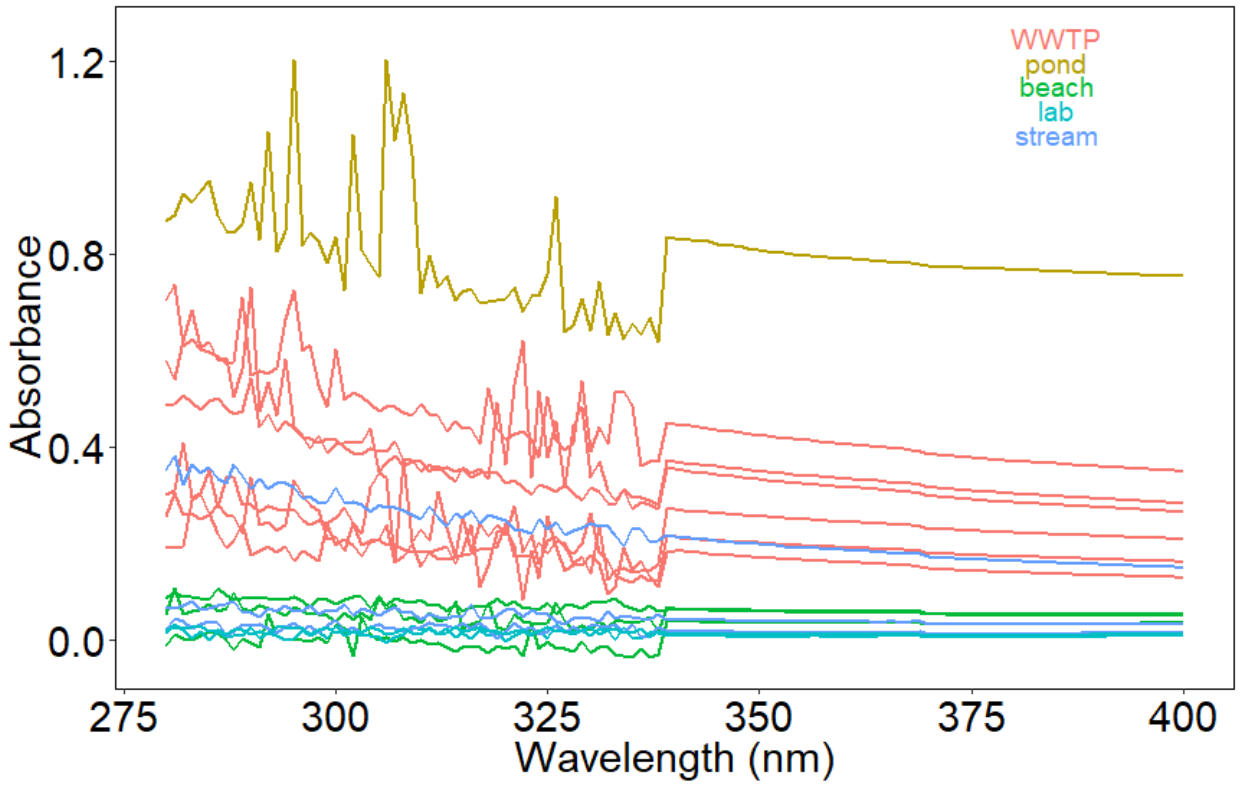
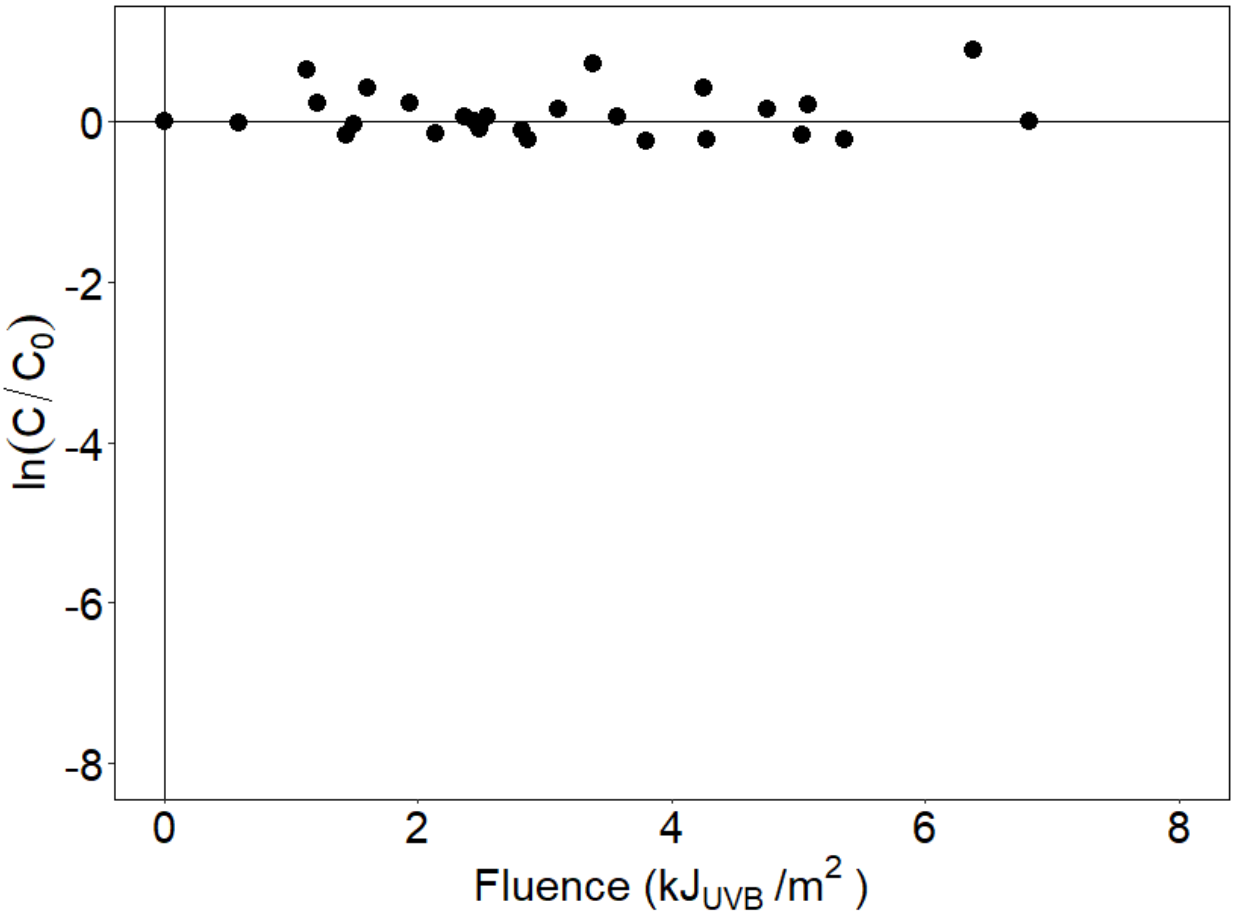


Figure S4 Absorbance of photoinactivation reactor solutions in the UVA and UVB range (280-400 nm). Colors correspond to sample type. There appears to be a discontinuity in the absorbance spectra ~340 nm that is particularly notable for the reactors seeded with WWTP and pond bacteria. The UV-Vis instrument uses two distinct light sources to measure absorbance below and above this wavelength. We did not note any problems with the cuvettes, or the blanks, nor did we note such a distinct discontinuity in the absorbances of the raw water samples (see Figure S3). We explored how this might have influenced our results as described in the ESI text.


0
-2
-4
-6
-8
ln(C / C0)
0
2
4
6
8
Fluence (kJUVB /m2)

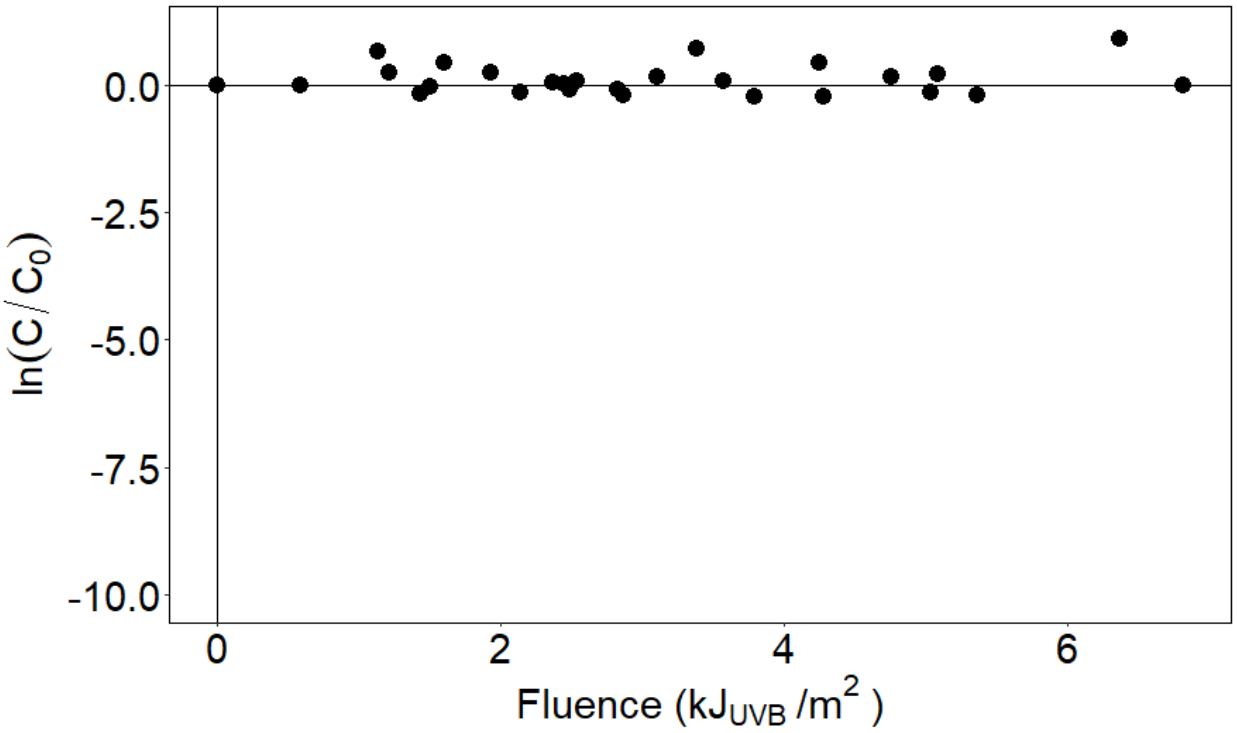

0.0
-2.5
-5.0
-7.5
-10.0
ln(C / C0)
0
2
4
6
Fluence (kJUVB /m2)

Figure S5 Data from dark control reactors from all wastewater and surface water sample photoinactivation experiments. $\ln(C/C_0)$ is the ln-transformed relative concentration of enterococci. Fluence was calculated in the UVB wavelengths. Note that samples here are controls and were not exposed to light; the fluence on the x-axis is the fluence experienced by comparable experimental reactors that were processed in parallel.