Electronic Supplementary Information

Effect of pH on Endogenous Sunlight Inactivation Rates of Laboratory Strain and Wastewater Sourced *E. coli* and Enterococci

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Measuring sunlight inactivation rates of overnight-mixed and re-washed bacteria

Control experiments were conducted to ensure that lysates and other potentially photoactive cellular secretions released during the overnight mixing of our bacterial suspensions did not confound our experimental results by facilitating exogenous inactivation processes. These experiments compared sunlight inactivation rates of bacteria that had been left to mix overnight before experiments, with those of bacteria that had been re-washed immediately prior to experiments. *E. coli* and *E. faecalis* cultures were grown as described in section 2.1 of the main manuscript, and indigenous wastewater bacteria were sourced and concentrated as described in section 2.2 of the main manuscript. After mixing overnight, a portion of the bacteria suspensions were re-washed by centrifuging at 9500 xg for 5 min for laboratory-cultured bacteria and 7700 xg for 5 min for wastewater-sourced bacteria, discarding the supernatant and resuspending the pellet in fresh, sterile PBS by vortexing. The washing steps were repeated twice. Bacteria from the unwashed overnight suspensions and the re-washed suspensions were then inoculated separately into sterile PBS with a pH of 7 at a volume ratio of 1:100 for laboratory-sourced bacteria and 1:10 for wastewater-sourced bacteria and allowed to mix for 20 min before the start of the experiment to ensure a completely mixed solution by stirring with magnetic stir bars at 100 rpm. Thirty milliliter aliquots of these bacterial mixtures were added to each experimental reactor; duplicate reactors were evaluated for each treatment, and dark controls were included that were covered with aluminum foil. The reactors were exposed to UVB-filtered simulated light for 8 h, and 240 µL subsamples were withdrawn intermittently from each reactor for enumeration of viable bacteria, as described in the main manuscript. Given an interest in evaluating whether the extracellular solution was contributing to exogenous inactivation, UVB-filtered sunlight was used to minimize direct endogenous processes contributing to observed inactivation, which would obscure the contribution of indirect photo-processes.

No difference was observed between sunlight inactivation rates of the two bacterial treatments for any of the four bacterial populations evaluated (Fig. S1), signifying that any potential contribution of exogenous photo-processes driven by extracellular constituents in the overnight bacterial suspension to our observed solar inactivation rates was insignificant.

Measuring photosensitized production of singlet oxygen in the extracellular matrix of bacterial overnight suspensions

Additional control experiments were conducted to measure photosensitized production of singlet oxygen in PBS solutions inoculated with the supernatant collected from overnight bacterial suspensions. *E. coli* and *E. faecalis* cultures were grown as described in section 2.1 of the main manuscript, and indigenous wastewater bacteria were sourced and concentrated as described in section 2.2 of the main manuscript. After being left to mix overnight, the bacteria suspensions were centrifuged at 9500 xg for 5 min for laboratory-cultured bacteria and 7700 xg for 5 min for wastewater-sourced bacteria, and the supernatant collected and filtered through a sterile 0.2 µm filter in order to isolate and collect the extracellular

constituents. The filtrate was inoculated into reactors containing sterile PBS of pH 4, 7 and 10 at volume ratios of 1:100 and 1:10 for laboratory-cultured and wastewater-sourced bacteria, respectively, which were the same ratios used for sunlight inactivation experiments. The solutions were magnetically stirred at 100 rpm for 10 min before being aliquoted into 30 mL reactors; duplicate reactors and a dark control (covered with aluminum foil) were evaluated for each separate pH and bacteria-source combination. The reactors were then inoculated with furfuryl alcohol (FFA) (a molecular probe for singlet oxygen ($^1O_2$)) at an initial concentration of 10 µM and allowed to equilibrate by magnetically stirring at 100 rpm for 5 min, before being exposed to UVB-filtered simulated sunlight for 8 h. Intermittent subsamples of 300 µL were collected over the duration of the experiment and the FFA concentrations in the subsamples measured using high-performance liquid chromatography (HPLC) (InfinityLab Poroshell HPH-C18 3.0 x 150 mm 4-Micron column with a 3.0 x 5 mm guard column of the same material; FFA was analyzed at λ = 219 nm using a 10% solution of acetonitrile in Millipore water). Negative controls consisted of reactors containing PBS only spiked with FFA. Solution pH was monitored intermittently during experiments and adjusted as needed by dropwise addition of 1M HCl or 1M NaOH

Steady-state singlet oxygen concentrations were estimated based on the pseudo first-order reaction between FFA and $^1O_2$ (Equation S1)

$$\frac{-d[FFA]}{dt} = k_{obs}[FFA] \qquad \text{(S1)}$$

Where k_{obs} is the observed pseudo first-order rate constant and the slope of $\ln([FFA]/[FFA]_0)$ plotted against time. k_{obs} is also the product of the second-order reaction rate constant of FFA and $^1O_2$ (k_2) and the steady state singlet oxygen concentration ($[^1O_2]_{ss}$) (Equation S2).

$$k_{obs}=k_2[^1O_2]_{ss} \qquad \text{(S2)}$$

$[^1O_2]_{ss}$ was calculated by dividing k_{obs} by k_2, which was previously reported[1] to be 1.00×10^8 $M^{-1}s^{-1}$ at 22 °C; adjusted to 25 °C , the value of k_2 is 1.06×10^8 $M^{-1}s^{-1}$.

Figures

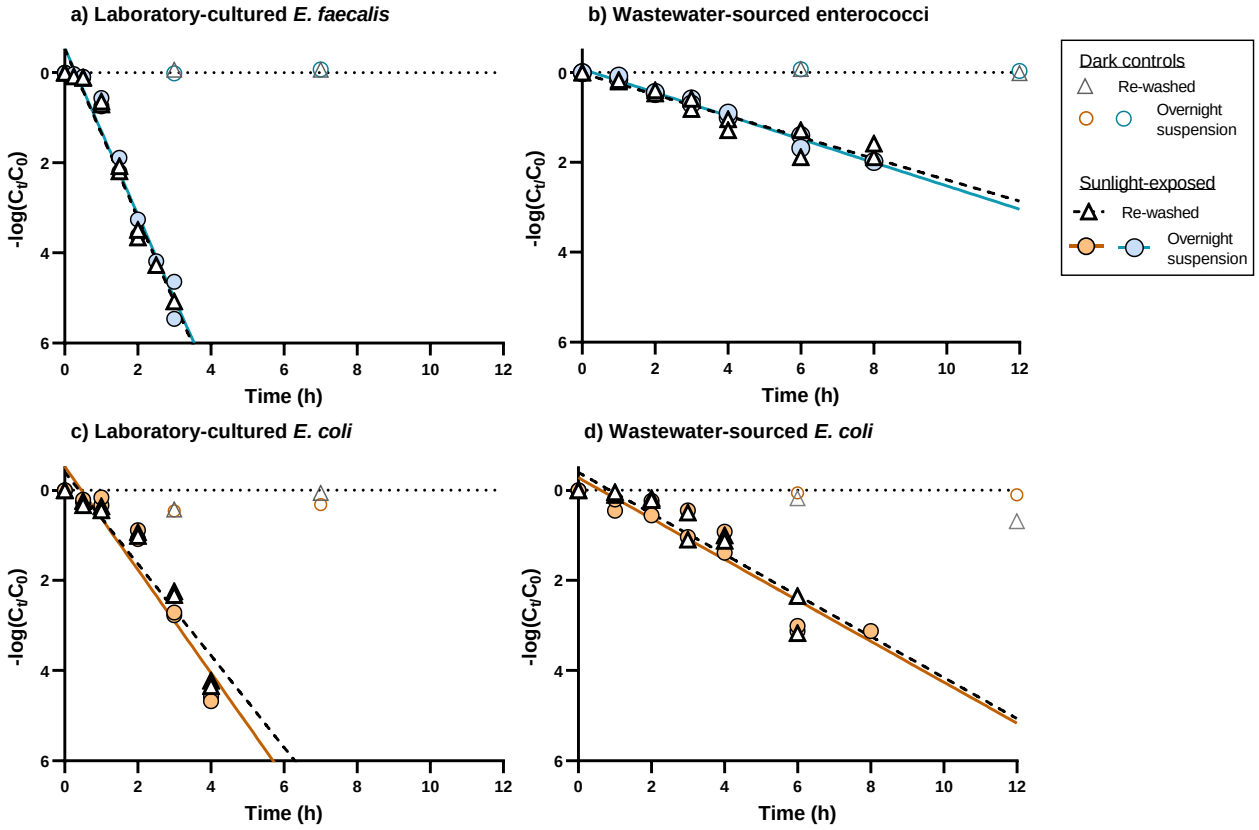

Fig. S1. Comparison of log-inactivation data of laboratory-cultured and wastewater-sourced *E. faecalis* (enterococci) and *E. coli* derived from bacterial suspensions that were left to mix overnight and from bacterial suspensions that were re-washed immediately prior to experiments (n=2). Inactivation was measured under UVB-filtered simulated sunlight at pH 7. Solid and dashed lines are the linear regression lines of the pooled log inactivation data of the overnight suspension and re-washed bacteria, respectively.

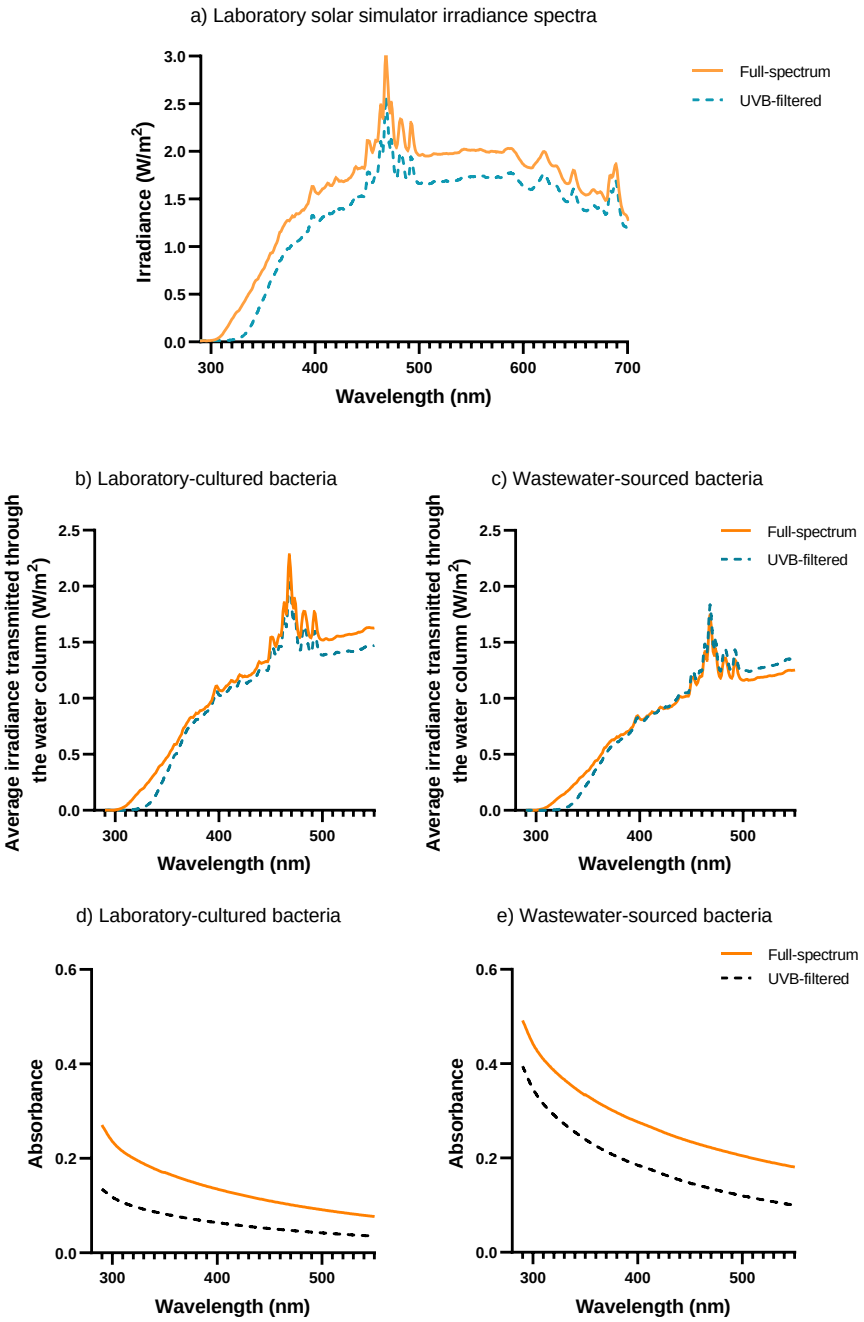

Fig. S2. **Top panel (a):** Average irradiance spectra emitted by the solar simulator with an air mass filter and atmospheric attenuation filter only (full-spectrum; n=6) and with an air mass filter, an atmospheric attenuation filter and UVB/C-blocking filter (UVB-filtered; n=3) **Middle panels:** Average irradiance transmitted through the water column, $\langle E_0(z, \lambda)\rangle$ (W m^{-2}), in experiments with (b) laboratory-cultured and (c) wastewater-sourced bacteria. **Bottom panels**: Average spectral absorbance of bacteria solutions measured during experiments with (d) laboratory-cultured and (e) wastewater-sourced bacteria during experiments performed under full-spectrum simulated sunlight and UVB-filtered simulated sunlight. The differences in the spectral absorbance measured during the two groups of experiments under different light conditions were a result of differences in the average starting bacterial concentrations during the separate experiments.

Laboratory-cultured *E. faecalis*

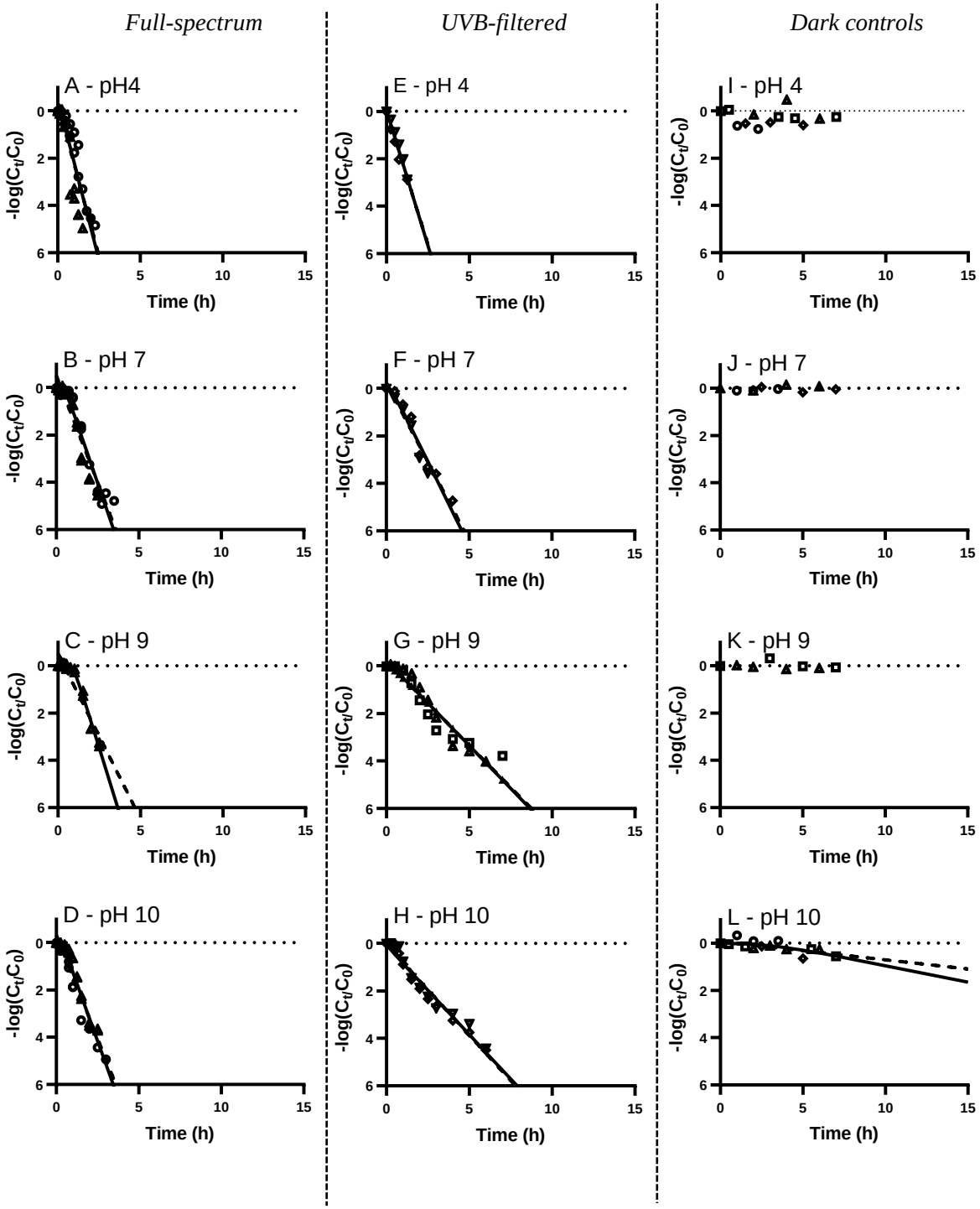

Fig. S3. Log-inactivation data for laboratory-cultured *E. faecalis* under different irradiance and pH conditions. A-D: under full-spectrum simulated sunlight (n=4 except pH 9 where n=2). E-H: under UVB-filtered simulated sunlight (n=2). I-L: dark controls (n=4 except pH 7 where n=3 and pH 9 where n=2). The dashed line is the linear regression line solved using the first-order decay model and the solid line is the best fit line solved using the multitarget decay model. Different symbols represent data from replicate experiments.

Laboratory-cultured *E. coli*

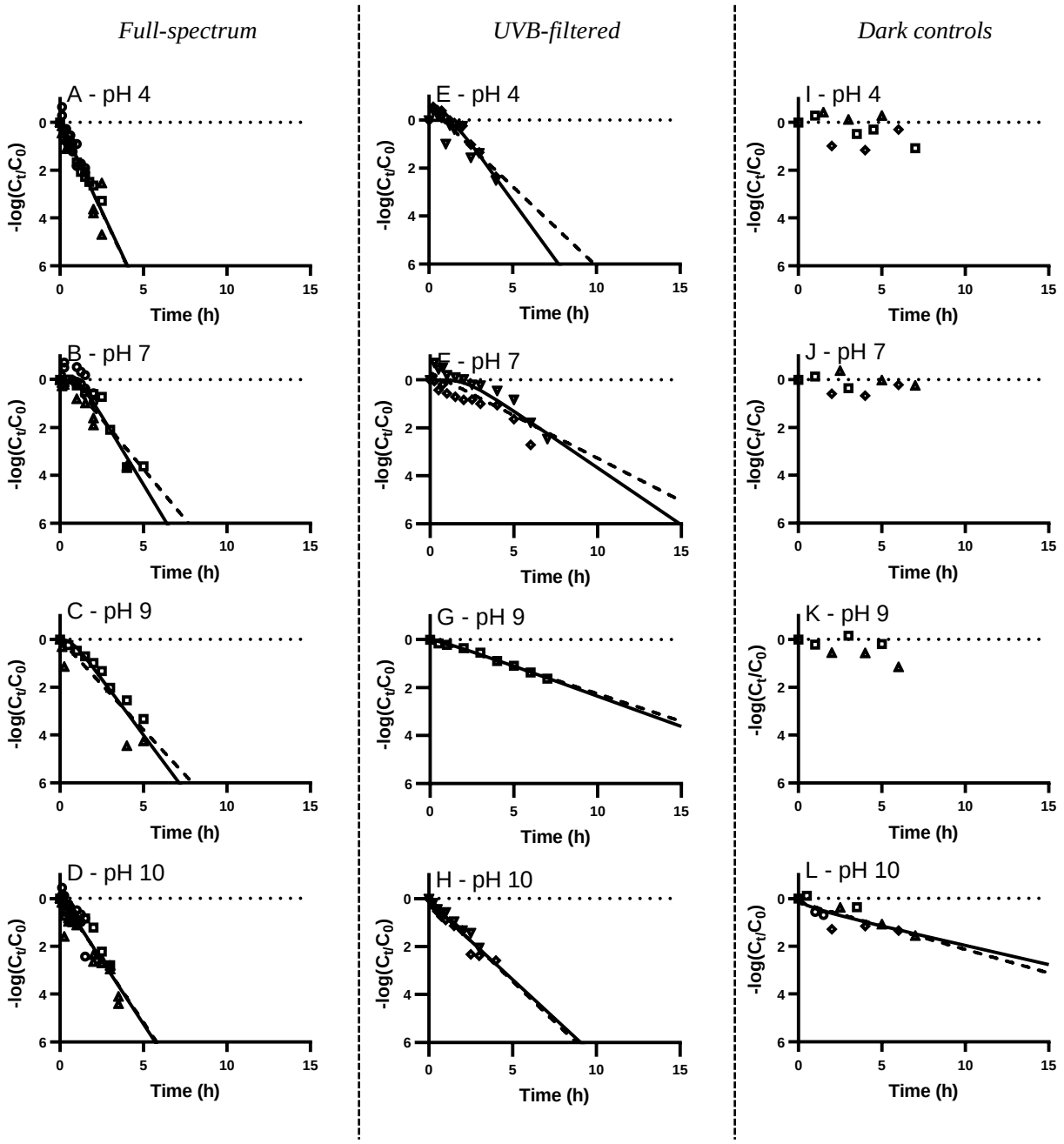

Fig. S4. Log-inactivation data for laboratory-cultured *E. coli* under different irradiance and pH conditions A-D: under full-spectrum simulated sunlight (n=5 except pH 9 where n=2). E-H: under UVB-filtered simulated sunlight (n=2 except pH 9 where n=1). I-L: dark controls (n=4 except pH 9 where n=2). The dashed line is the linear regression line solved using the first-order decay model and the solid line is the best fit line solved using the multitarget decay model. Different symbols represent data from replicate experiments.

Wastewater-sourced enterococci

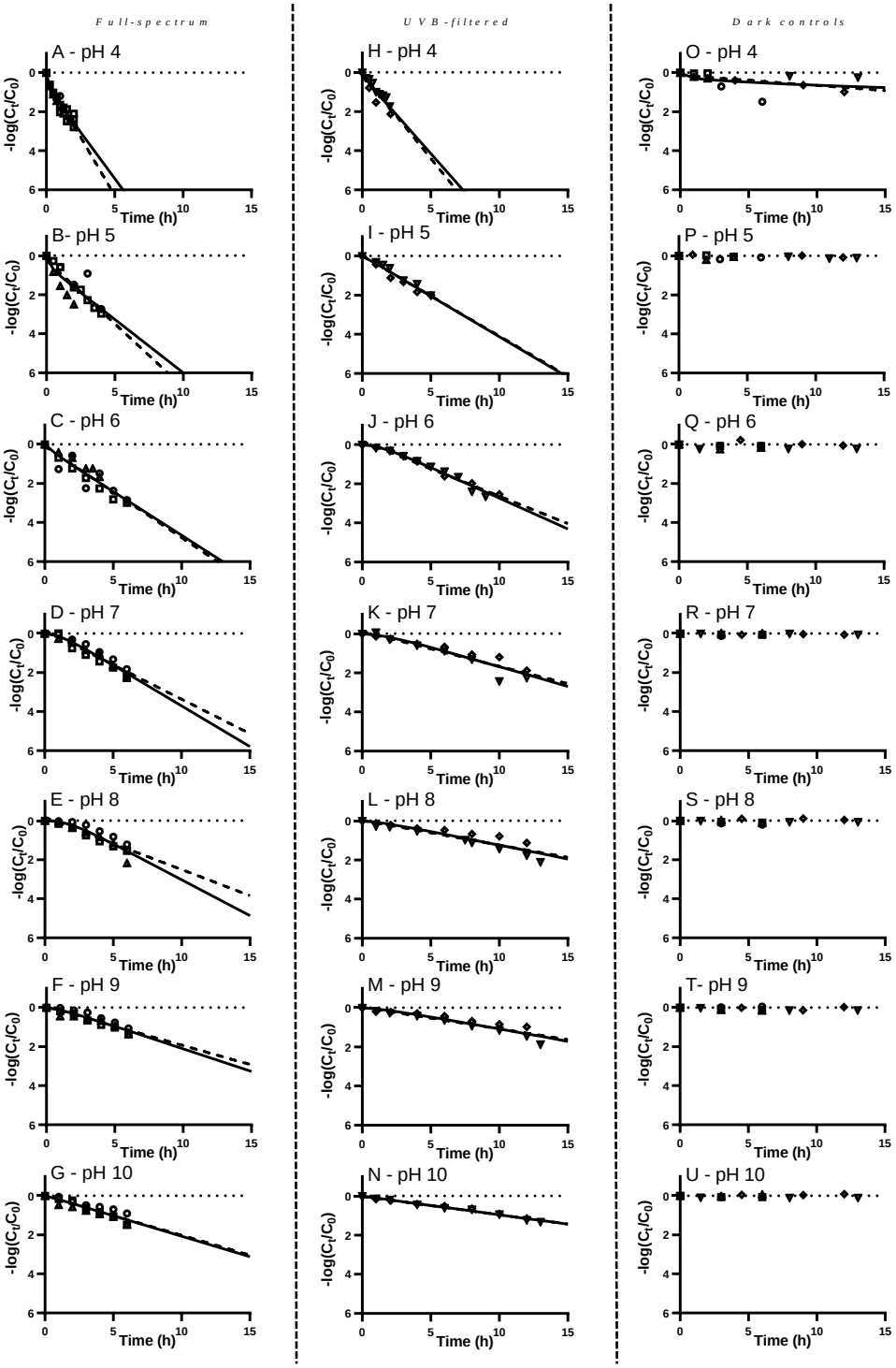

Fig. S5. Log-inactivation data for wastewater-sourced enterococci under different irradiance and pH conditions. A-G: under full-spectrum simulated sunlight (n=3). H-N: under UVB-filtered simulated sunlight (n=2). O-U: dark controls (n=5). The dashed line is the linear regression line solved using the first-order decay model and the solid line is the best fit line solved using the multitarget decay model. Different symbols represent data from replicate experiments.

Wastewater-sourced *E. coli*

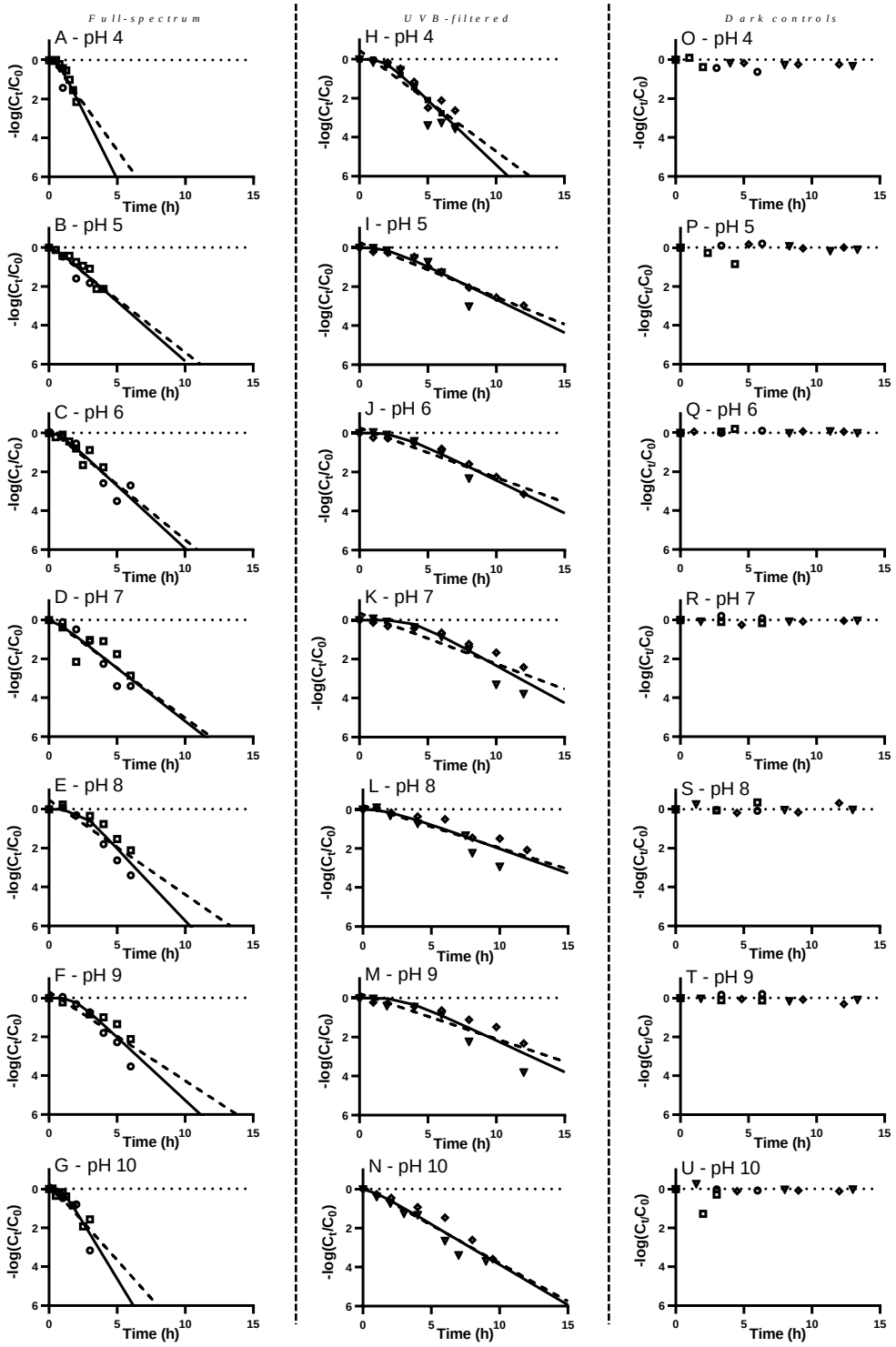

Fig. S6. Log-inactivation data for wastewater-sourced *E. coli* under different irradiance and pH conditions. A-G: under full-spectrum simulated sunlight (n=2). H-N: under UVB-filtered simulated sunlight (n=2). O-U: dark controls (n=4). The dashed line is the linear regression line solved using the first-order decay model and the solid line is the best fit line solved using the multitarget decay model. Different symbols represent data from replicate experiments.

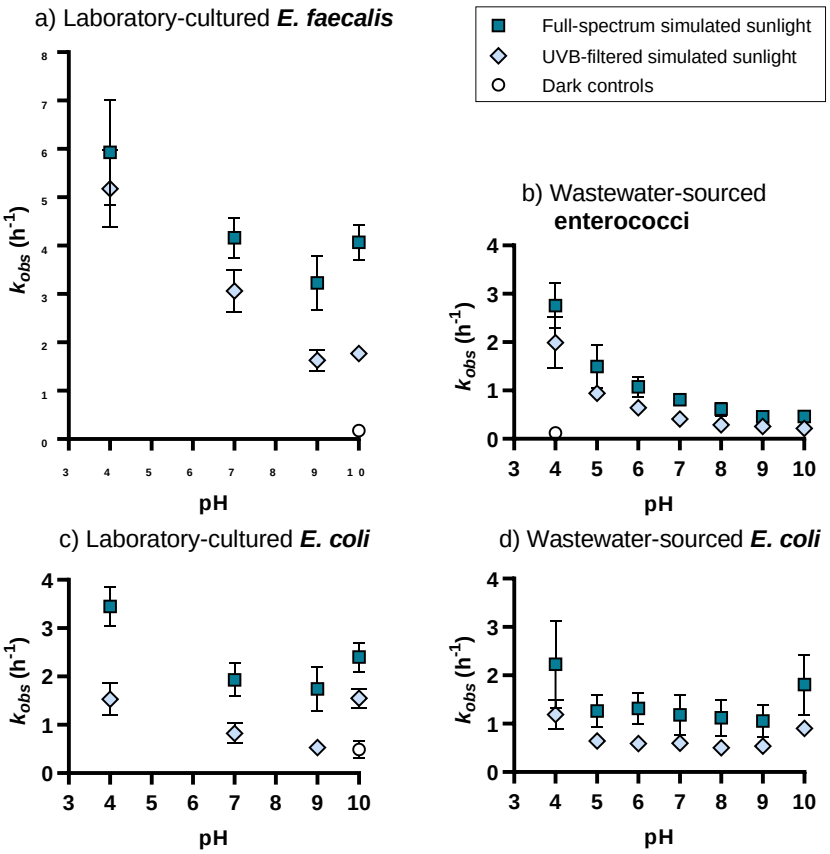

Fig. S7. Observed inactivation rate constants (k_{obs}) at pH ranging from 4 to 10 for laboratory-cultured and wastewater-sourced enterococci (or *E. faecalis*) and *E. coli* under full-spectrum and UVB-filtered simulated sunlight. Inactivation rate constants were calculated based on the log-linear decay model. Dark controls were conducted at the same pHs as the sunlight-exposed treatments, however, only the dark controls with k_{obs} values significantly different from zero are presented in the figure [*i.e.*, pH 10 in panels (a) and (c), and pH 4 in panel (b)]. Error bars represent 95% CI; some error bars are smaller than the data marker.

Tables

Table S1. Volumes of 0.1 M sodium phosphate monobasic (NaH_2PO_4), 0.1 M sodium phosphate dibasic (Na_2HPO_4) and DI water that were combined to produce 500 mL of 20 mM Modified PBS at pH ranging from 4 to 10.

Target pH	0.1 M NaH_2PO_4 volume (mL)	0.1 M Na_2HPO_4 volume (mL)	Deionized water volume (mL)
4	99.94	0.06	400
5	99.37	0.63	400
6	94.06	5.94	400
7	61.31	38.69	400
8	13.68	86.32	400
9	1.56	98.44	400
10	0.16	99.84	400

Table S2. First-order sunlight inactivation rate constants, k_{obs} (h^{-1}), calculated for the four bacterial populations at pH ranging from 4 to 10 under full-spectrum and UVB-filtered simulated sunlight. *n* represents the total number of replicates that produced the data used to calculate k_{obs}. Slashed-out cells belong to pH conditions that were not evaluated.

	pH	Laboratory-cultured				Wastewater-sourced			
		E. faecalis		***E. coli***		**Enterococci**		***E. coli***	
		k_{obs} ± 95 % CI	R^2 (*n*)	k_{obs} ± 95 % CI	R^2 (*n*)	k_{obs} ± 95 % CI	R^2 (*n*)	k_{obs} ± 95 % CI	R^2 (*n*)
Full-spectrum simulated sunlight	4	5.93 ± 1.09	0.80 (4)	3.45 ± 0.40	0.89 (5)	2.76 ± 0.47	0.89 (3)	2.23± 0.92	0.77 (2)
	5					1.50 ± 0.45	0.76 (3)	1.26 ± 0.92	0.86 (2)
	6					1.08 ± 0.21	0.87 (3)	1.32 ± 0.33	0.86 (2)
	7	4.17 ± 0.42	0.91 (4)	1.93 ± 0.34	0.81 (5)	0.81 ± 0.11	0.92 (3)	1.18 ± 0.41	0.77 (2)
	8					0.62 ± 0.14	0.82 (3)	1.12 ± 0.38	0.80 (2)
	9	3.23 ± 0.56	0.89 (2)	1.74 ± 0.46	0.85 (2)	0.46 ± 0.08	0.88 (3)	1.06 ± 0.33	0.81 (2)
	10	4.07 ± 0.37	0.94 (4)	2.40 ± 0.30	0.86 (5)	0.47 ± 0.10	0.84 (3)	1.81 ± 0.63	0.79 (2)
UVB-filtered simulated sunlight	4	5.18 ± 0.81	0.96 (2)	1.53 ± 0.33	0.83 (2)	1.99 ± 0.53	0.86 (2)	1.19 ± 0.30	0.84 (2)
	5					0.95 ± 0.13	0.96 (2)	0.65 ± 0.13	0.89(2)
	6					0.64 ± 0.06	0.97 (2)	0.59 ± 0.11	0.92 (2)
	7	3.06 ± 0.44	0.95 (2)	0.82 ± 0.21	0.75 (2)	0.41 ± 0.08	0.89 (2)	0.60 ± 0.16	0.82 (2)
	8					0.29 ± 0.07	0.85 (2)	0.50 ± 0.14	0.82 (2)
	9	1.63 ± 0.22	0.92 (2)	0.53 ± 0.05	0.99 (1)	0.25 ± 0.05	0.88 (2)	0.53 ± 0.16	0.80 (2)
	10	1.77 ± 0.15	0.97 (2)	1.55 ± 0.02	0.94 (2)	0.22 ± 0.02	0.98 (2)	0.90 ± 0.16	0.92 (2)
Dark controls	4	*0.06 ± 0.18ᵗ*	*0.04 (4)*	*0.27 ± 0.27ᵗ*	*0.24 (4)*	0.12 ± 0.11	0.30 (5)	*0.05 ± 0.06ᵗ*	*0.26 (4)*
	5					*0.012 ± 0.02ᵗ*	*0.11 (5)*	*-0.003±.08ᵗ*	*0.0005 (4)*
	6					*0.015 ± 0.035ᵗ*	*0.06 (5)*	*-0.007 ± 0.02ᵗ*	*0.06 (4)*
	7	*-0.01± 0.06ᵗ*	*0.01 (3)*	*0.11 ± 0.20ᵗ*	*0.11 (4)*	*0.006 ± 0.01ᵗ*	*0.11 (5)*	*0.009 ± 0.03ᵗ*	*0.03 (4)*
	8					*0.0007 ± 0.02ᵗ*	*0.0003 (5)*	*-0.01 ± 0.05ᵗ*	*0.02 (4)*
	9	*0.03 ± 0.2ᵗ*	*0.01 (2)*	*0.27 ± 0.41ᵗ*	*0.25 (2)*	*0.016 ± 0.02ᵗ*	*0.18 (5)*	*0.03 ± 0.04ᵗ*	*0.23 (4)*
	10	0.18 ± 0.09	0.50 (4)	0.49 ± 0.18	0.74 (4)	*-0.0018 ± 0.02ᵗ*	*0.003 (5)*	*-0.01 ± 0.11ᵗ*	*0.004 (4)*

ᵗ *Grayed-out values not significantly different from zero*

Table S3. Sunlight inactivation rate constants, k_{obs} (h^{-1}), calculated using the multitarget decay model for the four bacterial populations at pH ranging from 4 to 10 under full-spectrum and UVB-filtered simulated sunlight. *n* represents the total number of replicates that produced the data used to calculate k_{obs} and *m* represents the shoulder coefficient. Slashed-out cells belong to pH conditions that were not evaluated.

	pH	Laboratory-cultured				Wastewater-sourced			
		E. faecalis		*E. coli*		Enterococci		*E. coli*	
		k_{obs} (*m*)	R^2 (*n*)	k_{obs} (*m*)	R^2 (*n*)	k_{obs} (*m*)	R^2 (*n*)	k_{obs} (*m*)	R^2 (*n*)
Full-spectrum simulated sunlight	4	6.33 (3.61)	0.80 (4)	3.43 (1.03)	0.89 (5)	2.20 (0.22)	0.93 (3)	3.23 (7.44)	0.82 (2)
	5					1.26 (0.30)	0.75 (3)	1.41 (1.95)	0.87 (2)
	6					1.03 (0.68)	0.87 (3)	1.49 (3.42)	0.88 (2)
	7	4.71 (12.4)	0.94 (4)	2.66 (23.8)	0.88 (5)	0.96 (2.97)	0.94 (3)	1.26 (1.85)	0.77 (2)
	8					0.85 (4.59)	0.86 (3)	1.71 (49.6)	0.87 (2)
	9	5.23 (156)	0.99 (2)	2.17 (5.08)	0.86 (2)	0.53 (1.64)	0.88 (3)	1.50 (17.0)	0.85 (2)
	10	4.49 (5.17)	0.94 (4)	2.48 (1.31)	0.86 (5)	0.48 (1.12)	0.85 (3)	2.64 (13.6)	0.84 (2)
UVB-filtered simulated sunlight	4	5.27 (1.15)	0.96 (2)	2.21 (27.2)	0.85 (2)	1.83 (0.66)	0.86 (2)	1.53 (17.5)	0.87 (2)
	5					0.96 (1.11)	0.96 (2)	0.78 (5.44)	0.92 (2)
	6					0.72 (2.63)	0.98 (2)	0.78 (9.51)	0.96 (2)
	7	3.28 (3.09)	0.96 (2)	1.1 (12.3)	0.62 (2)	0.47 (2.24)	0.89 (2)	0.89 (33.3)	0.88 (2)
	8					0.33 (1.63)	0.85 (2)	0.58 (3.15)	0.83 (2)
	9	1.66 (1.83)	0.92 (2)	0.58 (1.44)	0.99 (1)	0.29 (1.63)	0.89 (2)	0.75 (11.9)	0.83 (2)
	10	1.72 (0.80)	0.97 (2)	1.49 (0.72)	0.94 (2)	0.22 (1.02)	0.98 (2)	0.96 (1.91)	0.92 (2)
Dark controls	4	*$1.87x10^{-6}$ † (0.05)*	*0.18 (4)*	*$4.88x10^{-5}$ † (0.10)*	*0.06 (4)*	0.04 (0.23)	0.39 (5)	*$1.69x10^{-3}$ † (0.17)*	*0.43 (4)*
	5					*$5.16x10^{-4}$ † (0.37)*	*0.15 (5)*	*$2.72x10^{-9}$ † (0.11)*	*0.01 (4)*
	6					*$6.95x10^{-8}$ † (0.11)*	*0.11 (5)*	*0.00 (0.9)*	*-0.77 (4)*
	7	*0.00 (0.05)*	*-0.04 (3)*	*0.07 † (0.73)*	*0.11 (4)*	*$4.12x10^{-6}$ † (0.26)*	*0.17 (5)*	*$3.13x10^{-5}$ † (0.27)*	*0.04 (4)*
	8					*$2.21x10^{-8}$ † (0.17)*	*0.02 (5)*	*0.00 (0.2)*	*-0.05 (4)*
	9	*0.00 (0.12)*	*-0.02 (2)*	*0.27 † (115)*	*-0.31 (2)*	*$2.04x10^{-3}$ † (0.47)*	*0.21 (5)*	*0.12 † (4.11)*	*0.28 (4)*
	10	0.33 (3.39)	0.53 (4)	0.37 (0.43)	0.76 (4)	*$1.09x10^{-11}$ † (0.16)*	*0.0019 (5)*	*$8.45x10^{-11}$ (0.05)*	*0.04 (4)*

† *Grayed-out values not significantly different from zero*

Table S4. Results of one-way ANOVA significance tests followed by Tukey multiple comparison test comparing first-order inactivation rate constants (k_{obs}) observed at pH ranging from 4 to 10 for laboratory-cultured and wastewater-sourced enterococci (or *E. faecalis*) and *E. coli* under full-spectrum and UVB-filtered simulated sunlight. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, ns: not significant. The slashes in the tables for the laboratory-cultured bacteria represent pH treatments that were not included in the study.

Laboratory-cultured

Enterococci

Full-spectrum simulated sunlight

pH	4	5	6	7	8	9	10
4		/	/	***	/	****	***
5			/	/	/	/	/
6				/	/	/	/
7					/	ns	ns
8						/	/
9							ns
10							

UVB-filtered simulated sunlight

pH	4	5	6	7	8	9	10
4		/	/	****	/	****	****
5			/	/	/	/	/
6				/	/	/	/
7					/	****	****
8						/	/
9							ns
10							

E. coli

Full-spectrum simulated sunlight

pH	4	5	6	7	8	9	10
4		/	/	****	/	****	****
5			/	/	/	/	/
6				/	/	/	/
7					/	ns	ns
8						/	/
9							ns
10							

UVB-filtered simulated sunlight

pH	4	5	6	7	8	9	10
4		/	/	***	/	****	ns
5			/	/	/	/	/
6				/	/	/	/
7					/	ns	***
8						/	/
9							****
10							

Wastewater-sourced

Enterococci

Full-spectrum simulated sunlight

pH	4	5	6	7	8	9	10
4		****	****	****	****	****	****
5			ns	**	****	****	****
6				ns	ns	**	**
7					ns	ns	ns
8						ns	ns
9							ns
10							

UVB-filtered simulated sunlight

pH	4	5	6	7	8	9	10
4		****	****	****	****	****	****
5			ns	***	****	****	****
6				ns	*	*	**
7					ns	ns	ns
8						ns	ns
9							ns
10							

E. coli

Full-spectrum simulated sunlight

pH	4	5	6	7	8	9	10
4		ns	ns	*	*	**	ns
5			ns	ns	ns	ns	ns
6				ns	ns	ns	ns
7					ns	ns	ns
8						ns	ns
9							ns
10							

UVB-filtered simulated sunlight

pH	4	5	6	7	8	9	10
4		***	****	****	****	****	ns
5			ns	ns	ns	ns	ns
6				ns	ns	ns	ns
7					ns	ns	ns
8						ns	*
9							*
10							

References

(1) E. Appiani, R. Ossola, D. E. Latch, P. R. Erickson and K. McNeill, Aqueous singlet oxygen reaction kinetics of furfuryl alcohol: effect of temperature, pH, and salt content, *Environ. Sci.: Processes Impacts*, 2017, **19**, 507–516.