

Titled: UV-C Peroxymonosulfate Activation for Wastewater Regeneration: Simultaneous Inactivation of Pathogens and Degradation of Contaminants of Emerging Concern

Authors: Ilaria Berruti¹, Samira Nahim-Granados ¹, María Jesús Abeledo-Lameiro¹, Isabel Oller^{1,2} and María Inmaculada Polo-López^{1,2,*}

Affiliations:

¹CIEMAT-PSA, Carretera de Senés Km 4, 04200, Tabernas (Almería), Spain

²CIESOL, Joint Centre of the University of Almería-CIEMAT, 04120 Almería, Spain

***Correspondence:** mpolo@psa.es; Tel.: + 34 950 387900

Supplementary Materials

Table S1. Physico-chemical and microbiological characterization of SUWW and UWW.

Parameter	SUWW	UWW
DOC (mg/L)	11.1±2.5	19.6±2.8
BOD ₅ (mgO ₂ /L)	-	16±5
COD ((mgO ₂ /L)	-	40±10
[HCO ₃ ⁻] (mg/L)	69.5±7.3	370.0±28.0
Turbidity (NTU)	2.5±0.2	9.1±9.3
pH	7.4±0.2	7.6±0.1
Conductivity (mS/cm)	1.5±0.1	2.4±0.1
Cl ⁻ (mg/L)	356.7±4.4	436.7±80.0
NO ₃ ⁻ (mg/L)	2.7±0.9	4.0±0.9
NO ₂ ⁻ (mg/L)	-	6.0±3.1
PO ₄ ³⁻ (mg/L)	4.0±1.6	3.4±1.5
Br ⁻ (mg/L)	-	2.8±0.1
SO ₄ ²⁻ (mg/L)	99.1±0.8	101.5±22.7
Na ⁺ (mg/L)	255.2±1.6	252.0±13.4
NH ₄ ⁺ (mg/L)	7.6±1.0	39.3±7.7
K ⁺ (mg/L)	9.5±2.3	27.8±1.0
Ca ²⁺ (mg/L)	18.6±3.4	88.5±11.5
Mg ²⁺ (mg/L)	13.3±1.6	46.7±7.5
<i>E. coli</i> (CFU/mL)	-	5.87·10 ³ ±1.02·10 ⁴
<i>Enterococcus</i> spp (CFU/mL)	-	1.41·10 ⁴ ±2.24·10 ⁴
<i>Pseudomonas</i> spp (CFU/mL)	-	3.60·10 ² ±5.03·10 ²
<i>Total coliforms</i> (CFU/mL)	-	1.43·10 ⁴ ±2.14·10 ⁴

Table S2. Conditions used in qPCR real-time assays.

Genes	Primer sequence	μM	bp	Conditions	Ref.
<i>16s rRNA</i>	FW: TCCTACGGGAGGCAGCAGT RV: ATTACCGCGGCTGCTGG	0.50	195	50°C - 1 min (1X); 95°C - 3 sec, 60°C - 30 sec (40X); 95°C - 15 sec; 60°C - 1 min; 95°C -15 sec	Modified from [19]
<i>intI1</i>	FW: GATCGGTCTGAATGCGTGT RV: GCCTTGATGTTACCCGAGAG	0.50	196	50°C - 1 min (1X); 95°C - 3 sec, 60°C - 30 sec (40X); 95°C - 15 sec; 60°C - 1 min; 95°C -15 sec	Modified from [19]
<i>qnrS</i>	FW: GACGTGCTAACTTGCGTG RV: TGGCATTGTTGGAAACTT	0.60	118	50°C - 1 min (1X); 95°C - 3 sec, 60°C - 30 sec (40X); 95°C - 15 sec; 60°C - 1 min; 95°C -15 sec	Modified from [19]
<i>sulI</i>	FW:CGCACCGGAAACATCGCTGCAC RV: TGAAGTTCGCCGCAAGGCTCG	0.30	162	50°C - 1 min (1X); 95°C - 3 sec, 60°C - 30 sec (40X); 95°C - 15 sec; 60°C - 1 min; 95°C -15 sec	Modified from [19]
<i>bla_{CTX-M32}</i>	FW: CGTCACGCTGTTGTTAGGAA RV: CGCTCATCAGCACGATAAAG	0.30	156	95°C - 10 min (1X); 95°C - 30 sec; 55°C - 1 min; 72°C - 1 min (40X); 72°C - 7 min; 60°C - 10 sec; 95°C - 10 sec (1X)	[20]
<i>Tet(M)</i>	FW: GCAATTCTACTGATTTCTGC RV:CTGTTTGATTACAATTTCCGC	0.20	186	50°C - 1 min (1X); 95°C - 3 sec, 60°C - 30 sec (40X); 95°C - 15 sec; 60°C - 1 min; 95°C -15 sec	Modified from [19]
<i>bla_{TEM}</i>	FW:TTCCTGTTTTTGCTCACCCAG RV:CTCAAGGATCTTACCGCTGTTG	0.20	113	50°C - 1 min (1X); 95°C - 3 sec, 60°C - 30 sec (40X); 95°C - 15 sec; 60°C - 1 min; 95°C -15 sec	Modified from [19]

Table S3. Inactivation kinetic constants of *E. coli*, *E. faecalis* and *P. aeruginosa* in SUWW.

[PMS] mmol/L	<i>E. coli</i>		<i>E. faecalis</i>		<i>P. aeruginosa</i>	
	k (min ⁻¹)	R ²	k (min ⁻¹)	R ²	k (min ⁻¹)	R ²
UV-C (Figure 1a)						
0	0.960±0.162	0.919	0.747±0.084	0.963	1.010±0.194	0.847
	0.016±0.002	0.866	0.010±0.002	0.753	0.008±0.002	0.648
0.01	1.130±0.210	0.884	0.921±0.036	0.995	1.170±0.206	0.913
	0.003±0.002	0.139	0.004±0.002	0.214	0.009±0.002	0.556
0.05	1.110±0.193	0.915	0.911±0.041	0.994	1.140±0.210	0.896
	0.008±0.002	0.375	0.007±0.002	0.691	0.009±0.001	0.833
0.1	1.120±0.191	0.918	0.911±0.017	0.999	1.170±0.241	0.881
	0.027±0.004	0.820	0.011±0.002	0.797	0.021±0.003	0.817
0.2	1.140±0.154	0.947	0.864±0.134	0.931	1.200±0.199	0.922
	0.022±0.005	0.724	0.013±0.002	0.774	0.023±0.003	0.776
0.3	1.000±0.189	0.900	0.752±0.110	0.938	1.030±0.237	0.856
	0.067±0.007	0.916	0.031±0.003	0.903	0.047±0.017	0.742
0.5	1.240±0.219	0.911	0.845±0.098	0.960	1.270±0.226	0.910
	0.062±0.005	0.944	0.040±0.005	0.871	0.057±0.011	0.790

DL= 1CFU/100mL *Shoulder length (SL)= 20 min ** SL= 50 min *** SL= 30 min

Table S4. Pseudo-first order degradation kinetic constants of Trimethoprim (TMP), Sulfamethoxazole and Diclofenac (DCF) in SUWW.

[PMS] mmol/L	TMP		SMX		DCF	
	k (min ⁻¹)	R ²	k (min ⁻¹)	R ²	k (min ⁻¹)	R ²
UV-C (Figure 1b)						
0	0.007±0.001	0.978	0.107±0.005	0.978	0.113±0.012	0.927
0.01	0.009±0.001	0.997	0.121±0.006	0.978	0.166±0.010	0.970
0.05	0.015±0.001	0.974	0.129±0.006	0.974	0.185±0.012	0.966
0.1	0.028±0.001	0.997	0.136±0.005	0.986	0.195±0.012	0.974
0.2	0.059±0.001	0.996	0.147±0.008	0.975	0.204±0.021	0.930
0.3	0.096±0.002	0.995	0.184±0.005	0.995	0.229±0.036	0.869
0.5	0.175±0.008	0.985	0.230±0.005	0.996	0.214±0.025	0.918

Table S5. Inactivation kinetic constants of *E. coli*, Total Coliforms, *Enterococcus* spp and *Pseudomonas* spp in UWW

[PMS] (mmol/L)	<i>E. coli</i>		Total Coliforms		<i>Enterococcus</i> spp		<i>Pseudomonas</i> spp	
	k (min ⁻¹)	R ²	k (min ⁻¹)	R ²	k (min ⁻¹)	R ²	k (min ⁻¹)	R ²
UV-C (Figure 2a)								
0	0.193±0.023	0.900	0.202±0.031	0.841	0.271±0.001	0.999	0.124±0.027	0.691
	0.003±0.000		0.007±0.001	0.876				
0.1	0.392±0.091	0.815	0.390±0.055	0.926	0.311±0.035	0.952	0.686±0.081	0.959
	0.035±0.015	0.681	0.026±0.005	0.937			0.085±0.017	0.926
0.5	0.464±0.046	0.952	0.416±0.045	0.933	0.379±0.062	0.860	1.075±0.083	0.976
	0.077±0.012	0.954	0.015±0.002	0.938				
0.75	0.519±0.084	0.902	0.518±0.096	0.876	0.485±0.032	0.979	0.624±0.068	0.944
	0.035±0.008	0.889	0.071±0.017	0.887				
1	0.416±0.076	0.851	0.896±0.082	0.975	0.530±0.053	0.952	0.958±0.056	0.987
	0.007±0.003	0.786	0.080±0.024	0.766				

Table S6. Pseudo-first order degradation kinetic constants of Trimethoprim (TMP), Sulfamethoxazole (SMX) and Diclofenac (DCF) in UWW

[PMS] (mmol/L)	TMP		SMX		DCF	
	k (min ⁻¹)	R ²	k (min ⁻¹)	R ²	k (min ⁻¹)	R ²
UV-C (Figure 2b)						
0	0.001±0.000	0.957	0.071±0.004	0.968	0.110±0.018	0.903
0.1	NA		0.088±0.004	0.974	0.119±0.013	0.925
0.5	NA		0.076±0.003	0.986	0.205±0.040	0.779
0.75	0.033±0.002	0.935	0.144±0.004	0.993	0.197±0.018	0.943
1	0.045±0.002	0.974	0.185±0.010	0.970	0.314±0.039	0.900

NA= no fitting;

Table S7. ARGs means and standard deviation of the ratio detected in this study.

	<i>16s rRNA</i>	<i>intI1</i>	<i>sulI</i>	<i>bla_{CTX-M32}</i>	<i>bla_{TEM}</i>	<i>tetM</i>	<i>qnrS</i>
Time (min)	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
UV-C (Figure 3b)							
0	1.00±0.01	0.67±0.01	0.52±0.00	0.38±0.01	0.48±0.00	0.51±0.01	0.40±0.00
20	0.87±0.01	0.51±0.00	0.49±0.01	0.36±0.01	0.45±0.01	0.37±0.00	0.37±0.01
40	0.73±0.01	0.45±0.00	0.44±0.00	QL	0.39±0.00	0.35±0.00	QL
60	0.67±0.01	0.41±0.00	0.40±0.00	QL	0.39±0.00	0.36±0.00	QL
PMS/UV-C (Figure 3b)							
0	1.00±0.04	0.64±0.02	0.54±0.02	0.44±0.01	0.41±0.01	0.38±0.01	0.37±0.01
20	0.64±0.02	0.49±0.02	0.42±0.01	0.31±0.01	0.34±0.01	QL	QL
40	0.63±0.02	0.41±0.02	0.41±0.01	0.32±0.01	0.32±0.01	QL	QL
60	0.52±0.02	0.35±0.01	QL	QL	QL	QL	QL

QL, quantification limit

Table S8. Treatment efficiencies achieved in continuous flow (after 2 min) and in batch mode (considering the treatment time to achieve *E. coli* ≤ 10 CFU/100 mL and 80% of removal of CECs) by UV-C and PMS/UV-C (0.5 mmol/L).

		Continuous Flow		Batch mode <i>E. coli</i> ≤ 10 CFU/100 mL 80% of removal of CECs	
		Time (min)	Log (C _i /C _f)	Time (min)	Log (C _i /C _f)
<i>E. coli</i>	UV-C	2	0.5	30	4.6
	PMS/UV-C	2	1.6	15	4.7
CECs	UV-C	2	0.009	180	0.6
	PMS/UV-C	2	0.119	12	0.7

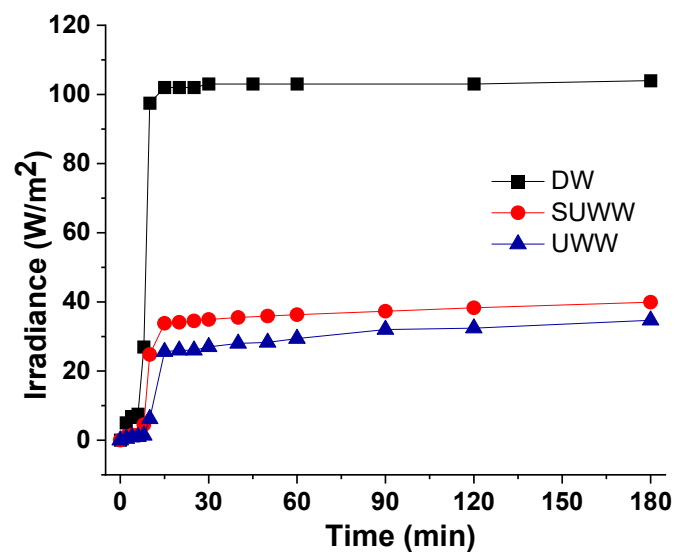


Figure S1. UV-C lamp irradiance (W/m²) profiles registered in demineralized water (as baseline), SUWW and UWW.

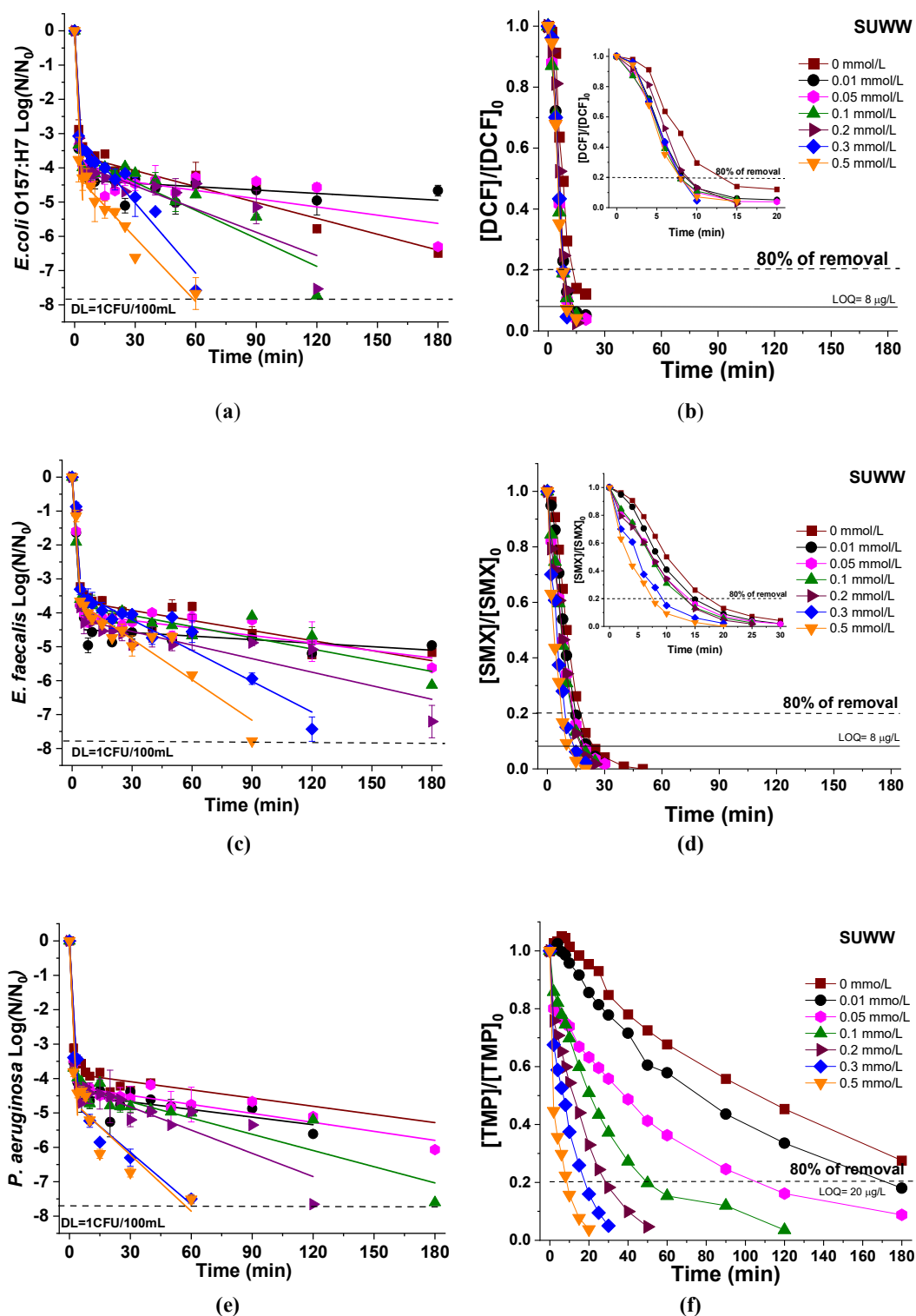


Figure S2. Inactivation profiles of *E. coli* (a), *E. faecalis* (c) and *P. aeruginosa* (e) and degradation profiles of DCF (b), SMX (d) and TMP (f) in the presence of increasing concentrations of PMS (0-0.5 mmol/L) and under UV-C irradiation in SUWW. Inserts: Enlargement of degradation curves.

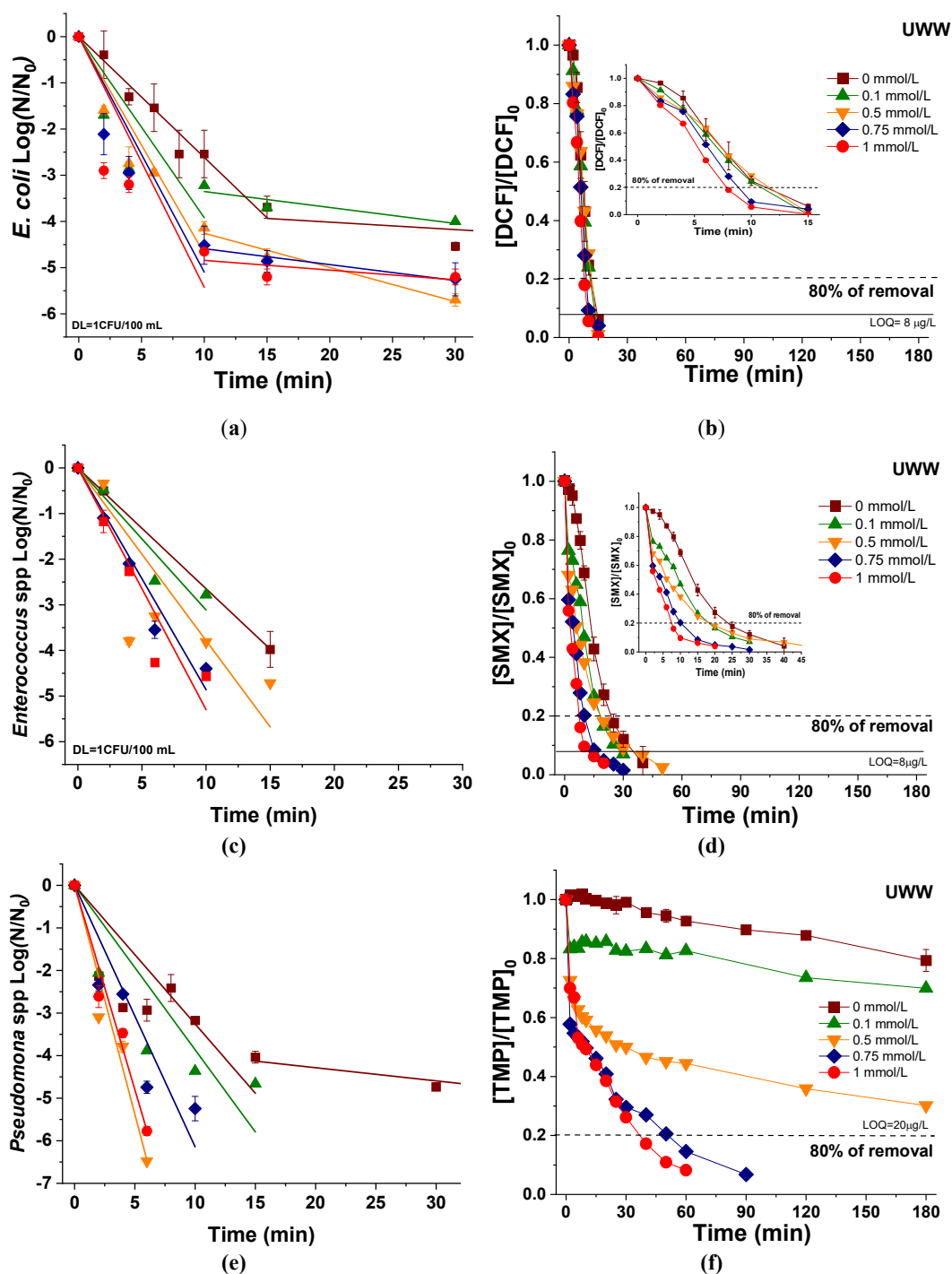


Figure S3. Inactivation profiles of *E. coli* (a), *Enterococcus* spp (c) and *Pseudomonas* spp (e) and degradation profiles of DCF (b), SMX (d) and TMP (f) in the presence of increasing concentrations of PMS (0-1 mmol/L) and under UV-C irradiation in UWW. Inserts: Enlargement of degradation curves.

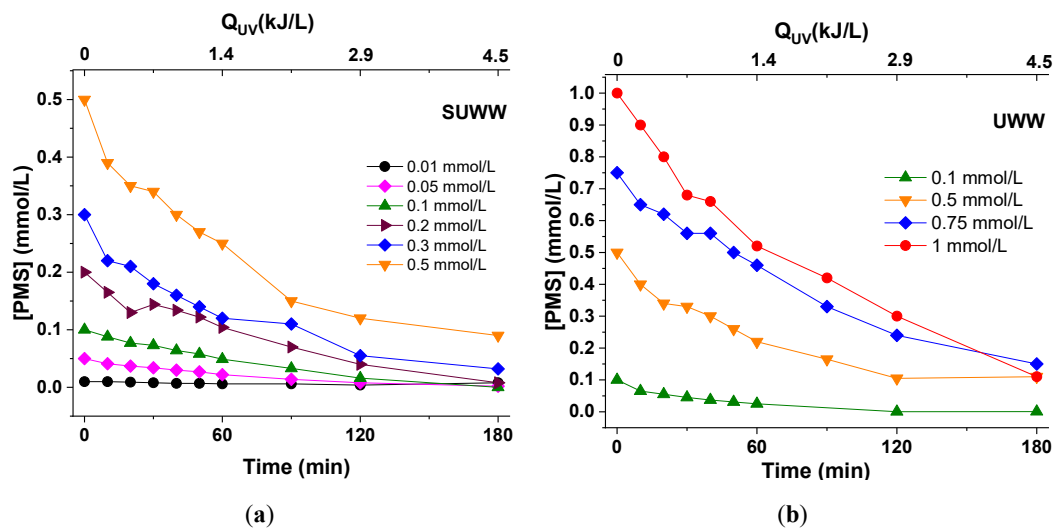


Figure S4. PMS concentration decrease in function of time (minutes) and accumulative UV energy Q_{UV} (kJ/L) under UV-C treatment at different PMS concentrations in SUWW (0.01-0.5 mmol/L) (a) and in UWW (0.1-1 mmol/L) (b).