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Supplementary Materials

Controlling Biofilm Growth and Its Antibiotic Resistance in Drinking Water by Combined UV and Chlorination Processes

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Text S1 Parallel factor (PARAFAC) analysis of fluorescence excitation-emission matrix

Fluorescence EEMs were measured with an excitation range 200–550 nm (at 5 nm intervals) and an emission range 200–550 nm (at 2 nm intervals). Readings were collected using a scanning rate of 1200 nm/min with a slit width of 10.0 nm band pass.

A total of 131 EEM spectra, including 6 samples of R, ST, SF respectively, 30 samples after UV/Cl, 28 samples after UV-Cl and 31 samples after Cl-UV, were collected and processed. Parallel factor (PARAFAC) analysis was conducted using MATLAB 2022a with the graphical user interface EEMlab [33], which employs the routines of drEEM toolbox for the pre-processing, modelling, and analysis of the data [34].

Briefly, the procedure consists of the following key steps: Blank corrections were performed based on the blank deionised water peaks (Ex at 350 nm). Raman and Rayleigh scattering removal, outlier samples removal and normalization were conducted prior to the PARAFAC modelling. Preliminary EEM-PARAFAC models with 2 to 7 components were computed for all samples in the exploratory analysis. Then, all models were tested using residual analysis and split-half analysis [34] before the determination of the number of EEM components. The fluorescent components were assigned based on the similarity >95% obtained by comparing with identified components earlier in other studies using the OpenFluor database (https://openfluor.lablicate.com/) [35].

In this study, a five-component model was adopted to describe the entire EEM data set, which could explain more than 97% of the variance. The results are showed in Table S2 and Figure S5.

Text S2 Detection of target genes by real-time PCR

The total volume of qPCR mixtures was 20 µl, which consisted of 10 µl of POWRUP SYBR MASTER MIX (Applied Biosystems, Singapore), 0.8 µL forward or reverse primer (0.4 µM), 2 µl of template DNA, and 6.4 µl DNA-free H_2O.

The qPCR program consisted of 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C, 30 s at the annealing temperature, extension for 30 s at 72 °C and a fluorescence acquisition step at 72 °C, then a final melt curve stage with temperature ramping from 60 to 95 °C. Negative control samples were measured using DNA-free H_2O as the template. Each sample was run in triplicate.

Text S3 Detection and quantification of target ARGs

The 2^(−ΔΔCt) method was adopted to compare relative abundance of ARGs between samples, the relevant factors were calculated based on the following equations:

$$\Delta Ct = Ct(ARG) - Ct(16s\ rRNA) \quad (1)$$

$$\Delta\Delta Ct = \Delta Ct(Exp) - \Delta Ct(Con) \quad (2)$$

where Ct is the threshold cycle of ARGs or 16s rRNA, Exp is the experimental group (i.e., reactor UV-Cl, Cl-UV and UV/Cl), Con is the control group (i.e., SF). Value of 2^(−ΔΔCt) > 1 suggests a more abundance target ARG compared to the control group, 2^(−ΔΔCt) < 1 suggests a less abundance target ARG compared to the control group.

Text S4 Analysis of ATR-FTIR results

The bands at 2930 cm^{-1} and 1450 cm^{-1} can be assigned to aliphatic C–H groups [38]. The band at 1000 cm^{-1} is assigned to C–O stretching of polysaccharide-like substances [36]. The peak at 1720 cm^{-1} is associated with C=O stretching of carbonyl group [39]. Bands at 1200 cm^{-1} and 1050 cm^{-1} are associated with C–O stretching while origins from relative complex (e.g., carboxylic groups, phenols, aromatic/unsaturated ethers) and simple (alcohols, aliphatic ethers) structures, respectively [37]. The band at 875 cm^{-1} is assigned to C–H vibration of aromatic ring [36].

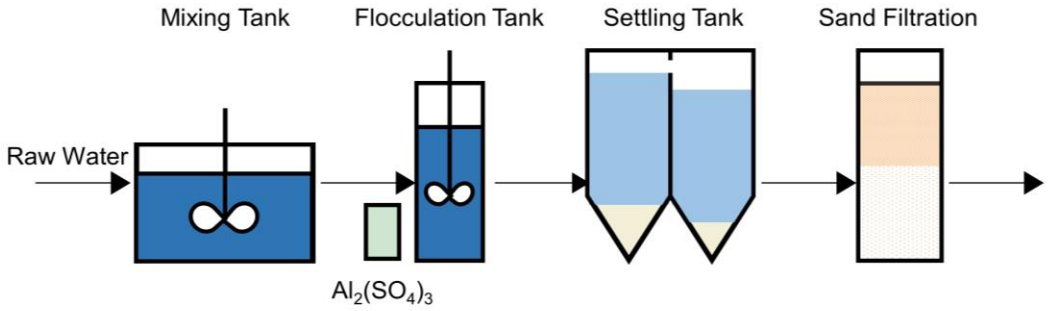

Figure S1. Illustration of pre-treatment processes.

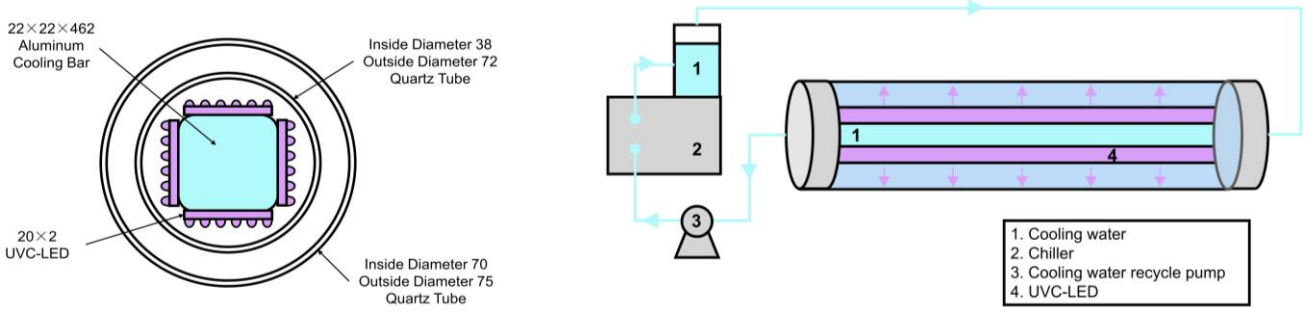

Figure S2. Schematic diagram of LED-UV lamp from top view (**a**) and side view (**b**).

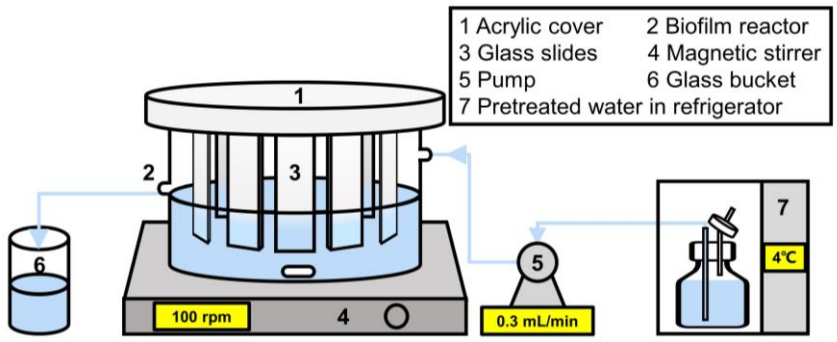

Figure S3. Schematic diagram of biofilm reactor.

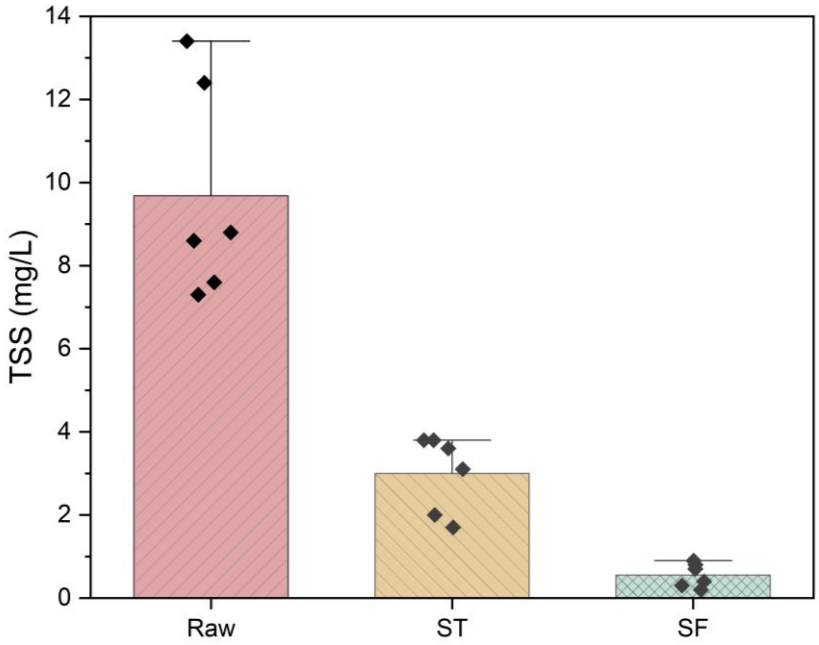

Figure S4. Removal of total suspended solids (TSS) in pre-treatment processes. Raw: raw water; ST: after settling tank; SF: after sand filtration.

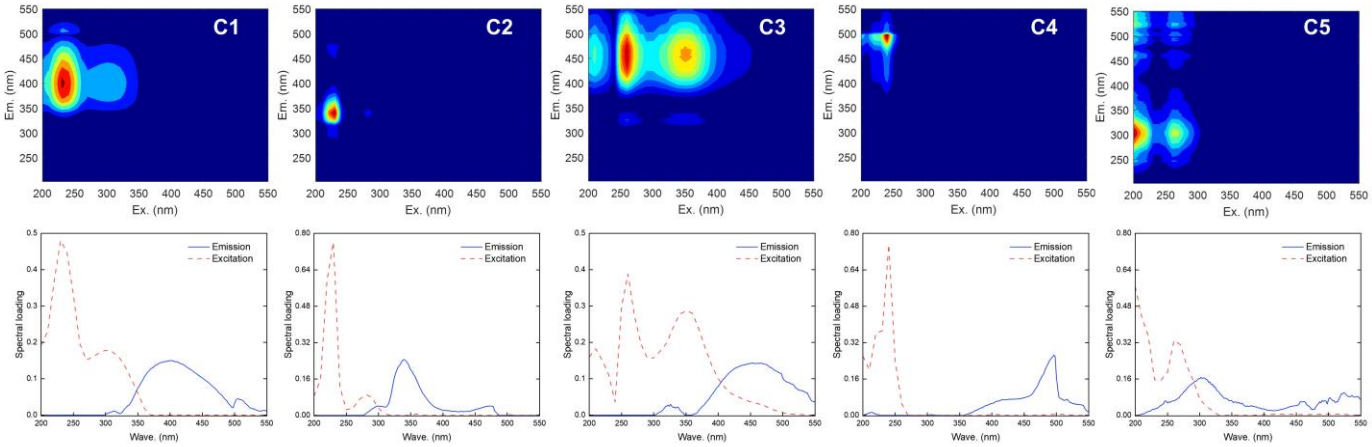

Figure S5. Identified EEM-PARAFAC components in water samples (C1-C5) and their spectral loadings.

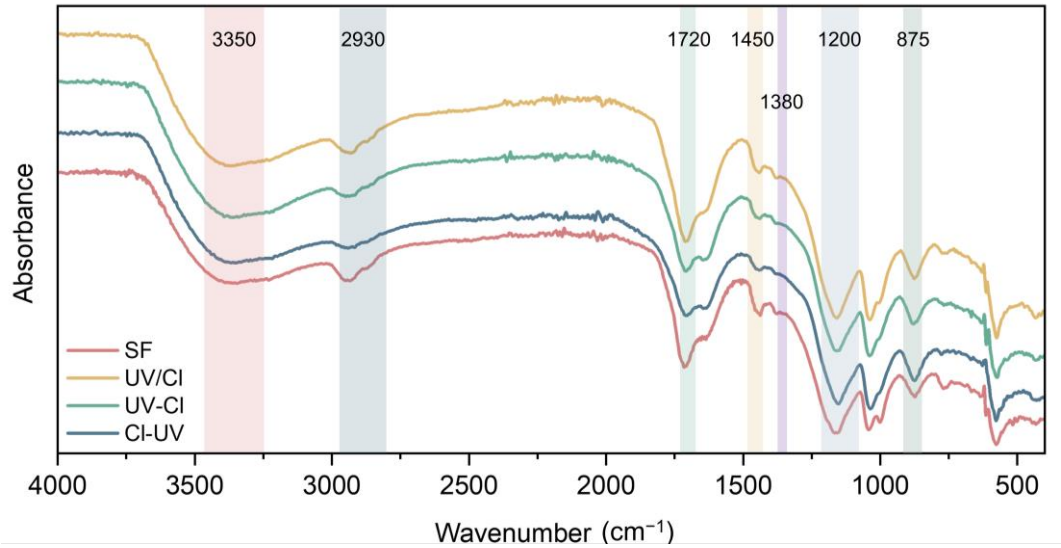

Figure S6. FTIR spectrum of organics after sand filtration (SF), UV-Cl, Cl-UV and UV/Cl.

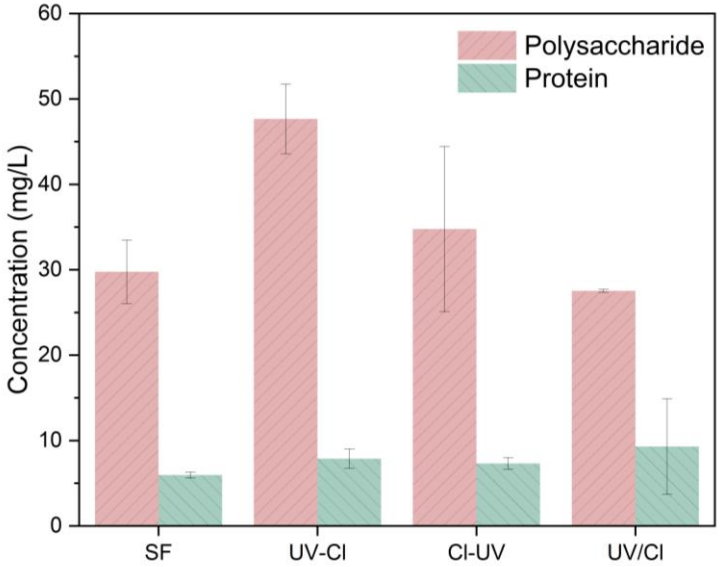

Figure S7. Polysaccharide and protein in extracellular polymeric substances of biofilm.

Table S1. Details of primers used in this study.

Target gene	Length (bp)	Primer set
16s rRNA	181	CCTACGGGAGGCAGCAG/ATTACCGCGGCTGCTGG
intI1	473	CTGGATTTCGATCACGGCACG/ACATGCGTGTAAATCATCGTCG
intI2	195	TGCTTTTCCCACCCTTACC/GACGGCTACCCTCTGTTATCTC
blaTEM-1	209	AATAAACCAGCCAGCCGGAA/TTGATCGTTGGGAACCGGAG
tetA	208	GCTACATCCTGCTTGCCTTC/TAGATCGCCGTGAAGAGGAG
mecA	212	CCACTTCATATCTTGTAACG/AGAATATAATAAATTAACCGAAGATA
qnrS	255	TCGGCACCACAACTTTTCAC/TCACACGCACGGAACTCTAT
oqxB	131	TCCTGATCTCCATTAACGCCCA/ACCGGAACCCATCTCGATGC
mexB	75	GGGATCGACAATCTGCGCTA/TTCGAAGGTCACGGTGATGG
adeA	72	CAGTTCGAGCGCCTATTTCTG/CGCCCTGACCGACCAAT
sul1	433	CGGCGTGGGCTACCTGAACG/GCCGATCGCGTGAAGTTCCG
sul2	435	CCTGTTTCGTCCGACACAGA/GAAGCGCAGCCGCAATTCAT

Table S2. PARAFAC components from 5-component model.

	Ex/Em peaks	**Source assignment**
C1	(230, 300)/(315, 400, 505)	terrestrial humic-like [43]
C2	(230, 280)/(300, 340, 480)	protein-like [27,44]
C3	(210, 260, 360)/(325, 455)	humic-like [45–47]
C4	240/495	fulvic acid-like [14]
C5	260/300	protein, tryptophan-like [48]