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Supporting Information

Effects of GAC and UV/H_2O_2-GAC filtration on bacterial community and opportunistic pathogens in drinking water systems

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Text S1. UV/H_2O_2 equipment

The UV/H_2O_2 treatment was carried out in a reactor with a volume of 500 mL. The reactor was equipped with an 11 W low-pressure Hg vapor lamp (Institution of Light Source, Beijing), emitting essentially monochromatic UV light at 254 nm. A quartz sleeve was inserted into the inner of reactor to isolate the lamp and reaction solutions. The external surface was covered with aluminum foil to reduce the influence of the sunlight. At the bottom of the reactor, a small stir bar was placed to ensure homogeneous UV exposure. The UV fluence (mJ/cm^2) was calculated by the exposure time multiplied by the average photon fluence rate. According to the article that has been reported by Canonica et al. (2008), atrazine was used to calculate the photon fluence rate. A photon fluence rate of 4.50×10^{-5} E m^{-1} s^{-1} was obtained, which corresponded to power output of 2.12 mW/cm^2. Before the experiment, a minimum of 30 min warm-up time ensured a stabilized UV emission output. 500 mJ/cm^2 UV dose and 1 mg/L H_2O_2 was used in this experiment.

Text S2. PMA method

In this study, PMA dye (Biotium, Inc., Hayward, CA) was diluted to form a 500 μM stock solution. The water and biofilm samples were subjected to PMA treatment by incubating the polycarbonate membrane filters in 40 μM PMA solution, respectively. PMA treated samples were incubated in the dark for 15 min at room temperature followed light exposure for 10 min at a distance of 20 cm from a 650-W halogen light source (Sachtler R651HS; Camera Dynamics, Inc., Valley Cottage, NY). The samples were laid horizontally on ice during the light exposure and rotated

periodically to avoid excessive heating. After PMA treatment, samples were centrifuged at 10000 g for 5 min to harvest pellets. The pellets were washed twice with ultrapure sterile water to remove residual PMA. The pellets were subjected to DNA extraction with FastDNA SPIN Kit (MP Biomedicals, Solon, OH, USA) following the manufacturer's instructions.

Text S3. Illumina Hiseq sequencing analysis

After sequencing, the sequences were analyzed including operational taxonomic unit (OTU) clustering and taxonomic classification. Paired-end reads from the original DNA fragments are merged by using FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/), and the paired-end reads was assigned to each sample according to the unique barcodes. Sequences were analyzed using QIIME (http://qiime.org/index.html) software package (V1.7.0, Quantitative Insights Into Microbial Ecology), and in-house Perl scripts are used to analyze alpha and beta diversity. Then, UPARSE pipeline (V7.0. 1001, http://drive5.com/uprase) is used to pick operational taxonomic units (OTUs) through making OTU table, and RDP classifier is used to assign taxonomic data to each representative sequence. Sequences were assigned to OTUs at 97% similarity. OTUs were used to calculate a Bray-Curtis distance matrix, based on which cluster analysis on samples was conducted using R software (version 3.0.1).

Text S4. qPCR process for different opportunistic pathogens

The 25 μL-SYBR Green-qPCR mixture was comprised of following: 12.5 μL SYBR Premix Ex taq (TaKaRa, China), 0.5 μL ROX (50 ×, TaKaRa, China), 9.5 μL

double-distilled H_2O, 0.25 μL of 10 μM forward and reverse primer, 2 μL template DNA. If there was probe, the 25 μL-TaqMan-qPCR mixture was comprised of 8 μL double-distilled H_2O, 0.5 μL of 10 μM forward and reverse primer, 1 μL of 3 μM probe, and the rest were the same. A melt curve analysis was conducted to verify the specificity of the primers in each run, which through increasing from 75 to 95 ºC with 20-sec holds.

Text S5. EPS extraction and analysis

EPS of bacteria in water and in biofilm was extracted by a heat extraction method (Wang et al., 2017). In order to extract the EPS in particle-associated bacteria and free-living bacteria, 1 L bulk water was filtered through 1.2 μm and 0.2 μm polycarbonate filter, sequentially. The two polycarbonate filters were transferred to 50 mL centrifuge tubes with 40 mL phosphate buffered solution (PBS, pH=7), respectively. In order to get the EPS in biofilm samples, the biofilm was also put into 50 mL centrifuge tubes. The two tubes were sonicated at 20 KHz and 40 W for 30 s, followed by heating in water bath at 70 ^{o}C for 1 hour, and then centrifuged at 8000 g for 20 minutes at 4 ^{o}C. The supernatant in the two tubes were filtered through 0.45 μm polycarbonate filter to collect EPS. The proteins in EPS were determined with the Lowry procedure using bovine serum albumin (BSA) (Sigma) as standard. The polysaccharide content was determined by the phenol-sulfuric acid method, with glucose as standard.

Reference

Canonica S, Meunier L, Gunten U V (2008). Phototransformation of selected

pharmaceuticals during UV treatment of drinking water. Water Research, 42：121–128

Wang X J, Li J, Li Y, Wei J, Zhang Y Z, Zhai J Y, Wang S Y, Zhang W G (2017). Diversity of bacteria and functional bacteria in MBR shortcut nitrification system treating late landfill leachate. Journal of Beijing University of Technology, 43(9): 1416–1425

Table S1 The characteristics of the water used in the experiment.

Parameter (Unit)	Value range of drinking water
pH	7.72~8.10
DO (mg/L)	7.92~8.27
DOC (mg/L)	1.45~1.92
NH_3-N(mg/L)	0~0.05
NO_2^- (mg/L)	0~0.58
NO_3^- (mg/L)	4.02~9.46
Cl^- (mg/L)	19.8~22.3
SO_4^{2-} (mg/L)	50.2~76.4
TDS (mg/L)	168~208
Hardness (mg $CaCO_3$/L)	197.9~212.8
Alkalinity (mg $CaCO_3$/L)	106.2~134.4
Turbidity (NTU)	0.07~0.20

Table S2 The primers, probes (if necessary) and q-PCR programs of 16S rRNA for total bacteria, *Mycobacterium avium*, *Legionella pneumophila* and *Pseudomonas aeruginosa*.

Target bacteria	Target gene	primer sequence	Bp	reaction conditions	reference
Total bacteria	16S rRNA	F: CGGTGAATACGTTCYCGG R: GGYTACCTTGTTACGACTT P: CTTGTACACACCGCCCGTC	124	95°C for 30 s, 35 cycles of 95°C for 15 s and 56°C for 60 s	(Wang et al., 2012)
Mycobacterium avium	16S rRNA	F：AGAGTTTGATCCTGGCTCAG R：ACCAGAAGACATGCGTCTTG	180	98°C for 2 min, 40 cycles of 98°C for 5 s and 68°C for 18 s	(Wang et al., 2012)
Legionella pneumophila	*mip*	F:AAAGGCATGCAAGACGCTATG R:GAAACTTGTTAAGAACGTCTTTCATTTG P:TGGCGCTCAATTGGCTTTAACCGA	78	95°C for 2 min, 40 cycles of 95°C for 5 s and 60°C for 10 s	(Wang et al., 2012)
Pseudomonas aeruginosa	*oprl*	F:GACGTACACGCGAAAGACCT R:GCCCAGAGCCATGTTGTACT	99	95°C for 5 min, 40 cycles: 95°C for 15 s, 60°C for 45 s	(Wang et al., 2018)

P: Premix Ex Taq, F: Forward primer, R: Reverse primer.

Reference:

Wang H, Edwards M, Falkinham III, J O, Pruden, A (2012). Molecular survey of the occurrence of legionella spp., mycobacterium spp., pseudomonas aeruginosa, and amoeba hosts in two chloraminated drinking water distribution systems. Applied and Environmental Microbiology 78: 6285–6294.

Wang H B, Hu C, Zhang S, Liu L Z, Xing X C (2018). Effects of O_3/Cl_2 disinfection on corrosion and opportunistic pathogens growth in drinking water distribution systems. Journal of Environmental Science (China). 73: 38–46.

Table S3 Quantification limits and amplification efficiencies for qPCR.

Target microorganism	Quantification limit (gene copies/µL DNA)	Slope	Amplification efficiency (%)	R^2
Legionella pneumophila	6	-3.411	96.43	0.9561
Mycobacterium avium	4	-3.388	97.33	0.9779
Pseudomonas aeruginosa	67	-3.420	96.06	0.9921
Total bacteria (16S rRNA)	8	-3.360	98.43	0.9910

Table S4 The observed species, Shannon, Chao 1 index and goods coverage of different samples.

	Sample	Observed species	Shannon	Chao1	Goods coverage
particle-associated bacteria	sand filtered water	319	4.159	350.017	0.998
	UV/H_2O_2	508	2.357	713.219	0.994
	SF DWDSs influents	439	3.566	613.69	0.995
	GAC DWDSs influents	335	1.955	396.552	0.997
	UV/H_2O_2-GAC DWDSs influents	331	1.866	395.225	0.997
	SF DWDSs effluents	415	5.296	467.697	0.997
	GAC DWDSs effluents	497	4.02	688.786	0.996
	UV/H_2O_2-GAC DWDSs effluents	303	3.773	366.382	0.997
free-living bacteria	sand filtered water	322	4.381	750.077	0.997
	UV/H_2O_2	322	3.139	414.5	0.997
	SF DWDSs influents	407	3.11	527.6	0.996
	GAC DWDSs influents	350	1.748	485.965	0.997
	UV/H_2O_2-GAC DWDSs influents	308	1.728	374.014	0.996
	SF DWDSs effluents	380	4.768	485.457	0.997
	GAC DWDSs effluents	371	4.551	511.909	0.996
	UV/H_2O_2-GAC DWDSs effluents	370	3.057	430	0.997
Biofilm	SF DWDSs	464	5.391	545.4	0.997
	GAC DWDSs	441	5.108	568	0.996
	UV/H_2O_2-GAC DWDSs	421	4.709	509.8	0.996

Table S5 The p value for the difference of the opportunistic pathogens in the influents, effluents and biofilm of the three simulated drinking water distribution systems

				M. avium	*L. pneumophila*	*P. aeruginosa*
influents	particle-associated bacteria	SF-UV/H_2O_2/GAC	FW5-FW9	$4.19*10^{-4}$	$3.41*10^{-5}$	$3.18*10^{-4}$
		SF-GAC	FW5-FW7	$7.47*10^{-4}$	$3.56*10^{-4}$	$3.55*10^{-3}$
		UV/H_2O_2/GAC-GAC	FW9-FW7	$3.32*10^{-2}$	$3.04*10^{-4}$	$4.56*10^{-3}$
	free-living bacteria	SF-UV/H_2O_2/GAC	FW6-FW10	$1.63*10^{-4}$	$3.21*10^{-4}$	$3.81*10^{-4}$
		SF-GAC	FW6-FW8	$1.52*10^{-3}$	$6.19*10^{-4}$	$1.25*10^{-3}$
		UV/H_2O_2/GAC-GAC	FW10-FW8	$1.48*10^{-3}$	$4.48*10^{-2}$	$3.46*10^{-2}$
effluents	particle-associated bacteria	SF-UV/H_2O_2/GAC	TW.1.1-TW.1.2	$7.24*10^{-3}$	$1.41*10^{-4}$	$1.05*10^{-6}$
		SF-GAC	TW.1.1-TW.1.3	$5.48*10^{-2}$	$1.00*10^{-2}$	$6.67*10^{-5}$
		UV/H_2O_2/GAC-GAC	TW.1.2-TW.1.3	$2.22*10^{-3}$	$5.20*10^{-4}$	$2.55*10^{-5}$
	free-living bacteria	SF-UV/H2O2/GAC	TW.0.1-TW.0.2	$2.44*10^{-3}$	$3.37*10^{-4}$	$1.24*10^{-5}$
		SF-GAC	TW.0.1-TW.0.3	$2.00*10^{-2}$	$6.47*10^{-3}$	$1.44*10^{-5}$
		UV/H_2O_2/GAC-GAC	TW.0.2-TW.0.3	$9.21*10^{-2}$	$2.91*10^{-3}$	$2.07*10^{-3}$
biofilm	□	SF-UV/H_2O_2/GAC	DWDS.1-DWDS.2	$2.65*10^{-5}$	$1.77*10^{-4}$	$3.23*10^{-3}$
		SF-GAC	DWDS.1-DWDS.3	$4.51*10^{-5}$	$6.25*10^{-1}$	$3.97*10^{-3}$
		UV/H_2O_2/GAC-GAC	DWDS.2-DWDS.3	$2.27*10^{-4}$	$1.92*10^{-4}$	$2.32*10^{-2}$

Table S6 The p value for the difference of the EPS composition in the influents, effluents and biofilm of the three simulated drinking water distribution systems

		proteins (PN)	polysaccharides (PS)	PN/PS
influents	SF-GAC	$8.36*10^{-2}$	$2.20*10^{-4}$	$1.86*10^{-1}$
	SF-UV/H_2O_2/GAC	$3.94*10^{-2}$	$5.95*10^{-4}$	$1.47*10^{-1}$
	UV/H_2O_2/GAC-GAC	$5.82*10^{-1}$	$3.71*10^{-2}$	$9.27*10^{-1}$
effluents	SF-GAC	$2.90*10^{-2}$	$2.76*10^{-5}$	$1.88*10^{-1}$
	SF-UV/H_2O_2/GAC	$4.37*10^{-3}$	$3.86*10^{-4}$	$8.38*10^{-3}$
	UV/H_2O_2/GAC-GAC	$1.28*10^{-1}$	$5.75*10^{-4}$	$4.55*10^{-2}$
biofilm	SF-GAC	$1.57*10^{-2}$	$3.89*10^{-4}$	$4.71*10^{-1}$
	SF-UV/H_2O_2/GAC	$1.76*10^{-4}$	$8.98*10^{-4}$	$9.10*10^{-3}$
	UV/H_2O_2/GAC-GAC	$2.38*10^{-4}$	$4.21*10^{-3}$	$2.43*10^{-2}$

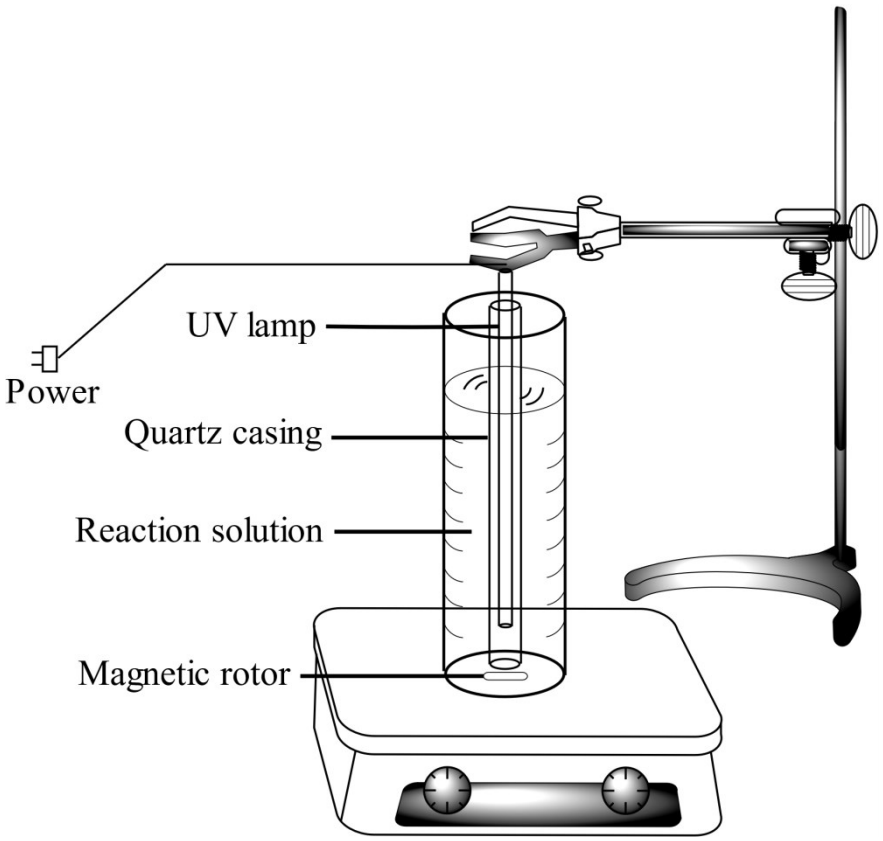

Fig. S1 The sketch map of UV/H_2O_2 equipment.

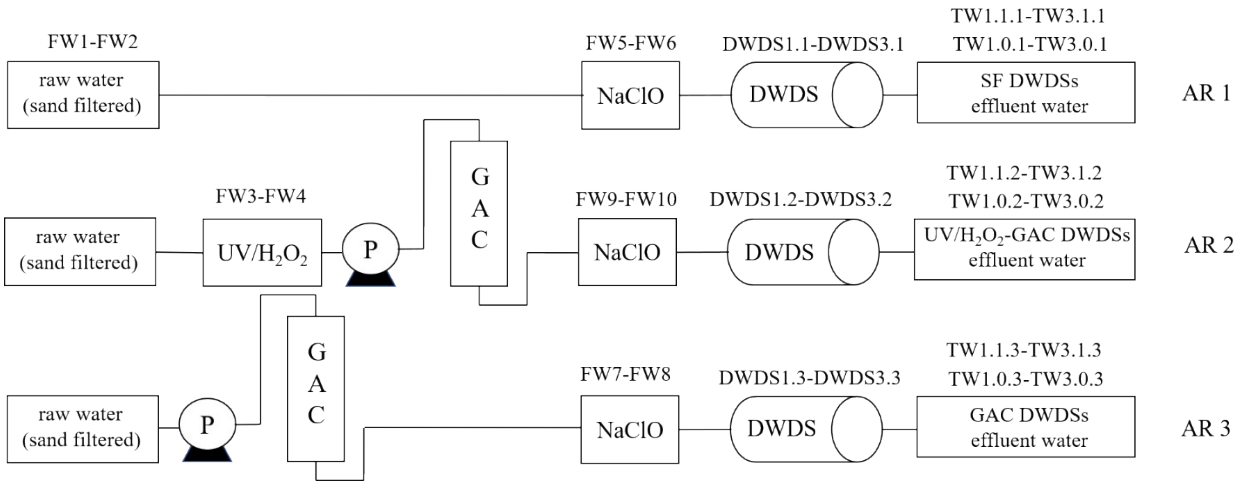

Fig. S2 Annular reactors set-up.

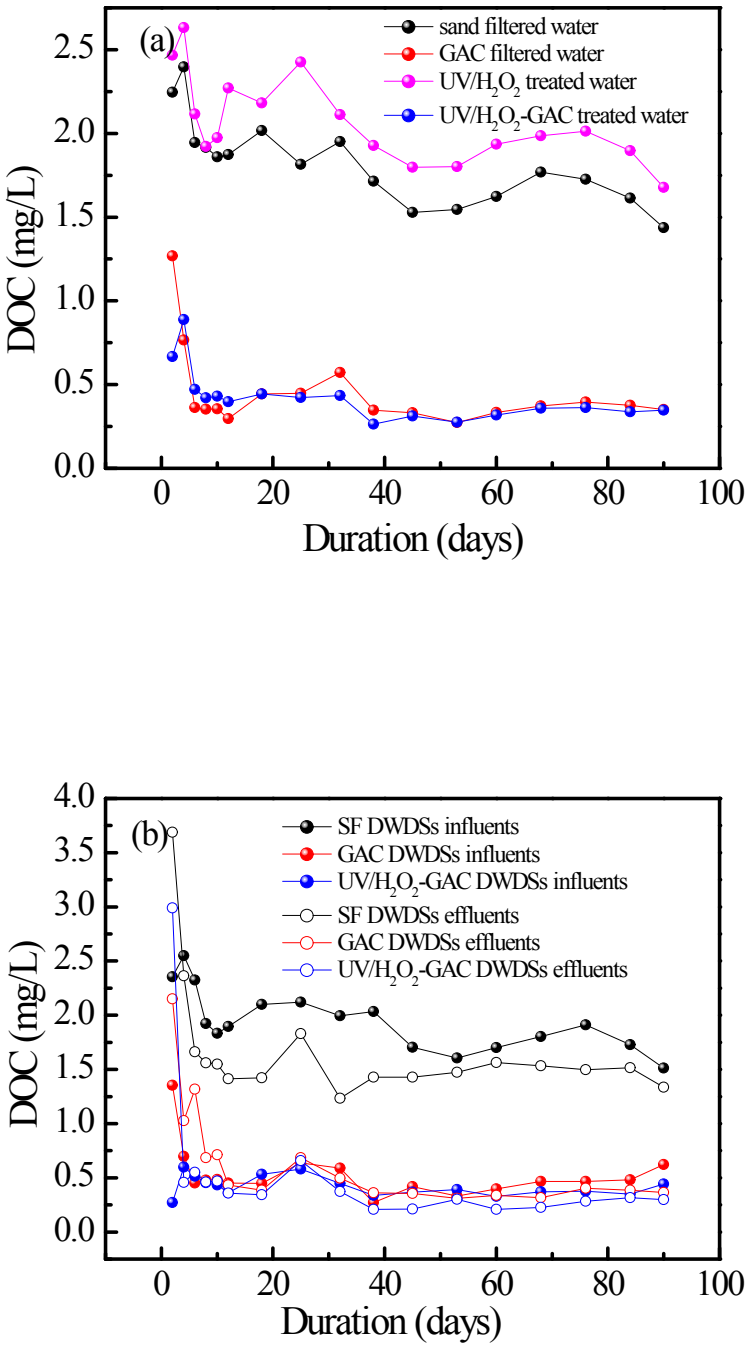

Fig. S3 The DOC concentration in the effluents of different water treatment processes (a), and influents and effluents of the three drinking water distribution systems.

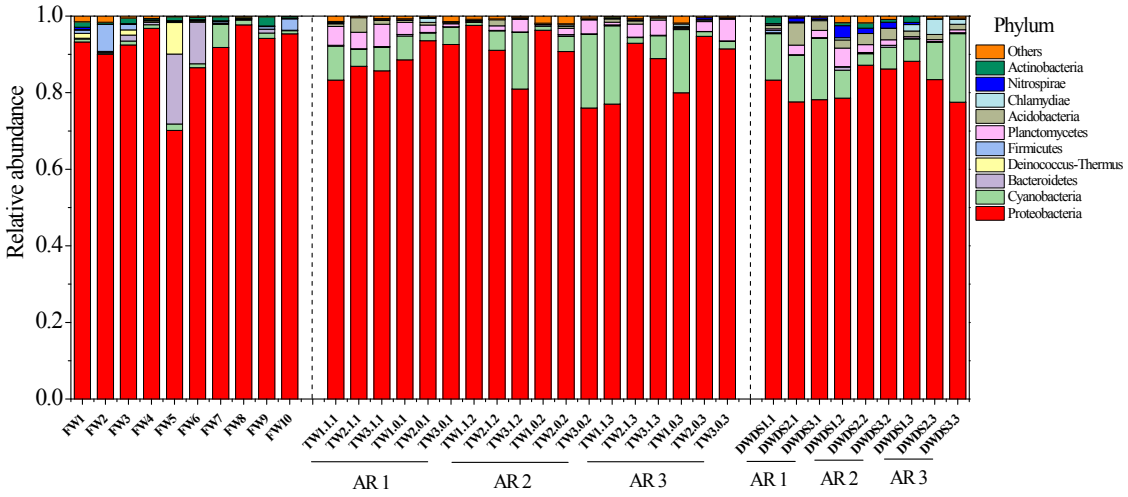

Fig. S4 The relative abundance of bacterial phylum in different samples.

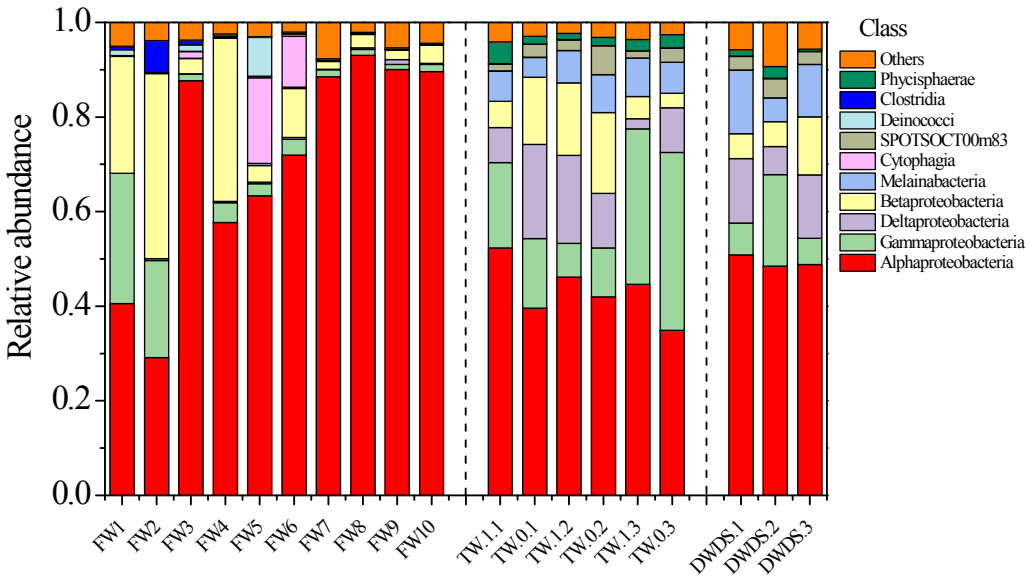

Fig. S5 The mean relative abundance of bacterial class in different samples. In sand filtered DWDSs, TW.1.1 showed the mean value of TW1.1.1, TW2.1.1 and TW3.1.1, TW.0.1 showed the mean value of TW1.0.1, TW2.0.1 and TW3.0.1, DWDS.1 showed the mean value of DWDS1.1, DWDS2.1 and DWDS3.1. Other symbols in GAC DWDSs and UV/H2O2-GAC DWDSs showed the same meaning with that in sand filtered DWDSs.

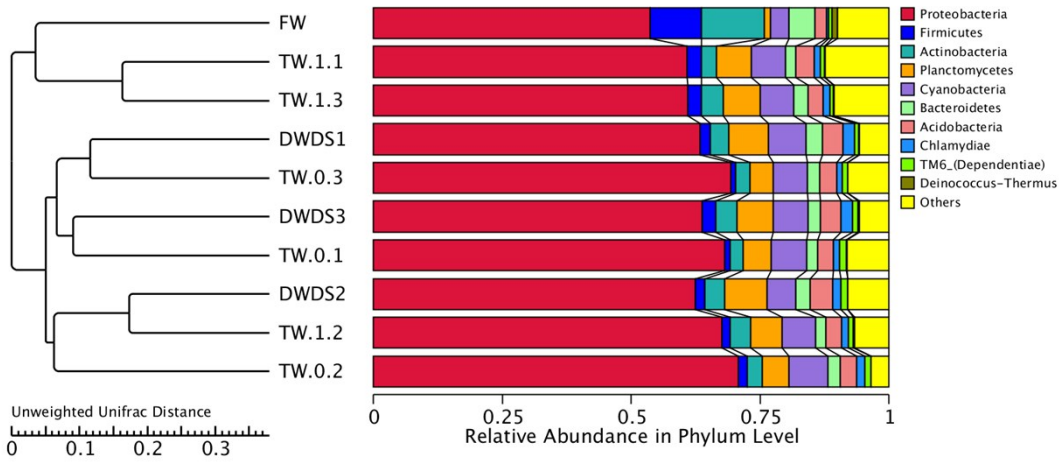

Fig. S6 Unweighted pair-group method with arithmetic mean (UPGMA) analysis of bacterial community at phylum level. DWDS1: SF DWDSs; DWDS2: UV/H_2O_2-GAC DWDSs; DWDS3: GAC DWDSs.

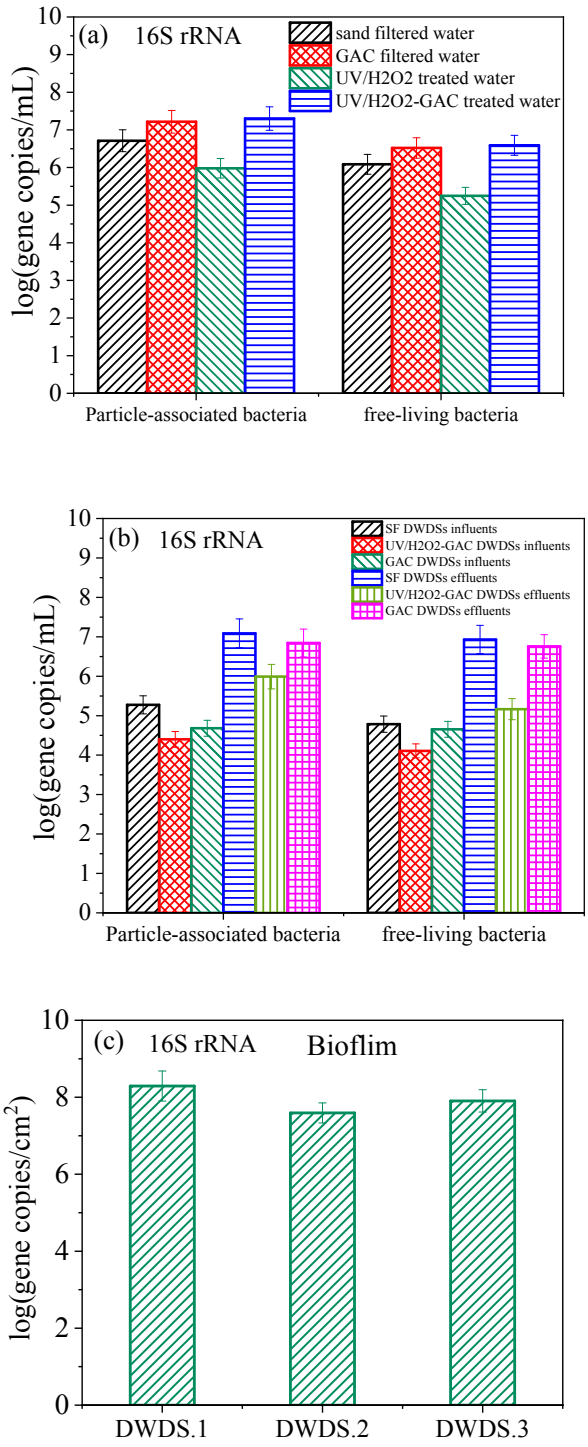

Fig. S7 The qPCR results of 16S rRNA in effluents of different water treatment processes (a), influents and effluents (b) and biofilm (c) of different drinking water distribution systems.