Effects of BAC-filtration, disinfection, and temperature on water quality in simulated reclaimed water distribution systems

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Supplementary Information

Section 1: Tables

SI Table 1 Summary of samples from the simulated RWDSs rigs selected for metagenomic sequencing. There were three sequencing events. The first two focused on the **unfiltered** samples and the third sequencing event included the filtered samples. Sampling ports (P1-P5) refer to those defined in Table 1. W= bulk water sample; B= biofilm sample**.**

	First sequencing HiSeq 2500		Second sequencing HiSeq 2500		Third sequencing NextSeq		
Condition	14 °C-1		22 °C-1	30 °C-1	30 °C-2		30 °C-3
Unfiltered Chlorine	P2_W, P5_W, P2_B, P5_B	P2_W, P5_W, P2_B, P5_B	P2_W, P5_W, P2_B, P5_B	P2_W, P5_W, P2_B, P5_B	P2_W, P5_W, P2_B, P5_B		P2_W, P5_W, P2_B, P5_B
Unfiltered Chloramine	P1_W, P5_W, P1_B, P5_B		P2_W, P5_W, P2_B, P5_B		P2_W, P5_W, P2_B, P5_B	P2_W, P5_W, P2_B, P5_B	P2_W, P5_W, P2_B, P5_B
Unfiltered No Residuals	P1_W, P5_W, P1_B, P5_B		P2_W, P5_W, P2_B, P5_B		P2_W, P5_W, P2_B, P5_B	P2_W, P5_W, P2_B, P5_B	P2_W, P5_W, P2_B, P5_B
Filtered Chlorine							P2_W, P5_W, P2_B, P5_B
Filtered Chloramine					P2_W, P5_W, P2_B, P5_B		P2_W, P5_W, P2_B, P5_B
Filtered No Residuals					P2_W, P5_W, P2_B, P5_B		P2_W, P5_W, P2_B, P5_B

SI Table 2 Summary of DO losses between the reservoir and the final sample port, P5, in the RWDSs at the five temperature phases. Positive numbers indicate decreases/loss of DO along the RWDSs.

	Unfiltered Chlorine	Unfiltered Chloramine	Unfiltered NoResiduals	Filtered Chlorine	Filtered Chloramine	Filtered NoResiduals
14°C-1	0.695	1.73	0.33	0.810	1.85	2.40
22°C-1	1.80	6.30	4.24	0.085	4.87	3.75
30°C	3.82	6.71	5.24	1.58	4.18	3.57
22°C-2	3.13	6.30	6.30	0.925	3.43	2.42
14°C-2	-0.790	-0.790	1.55	2.54	1.26	1.02
Mean	**1.73**	**4.05**	**3.53**	**1.19**	**3.12**	**2.63**

SI Table 3a. A summary of ANOSIM R values of the metagenomic taxonomy profiles for all experimental condition factors at 14 °C_1

|		14 °C_1					
		One-factor					
Disinfectants		0.261*					
Water/Biofilm		0.381**					
Water age		0.103					
		Two-factor crossed					
		Across					
		Disinfectants	Water/Biofilm	Water age			
Factor	Disinfectants		0.419*	0.431*			
	Water/Biofilm	0.557**		0.25			
	Water Age	0.34	-0.001				
		Crossed between disinfectants					
		Water/Biofilm			Water age		
		Chlorine	Chloramine	No Residual	Chlorine	Chloramine	No Residual
Disinfectants	Chlorine						
	Chloramine	0.429			0.5*		
	No Residual	0.393	0.25		0.339	0.25	

SI Table 3b. Summary of ANOSIM R values of the metagenomic taxonomy profiles for each experimental condition factor 22 °C_1.

|		22 °C_2					
		One-factor					
Disinfectants		0.222**					
Water/Biofilm		0.106					
Water age		-0.089					
		Two-factor crossed					
		Across					
		Disinfectants	Water/Biofilm	Water age			
	Disinfectants		0.778**	0.143*			
Factor	Water/Biofilm	0.667*		0.054			
	Water Age	-0.186	-0.223				
		Two-factor crossed between disinfectants					
		Water/Biofilm			Water age		
		Chlorine	Chloramine	No Residual	Chlorine	Chloramine	No Residual
	Chlorine						
Disinfectants	Chloramine	0.75			0.25		
	No Residual	0.75	0.875		0.134	0.193	

SI Table 3c. Summary of ANOSIM R of the metagenomic taxonomy profiles for each experimental condition factor at 30°C.

	30 °C			
	One-factor			
Disinfectants	0.24***			
Water/Biofilm	0.321**			
Water age	-0.043			
Filtration	0.133**			
	Two-factor crossed			
	Across			
	Disinfectants	Water/Biofilm	Water Age	Filtration
Factor Disinfectants		0.456***	0.275***	0.374***
Water/Biofilm	0.459***		0.328***	0.408***
Water Age	-0.046	-0.006		-0.059
Filtration	0.344***	0.273***	0.152**	

		Two-factor crossed between disinfectants					
		Water/Biofilm		Water age		Filtration	
		Chlorine	Chloramine	Chlorine	Chloramine	Chlorine	Chloramine
Disinfectants	Chlorine						
	Chloramine	0.483***		0.334**		0.393***	
	No Residual	0.505***	0.295***	0.368***	0.16*	0.525***	0.212*

**Section 2: Supplementary Figures**
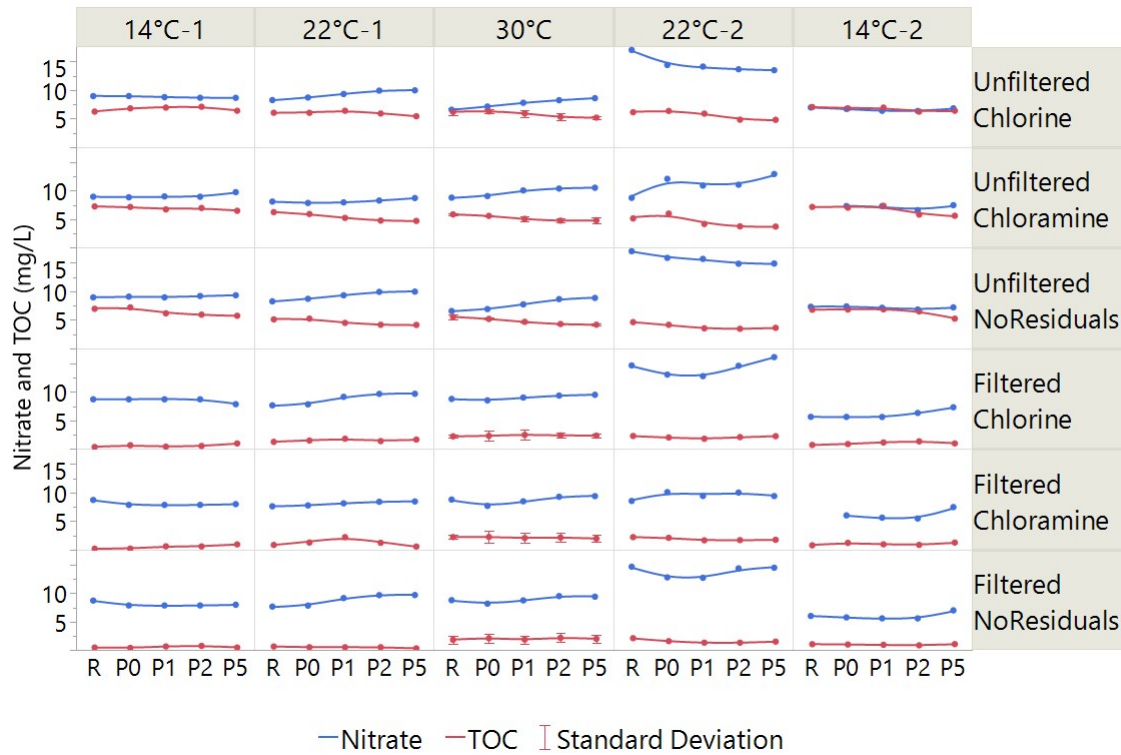
SI Figure 1 Levels of nitrate and TOC in the simulated RWDSs at each temperature phase. TOC at 30°C are mean and standard deviations of the three independent water chemistry sampling measurements.
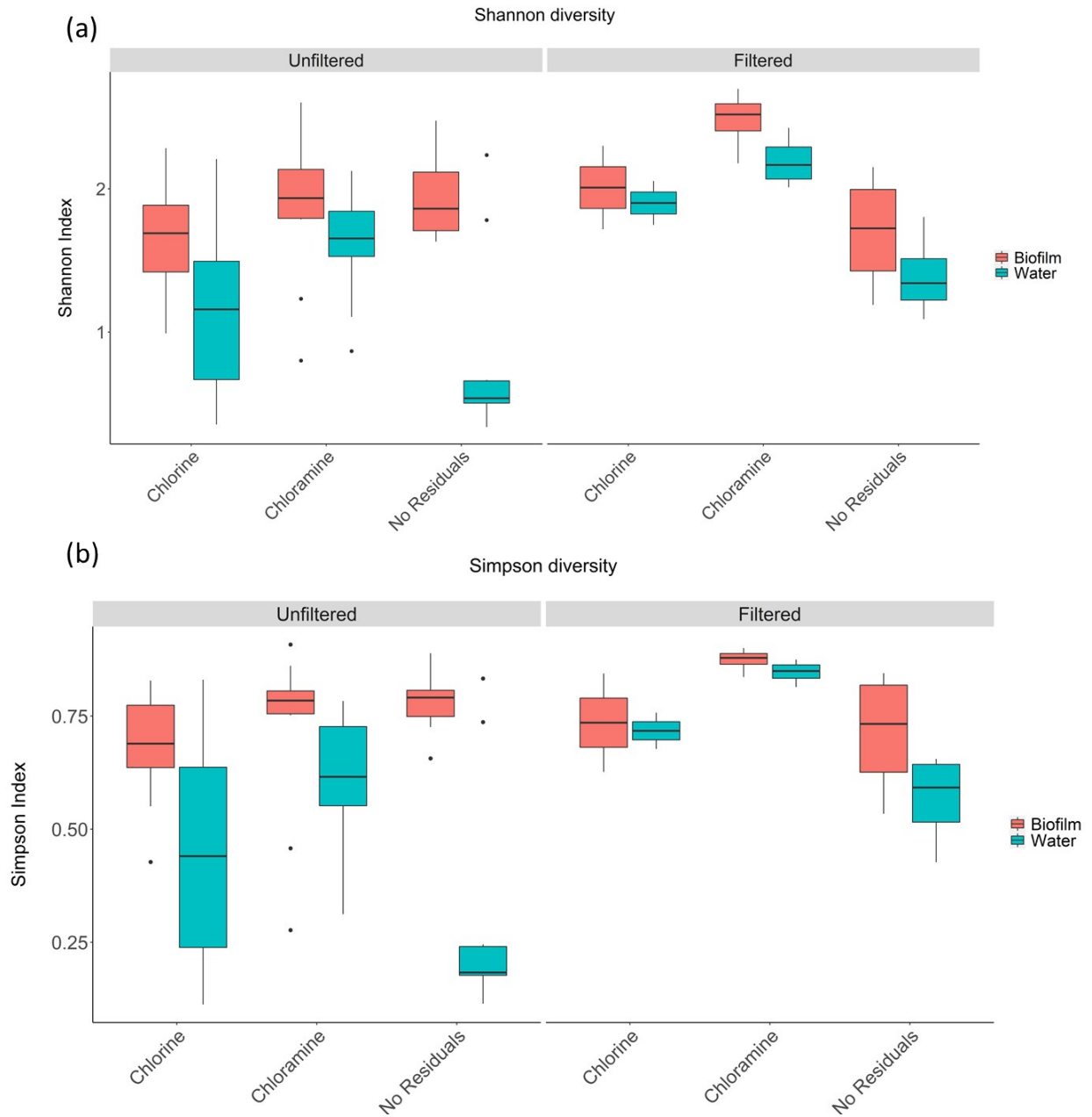
SI Figure 2. Distribution of microbial diversity indices (a) Shannon diversity and (b) Simpson diversity across three disinfectant conditions in water and biofilm samples
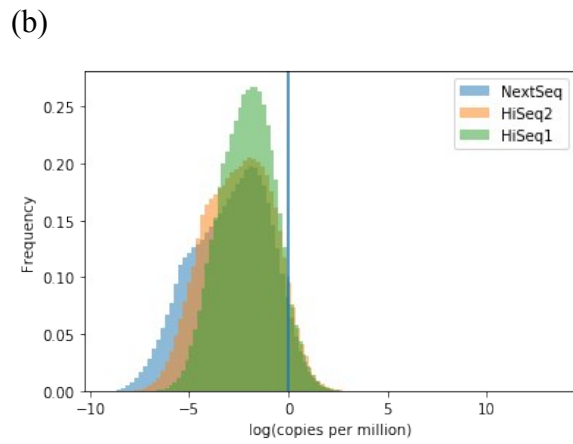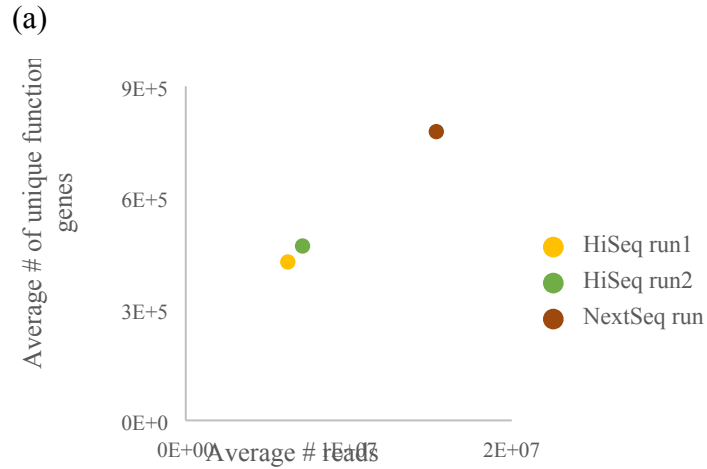
SI Figure 3. (a) Plot of average number of unique UniRef50 functional gene families identified vs. the average the number of reads in the two HiSeq and one NextSeq runs. (b) Histograms of log of mean abundance of each unique functional gene family in copies per million. The platforms diverged below a cutoff value (vertical blue line). For comparison of functional gene families across sequencing platforms only functional genes with abundances above the cutoff value were considered.
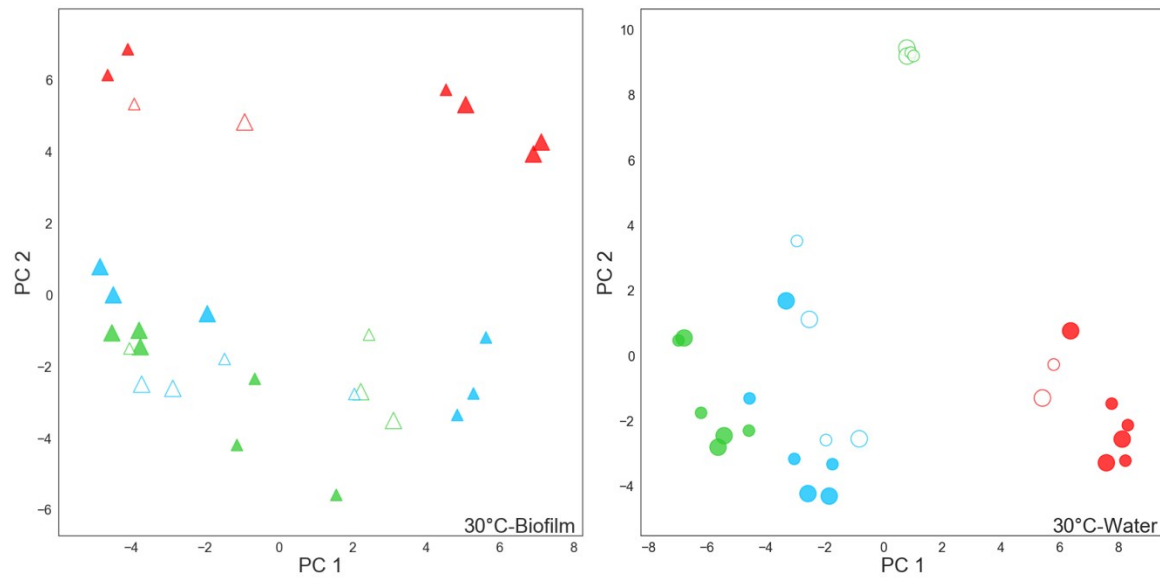
SI Figure 4. RWDS microbiome comparisons based on composition of functional gene family UniRef50 annotations at 30°C. The plots show the largest two principal components, PC1 and PC2. 30°C-Biofilm: PC1 and PC2 explain 31% of variance among samples, 30°C-Water: PC1 and PC2 explain 36% of variance among samples. Metagenomic reads were mapped to the UniRef50 database for functional gene family annotations using the HUMAnN2 pipeline (Franzosa et al. 2018).