Supplementary Material

The Reduction of SARS-CoV-2 RNA Concentration in the Presence of Sewer Biofilms

Shuxin Zhang [1], Elipsha Sharma [1], Ananda Tiwari [2], Yan Chen [1], Samendra P. Sherchan [3,4], Shuhong Gao [5], Xu Zhou [5], Jiahua Shi [6] and Guangming Jiang [1,6,*]

[1] School of Civil, Mining and Environmental Engineering, University of Wollongong, Wollongong, 2520, Australia; sz877@uowmail.edu.au (S.Z.); es788@uowmail.edu.au (E.S.); yc742@uowmail.edu.au (Y.C.)
[2] Finnish Institute for Health and Welfare, Helsinki, 00271, Finland; ananda.tiwari@thl.fi
[3] Department of Environmental Health Sciences, Tulane University, New Orleans, 70118, LA, USA; samendra.sherchan@morgan.edu
[4] BioEnvironmental Science Program, Morgan State University, Baltimore, 21251, MD, USA
[5] State Key Laboratory of Urban Water Resource and Environment, School of Civil & Environmental Engineering, Harbin Institute of Technology, Shenzhen, 518055, China; gaoshuhong@hit.edu.cn (S.G.); zhouxu@hit.edu.cn (X.Z.)
[6] Illawarra Health and Medical Research Institute (IHMRI), University of Wollongong, Wollongong, 2520, Australia; jiahua@uow.edu.au
* Correspondence: gjiang@uow.edu.au; Tel.: +61-02-4221-3792

Table S1. RT-qPCR primer-probe sets used in this study.

Institute	Target	Primer/Probe	Sequence	Position on reference gene*	Amplicon size (bp)	Conditions	Reference
China CDC	N	CCDC-N-F CCDC-N-R CCDC-N-P	GGG GAA CTT CTC CTG CTA GAA T CAG ACA TTT TGC	28885 – 28906 28962 – 28983 28938 – 28957	98	A 20 µL reaction system included 10 µL of 2 × one-step reaction mix (iTaq Universal Probes One-Step Kit, catalogue No. 1725141, Bio-Rad Laboratories,	(Vogels et al., 2020)

			TCT CAA GCT G VIC-TTG CTG CTG CTT GAC AGA TT-QSY			Richmond, CA), 600 nM of forward primer, 800 nM of reverse primer, 200 nM of probe, 0.5 µL of iScript reverse transcriptase and 5 µL extracted RNA of each sample.	
Germany Charité	E	E_Sarbeco_F E_Sarbeco_R E_Sarbeco_P	ACA GGT ACG TTA ATA GTT AAT AGC GT ATA TTG CAG CAG TAC GCA CAC A VIC-ACA CTA GCC ATC CTT ACT GCG CTT CG-QSY	26273 – 26298 26364 – 26385 26336 – 26361	112	A 20 µL reaction system included 5 µL of 4 × one-step reaction mix (TaqPath™ 1-Step Multiplex Master Mix, catalogue No. A28525, Thermo Fisher Scientific, Waltham, MA, USA), 600 nM of forward primer, 800 nM of reverse primer, 200 nM of probe and 5 µL extracted RNA of each sample.	(Corman et al., 2020)

*Reference gene: MT276598.1 (GenBank accession number)

Table S2. Characteristics of the influent wastewater used as the feed for the sewer reactors.

Parameter	**Unit**	**Value (Avg+_ std. dev)**
Ph	-	7.2 ± 0.27
Dissolved oxygen	mg/L	0.67 ± 0.12
Total suspended solids	mg/L	142 ± 69

Table S3. Characteristics of wastewater in sewer reactors over a cycle of 6 hours.

Reactor	Timepoint (hour)	Sulfate Content (mg/L)	Dissolved oxygen (DO) (mg/L)	pH	Soluble COD (mg/L)	Total COD (mg/L)
RM	0	27	-	6.8	116	524
	0.5	26	-	-	-	-
	1	19	-	6.8	92	251
	2	14	-	6.8	102	199
	3	-	-	6.8	91	156
	4	-	-	6.8	83	172
	5	-	-	6.8	88	147
	6	-	-	6.8	84	135
GS	0	25	0.69	7.1	108	-
	0.5	25	-	-	-	-
	1	21	0.61	6.9	67	246
	2	23	0.60	6.9	98	217
	3	-	0.61	7.0	90	180
	4	-	0.63	7.0	90	161
	5	-	0.62	7.0	80	166
	6	-	0.61	7.0	86	299

"-" Not detected.

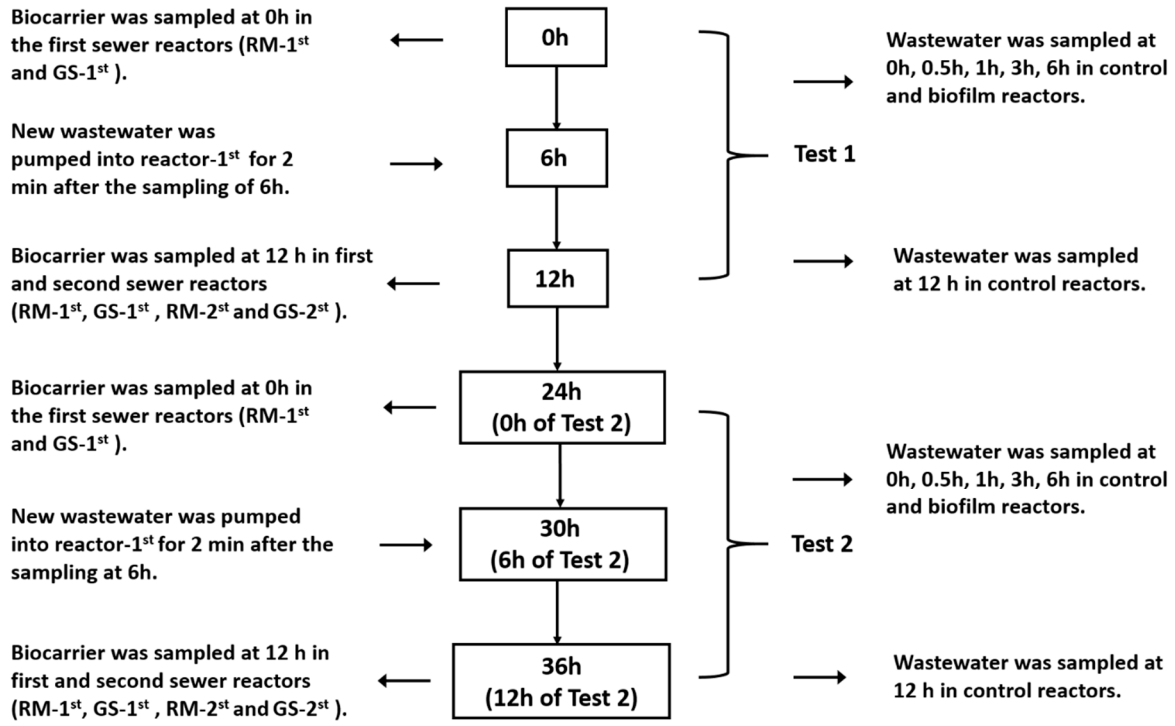

Figure S1. Experimental procedures of duplicate batch tests.

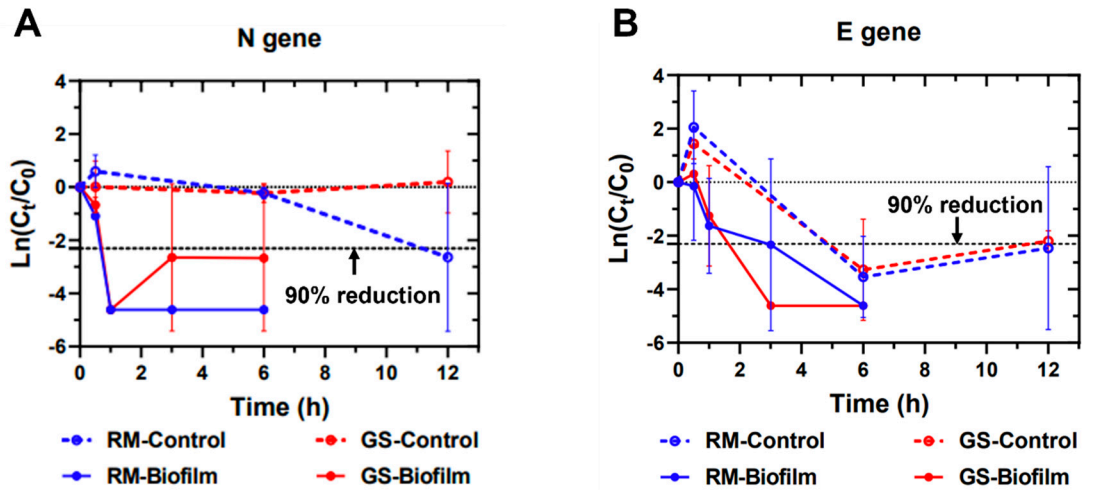

Figure S2. Variation of SARS-CoV-2 E and N gene concentration (mean ± SD) in gravity (GS) and rising main (RM) control and sewer reactors, respectively.

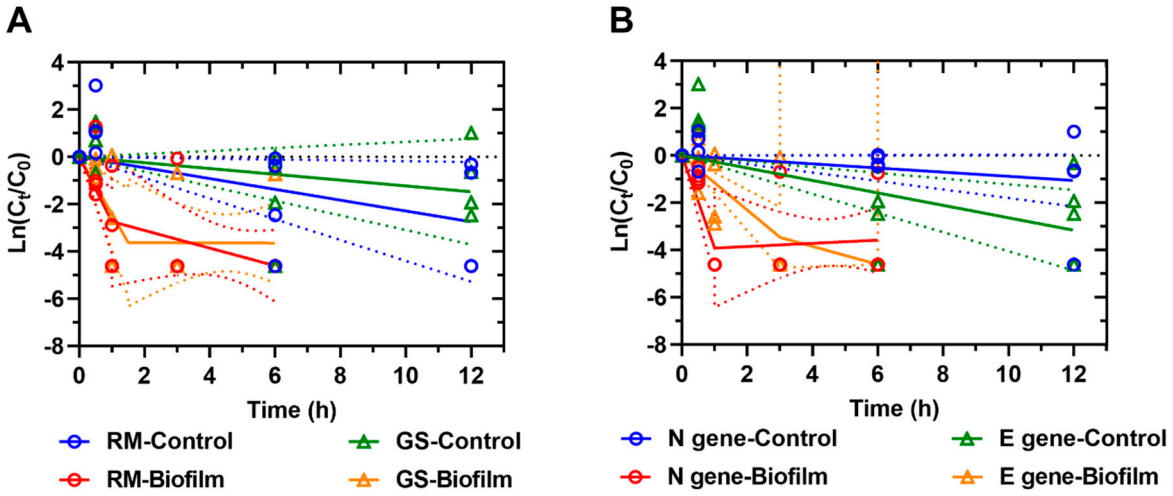

Figure S3. The in-sewer reduction kinetics of SARS-CoV-2 RNA (N and E gene) in different sewer reactors (RM and GS) with and without biofilms. Figure A combined the results of N and E genes. Figure B combined the results of RM and GS reactors. Lines of control reactor (blue and green lines) are fitted with monophasic first-order kinetic model. Lines of biofilm reactor (red and orange lines) are fitted with biphasic first-order kinetic model.

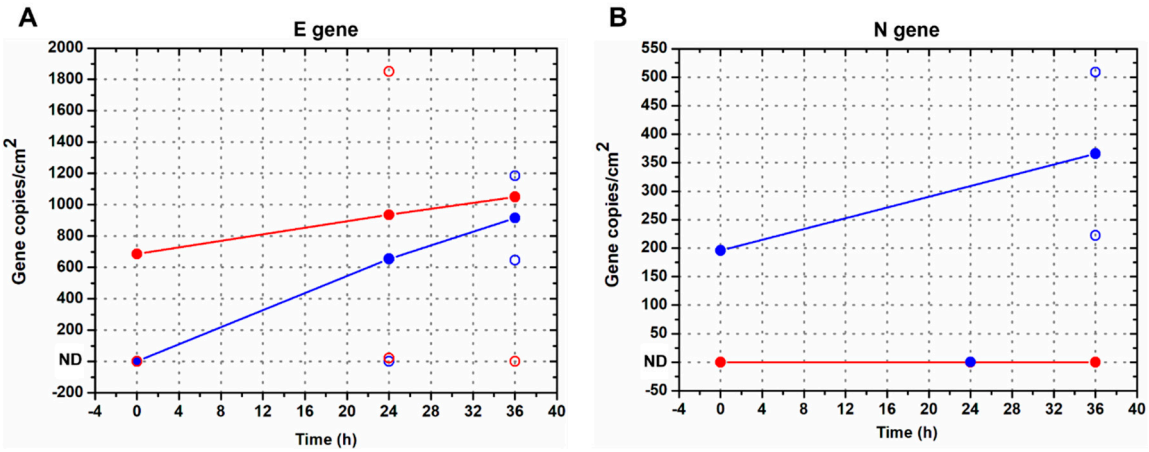

Figure S4. Short-term SARS-CoV-2 RNA accumulation observed in biofilm reactors during duplicate sampling days. The blue symbols represent the results of RM reactor. The red symbols represent the results of GS reactor. The empty circles represent the results of each test. The solid symbols represent the mean of duplicate tests.

Reference

1. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance 2020; 25: 2000045.

2. Vogels CBF, Brito AF, Wyllie AL, Fauver JR, Ott IM, Kalinich CC, et al. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT-qPCR primer-probe sets. Nat Microbiol 2020; 5: 1299-1305.