

Supporting Information for

Adsorption of Respiratory Syncytial Virus (RSV), Rhinovirus, SARS-CoV-2, and F+ bacteriophage MS2 RNA onto wastewater solids from raw wastewater

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Number of pages: 10

Number of Tables: 5

Number of Figures: 5

Linear and Langmuir models

The Linear and Langmuir isotherm equations are defined as

$$\text{Linear:} \quad K_d = q_e/C_e \quad (1)$$

$$\text{Langmuir:} \quad q_e = \frac{q_{\max}K_L C_e}{1 + K_L C_e} \quad (2)$$

where q_e is the equilibrium concentration of viral genomes in solids (cp/g), C_e is the equilibrium concentration of viral genomes in the liquid fraction of wastewater, q_{\max} is the maximum adsorption capacity (cp/g), K_L is the Langmuir constant, and K_d is the distribution coefficient.

Additional details related to EMMI¹ guidelines

The average number of droplets per two merged wells was 40,476 (standard deviation = 3,184). The machine vendor reports the droplet size as 0.00085 μL . Average number of copies per partition (λ) (standard deviation) for SARS-CoV-2 N gene was 0.027 (0.0008) and for BCoV was 0.238 (0.004). λ for other human viruses was similar to SARS-CoV-2.

Table S1: Primers and probes for RT-ddPCR assays

Target	Sequence	Reference
SARS-CoV-2 (N gene)	Forward primer: 5'-CATTACGTTTGGTGGACCCT-3' Reverse primer: 5'-CCTTGCCATGTTGAGTGAGA-3' Probe: CGCGATCAAAACAACGTCGG (5' FAM/ZEN/3' IBFQ)	2
RSV (N gene)	Forward primer: 5'- CTCCAGAATAYAGGCATGAYTCTCC-3' Reverse primer: 5'- GCYCTYCTAATYACWGCTGTAAGAC-3' Probe: TAACCAAATTAGCAGCAGGAGATAGATCAG (5' HEX/ZEN/3' IBFQ)	3
RV	Forward primer: 5'-GCCYGCGTGGCKGCC-3' Reverse primer: 5'-GAAACACGGACACCCAAAG-3' Probe: TCCTCCGGCCCCCTGAATG (5' FAM/ZEN/3' IBFQ)	3
MS2	Forward: 5'-GTCCATACCTTAGATGCGTTAGC-3' Reverse: 5'-CCGTTAGCGAAGTTGCTTGG-3' Probe: ACGTCGCCAGTTCGCCATTGTCTG (5' FAM/ZEN/3' IBFQ)	4,5
BCoV (Extraction control)	Forward primer: 5'-CTGGAAGTTGGTGGAGTT-3' Reverse primer: 5'-ATTATCGGCCTAACATACATC-3' Probe: CCTTCATATCTATACACATCAAGTTGTT (5' FAM/ZEN/3' IBFQ)	6

All probes contained fluorescent molecules and quenchers (5' FAM and or HEX/ZEN/3' IBFQ); FAM, 6-fluorescein amidite; HEX, hexachloro-fluorescein; ZEN, a proprietary internal quencher from Integrated DNA Technologies (Coralville, IA, USA); and IBFQ, Iowa Black FQ.

Table S2: Thermal cycling conditions for SARS-CoV-2, RSV, RV, MS2, and BCoV

Cycling Step	Temperature °C	Time	Number of Cycles
Reverse transcription	50	60 min	1
Enzyme activation	95	10 min	1
Denaturation	95	30 sec	40
Annealing/extension	SARS-CoV-2 and RSV: 59 RV: 61 MS2: 60 BCoV: 56	1 min*	40
Enzyme deactivation	98	10 min	1
Hold	4	Infinite	1

*Ramp rate set to 2°C/sec

Table S3: Served population and annual average daily flow (MGD) of wastewater treatment plants

Plant	Location	Approximate number of people served in the sewershed [†]	Annual Average daily flow (MGD)	Min and Max TSS reported in 2022 (mg/L)	Min and Max pH reported in 2022 [-]
Oceanside Water Pollution Control Plant (OS)	San Francisco	250,000	21.7	33–1,630	*
Southeast Water Pollution Control Plant (SE)	San Francisco	580,000	56.9	26–727	7.0–7.9
Silicon Valley Clean Water Wastewater Treatment Plant (SV)	Redwood City	199,000	12.4	160–400	7.0–7.6
Sunnyvale Water Pollution Control Plant (SU)	Sunnyvale	161,021	12.6	164–376	6.4–7.2
San Jose-Santa Clara Regional Wastewater Facility (SJ)	San Jose	1,419,393	90.7	250–400	7.5–7.9
South County Regional Wastewater Treatment Plant (GI)	Gilroy	105,394	8.5	152–552	6.5–7.3

Notes:

[†] Based on the most recent National Pollution Discharge Elimination System (NPDES) permit and U.S. Census Bureau, 2020 American Community Survey block data

* Wastewater treatment plant does not measure pH in raw influent

Table S4: Langmuir and Linear isotherm parameters for SARS-CoV-2, RSV-A, RV-B, and MS2 into wastewater solids and their respective average relative error (ARE).

Virus	Temp. (°C)	Langmuir model			Linear model	
		K_L (g·ml ⁻¹)	q_{max} (cp·g ⁻¹)	ARE	K_d (g·ml ⁻¹)	ARE
SARS-CoV-2	4	4.5×10^{-4}	2.6×10^7	0.52	7,460	0.45
	22	1.0×10^{-3}	9.5×10^7	0.94	46,044	3.83
RSV-A	4	6.2×10^{-4}	2.6×10^8	3.72	136,809	2.97
	22	2.6×10^{-3}	2.5×10^8	3.76	425,372	4.49
RV-B	4	3.3×10^{-4}	2.3×10^7	0.15	3,396	0.41
	22	4.0×10^{-6}	1.6×10^7	0.73	1,583	0.99
MS2	4	4.1×10^{-5}	6.1×10^7	0.65	1,260	0.42
	22	3.3×10^{-5}	1.1×10^7	0.68	148	0.93

Table S5: Background concentration of endogenous of SARS-CoV-2, RSV, RV, and F+ coliphage/MS2 in wastewater influent samples stored at 4°C and 22°C

Virus	4°C experiment		22°C experiment	
	Conc. in liquid fraction (cp/ml)	Conc. in solid fraction (cp/g)	Conc. in liquid fraction (cp/ml)	Conc. in solid fraction (cp/g)
SARS-CoV-2	0.50	13,451	0.33	93,752
RSV	0.67	4,208	0.97	22,190
RV	0.88	90,729	0.87	22,580
F+ coliphage/ MS2	0.22	28,731	0.25	4,121

Environmental Microbiology Minimum Information Checklist

Study Description

Study: SCAN
Date: March 2023
Completed by: Alexandria Boehm

Environmental Sampling

Described in methods section

Sample Treatment

Performed
 No sample treatment performed

Sample Reduction

Performed
Centrifugation was used, as described in the methods

Nucleic Acid Extraction

Methods provided in the paper.

Reverse Transcription

Performed
One Step RT-PCR

PCR Detection

qPCR dPCR
All methods provided

Analysis

Provided in methods

Control Checklist

	Environmental Sampling	Sample Treatment	Sample Reduction	Nucleic Acid Extraction	Reverse Transcription	PCR Detection	
Step performed	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Step has control info	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Negative Controls
# control replicates				5	3	3	
Control result reported	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Data handling reported	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Control introduced	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Positive Controls
Internal/External	N/A	N/A	Internal	External	External	External	
Independent/Parallel	N/A	N/A	Parallel	Independent	Independent	Independent	
Step has control info	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
# control replicates				5	3	3	
Control result reported	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Data Handling reported	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	

Process Checklist

Environmental Sampling

- Sampling Procedure
- Number of samples
- Sample amount, mean, range
- Sampling locations, dates, times

Sample Reduction

- Performed
- Reduction procedure
- Reagents
- Concentration Factor

Nucleic Acid Extraction

- Extraction procedure
- Amount extracted, amount obtained
- Extract storage conditions

qPCR or dPCR

- Target gene name, amplicon length
- Thermocycling temperatures and times
- Master mix: composition, vendors, concentrations
- Additives: vendors, concentrations
- Template amount added, pre-treatment (if any)
- Primers: sequences, concentrations, vendors, references
- Amplicon confirmation method (probe, melt curve, etc)
- Probe sequence, concentration, vendor, reference
- Instrumentation
- Equivalent volume of sample analyzed by PCR
- Inhibition assessment procedure
- Inhibition control description (if used)
- Number samples tested and found inhibited

Reverse Transcription

- Performed
- One or two step
- cDNA storage conditions (if two step)
- Reaction temperatures and times
- Reaction reagents and concentrations
- Priming method
- Reaction volume, added template amount
- Inhibition assessment procedure
- Inhibition control description (if used)
- Number samples tested and found inhibited

Analysis – dPCR

- Threshold settings
- Technical replicates, number, well merging
- Partitions measured, number, mean, variance
- Partition volume
- Target copies per partition, mean, variance
- Program used for dPCR analysis
- Explanation of control results, example plots

Analysis – qPCR

- Method for handling failed negative controls
- Technical replicates, number, calculations
- Calibration standards: description and source
- Method of quantifying standards
- Calibration curve slope
- Calibration curve R2
- Lowest standard measured or 95% LOD
- Cq value determination method

Figure S1. EMMI¹ checklist for reporting.

S7

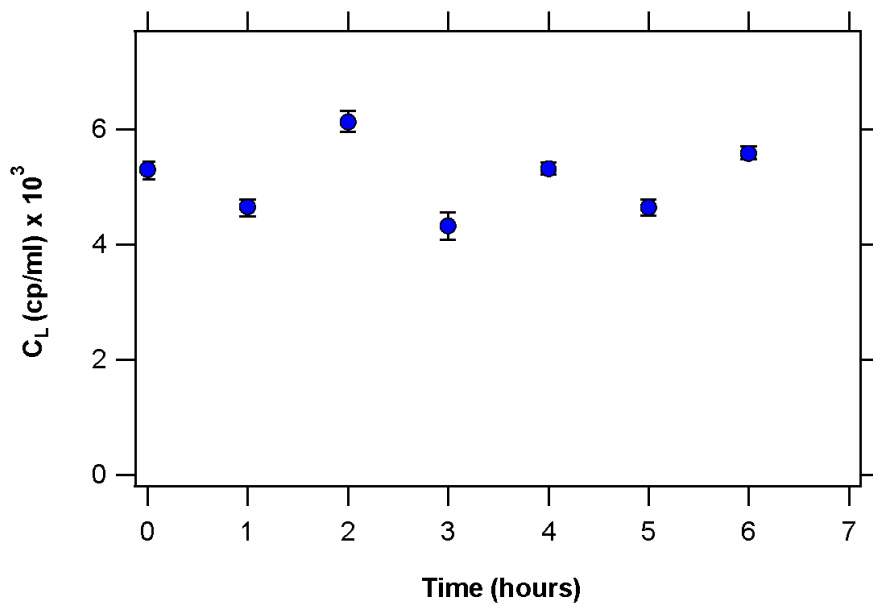


Figure S2: MS2 RNA concentration in liquid fraction at $t=0,1,2,3,4,5,$ and 6 hours. Error bars represent the 68% confidence interval from the ddPCR. Background concentration: ND

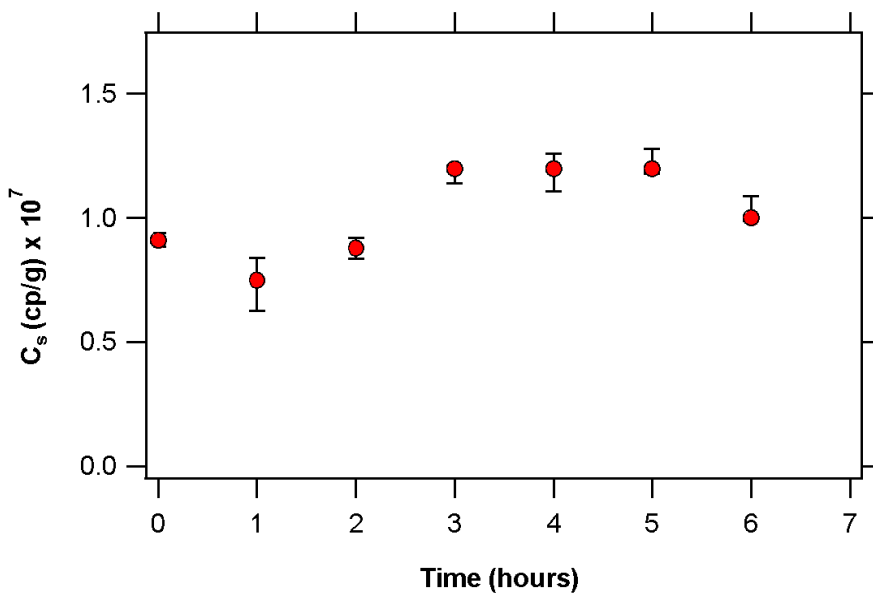


Figure S3: MS2 RNA concentration in solid fraction at $t=0,1,2,3,4,5,$ and 6 hours. Error bars represent the 68% confidence interval from the ddPCR. Background concentration $\sim 3.6 \times 10^4$ gc/g dry.

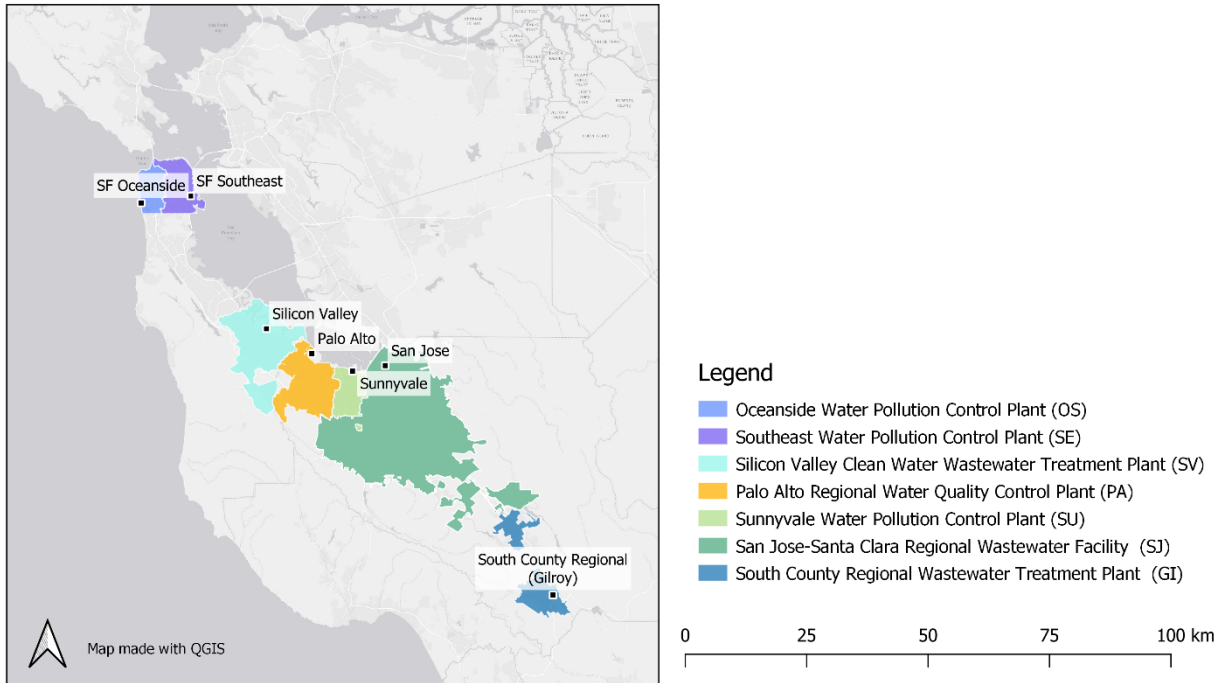


Figure S4: Outline of wastewater treatment plant service area (sewersheds)

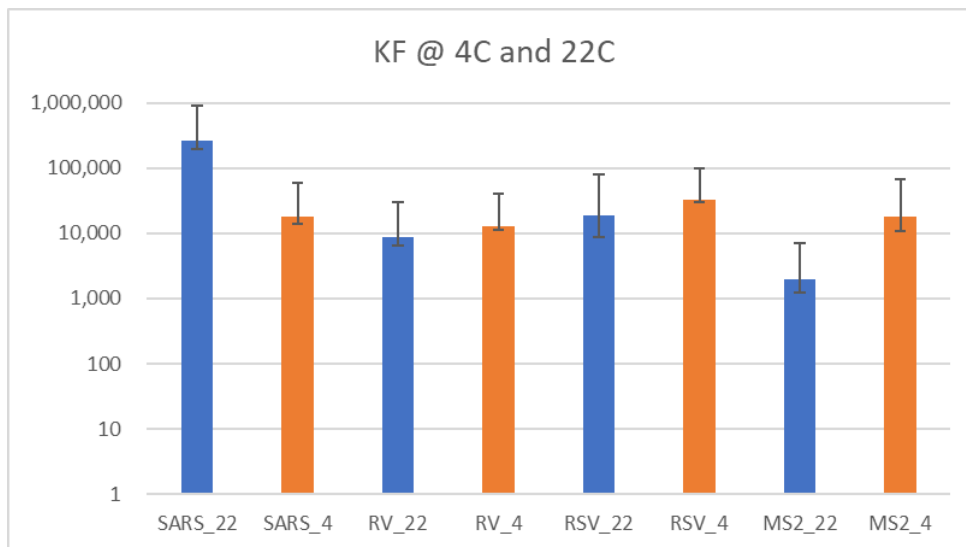


Figure S5: Partition coefficient (K_F) for SARS-CoV-2, RV, RV, and MS2 in wastewater influent samples stored at 4°C and 22°C. Standard errors (SE) were obtained from the linear regression of the Freundlich model in R (summary function).

References

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- (6) Decaro, N.; Elia, G.; Campolo, M.; Desario, C.; Mari, V.; Radogna, A.; Colaianni, M. L.; Cirone, F.; Tempesta, M.; Buonavoglia, C. Detection of Bovine Coronavirus Using a TaqMan-Based Real-Time RT-PCR Assay. *Journal of Virological Methods* **2008**, *151* (2), 167–171. <https://doi.org/10.1016/j.jviromet.2008.05.016>.