SUPPORTING INFORMATION

Partitioning of Viruses in Wastewater Systems and Potential for Aerosolization

*Mari Titcombe Lee, Amy Pruden, Linsey C. Marr**

Department of Civil and Environmental Engineering, Virginia Tech, 418 Durham Hall, Blacksburg, VA 24061, United States

*Corresponding author phone: (540) 231-6071, fax: (540) 231-7916, e-mail: lmarr@vt.edu

This supporting information document contains the following information:

- Comparison of virus properties
- Sludge properties
- Details about containers
- Schematic of virus, sludge, and material combinations.
- Primers and qPCR conditions
- Experimental controls
- Mass balance on virions
- Partitioning of viruses in each fraction by percentage

Number of pages: 6

Number of figures: 2

Number of tables: 5

Table S1. Characteristics of Ebola virus and surrogates MS2 and Phi-6.

virus	genome type	genome size (kb)	enveloped?	capsid structure	dimensions
Ebola	(-)ssRNA	19	yes	helical (filamentous)	diameter: 80 nm length: 800-14000 nm
MS2	(+)ssRNA	3.5	no	icosahedral (spherical)	27-34 nm
Phi6	dsRNA	13.5	yes	icosahedral (spherical)	75-85 nm

Table S2. Properties of real, anaerobically digested sludge at the wastewater treatment plant when we collected our sample.

property	value
pH	7.3
alkalinity	3880 mg L^{-1} as $CaCO_3$
volatile fatty acids	33 mg L^{-1}
total suspended solids	1.60%[a]
volatile suspended solids	1.20%[a]
temperature	35.6 °C

[a]Differs from the value appearing in the main document, which was for our specific sample.

Methods

Description of containers. We used 140 mL porcelain dishes (Fisher Scientific) that measured 8.1 cm in diameter across the top, 6.0 cm across the bottom, and 4.5 cm tall. We purchased polyvinyl chloride (PVC) pipe caps measuring 6.2 cm in diameter and 4.0 cm tall, with an interior surface area of 108 cm2, from a local hardware store. The autoclavable polypropylene jars (McMaster-Carr) had a capacity of 125 mL each and measured 7.0 cm in diameter and 6.0 cm tall. We fabricated concrete bowls from type I/II Portland cement, natural sand (passes #4, 4.76-mm sieve) as the fine aggregate, silica sand (mostly retained on #10, 2-mm sieve) as the coarse aggregate, and water reducer (Sika Plastocrete 161). The bowls measured 9.6 cm in diameter across the top, 7.4 cm across the bottom, and 2.4 cm tall. We allowed the bowls to cure for one month and applied an acrylic sealant (Quikrete 8730-02) before use.

Prior to each experiment, we cleaned all containers with ultrapure water, ethanol, and RNase Away and DNA Away (Thermo Scientific), and we autoclaved the porcelain, concrete and polypropylene bowls. PVC cannot be autoclaved, so we used new PVC pipe caps for each experiment.

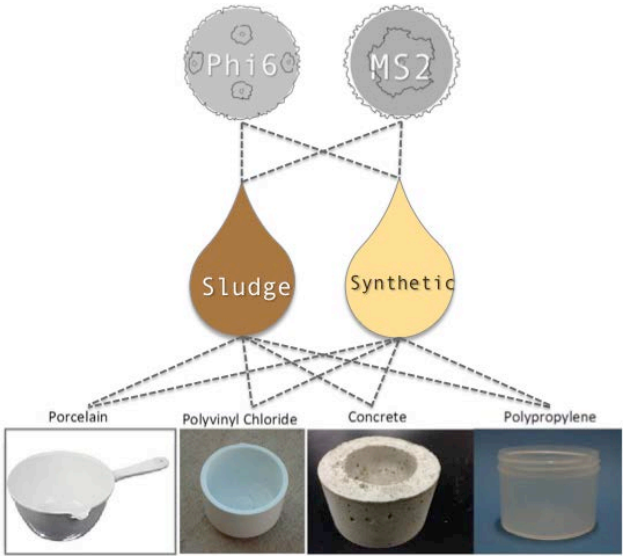

Figure S1. Schematic of virus, sludge, and material combinations investigated.

Table S3. Primers and probes used in qPCR.[1]

bacteriophage	purpose	sequence
MS2	forward primer	5'-GTCCATACCTTAGATGCGTTAGC-3'
	reverse primer	5'-CCGTTAGCGAAGTTGCTTGG-3'
	probe	5'-ACGTCGCCAGTTCCGCCATTGTCG-3'
Phi6	forward primer	5'-TGGCGGCGGTCAAGAGC-3'
	reverse primer	5'-GGATGATTCTCCAGAAGCTGCTG-3'
	probe	5'-CGGTCGTCGCAGGTCTGACACTCGC-3'

Table S4. qPCR conditions on a Bio-Rad CFX96 real-time system and C1000 thermal cycler.

MS2 and Phi6 SYBR green	MS2 probe	Phi6 probe
hold at 94 °C for 3 min	hold at 94 °C for 3 min	hold at 94 °C for 3 min
40 cycles at 94 °C for 15 s	35 cycles at 94 °C for 15 s	40 cycles at 94 °C for 15 s
hold at 60 °C for 1 min	hold at 60 °C for 1 min	hold at 60 °C for 1 min
N/A	final melt ramp from 65 °C to 95 °C with a 5-s hold at 65 °C and 0.5 °C/5 s ramp rate	final melt ramp from 65 °C to 95 °C with a 5-s hold at 65 °C and 0.5 °C/5 s ramp rate

Negative controls. We investigated the potential for RNA and DNA contamination from containers by mimicking the sampling procedure (sample volume, swabbing, contact time, etc.) using autoclaved, ultrapure water as a sample blank, in clean/autoclaved containers. We analyzed

sample blanks by RNA extraction, cDNA synthesis, and qPCR analysis as previously detailed. There was no amplification in the sample blanks after 35 PCR cycles.

We investigated the potential for RNA and DNA contamination from all stock solutions, working fluids, and bacterial and viral stocks, individually, using the previously described RNA extraction and cDNA conversion methods. We sequenced the samples to verify specificity of primers and probes for each bacteriophage. We amplified the converted cDNA samples using the previously specified qPCR reaction mixtures and volumes (intercalating dye or probe-based method), with a modified PCR machine protocol that omitted the final melt curve temperature ramp. We immediately removed amplified products from the PCR machine and further processed them or stored them at 4 °C for no more than 2 h before further processing. We analyzed aliquots of amplified PCR products by gel electrophoresis to verify base pair length and check for DNA carry over. We measured DNA concentration (Qubit fluorometer (Invitogen)) to determine volume of amplification product required for Sanger sequencing. We then cleaned amplified products by enzymatic treatment in sterile, optically clear vials, using Affymetrix ExoSAP-IT®, and incubated according to product recommendations on a Bio-Rad thermocycler. We submitted enzymatically cleaned, amplified samples, and primer and probe aliquots, to the Virginia Tech Biocomplexity Institute Genomics Research Institute for Sanger sequencing. We evaluated resulting chromographs using 4Peak ABI chromatogram freeware and identified DNA sequences by NCBI Nucleotide BLAST. Detectable background concentrations of bacteriophages in working fluids and bacterial stock solutions were at least four orders of magnitude below experimental sample concentrations.

Results

Table S5. Number of virions (median PFU equivalents measured by qPCR) recovered from each fraction (liquid, solids, surface) and total across all fractions for each combination of virus, sludge, and material surface. In each case, the amount seeded into the sludge was 10^8 PFU.

	porcelain	**concrete**	**PVC**	**polypropylene**
MS2 in synthetic sludge				
liquid	1×10^9	8×10^8	7×10^8	9×10^8
solids	6×10^6	1×10^7	1×10^7	7×10^6
surface	3×10^6	6×10^6	3×10^6	3×10^6
total	1×10^9	8×10^8	7×10^8	9×10^8
Phi6 in synthetic sludge				
liquid	2×10^9	6×10^9	1×10^9	6×10^8
solids	4×10^7	9×10^7	5×10^7	2×10^7
surface	9×10^7	9×10^6	1×10^6	1×10^6
total	3×10^9	6×10^9	1×10^9	6×10^8
MS2 in real sludge				
liquid	4×10^8	2×10^8	5×10^9	2×10^8
solids	2×10^5	2×10^5	4×10^5	1×10^4
surface	4×10^6	2×10^4	8×10^6	1×10^7
total	4×10^8	2×10^8	5×10^9	2×10^8
Phi6 in real sludge				
liquid	4×10^{10}	2×10^{10}	7×10^{11}	3×10^{11}
solids	1×10^7	1×10^8	1×10^6	3×10^6
surface	1×10^7	4×10^8	4×10^8	6×10^9
total	4×10^{10}	2×10^{10}	7×10^{11}	3×10^{11}

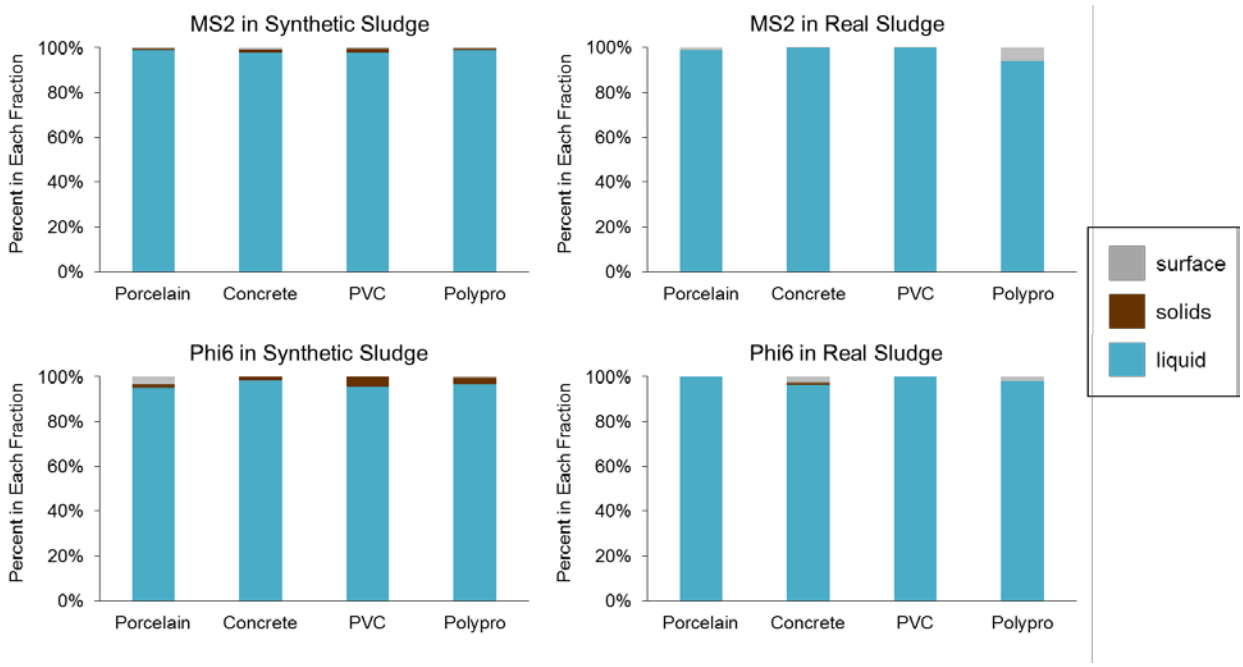

Figure S2. Partitioning of MS2 and Phi6 in synthetic sludge and anaerobically digested sludge between liquid, wetted solids, and material surface of porcelain, concrete, PVC, and polypropylene (polypro), in terms of percent of total virus recovered, based on median concentrations in each fraction.

References

1. Gendron, L.; Verreault, D.; Veillette, M.; Moineau, S.; Duchaine, C., Evaluation of Filters for the Sampling and Quantification of RNA Phage Aerosols. *Aerosol Sci. Technol.* **2010,** *44*, 893-901.