SUPPLEMENTAL DATA

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Viral Surrogates in Potable Reuse Applications: Evaluation of a Membrane Bioreactor and Full Advanced Treatment

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Running title: Viral surrogates in potable reuse applications

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Figure S1. Flow diagram of the full-scale membrane bioreactor (MBR) facility in Nevada. As denoted by the red stars, samples were collected after (1) fine screening (i.e., MBR feed) and (2) membrane filtration (i.e., MBR filtrate).

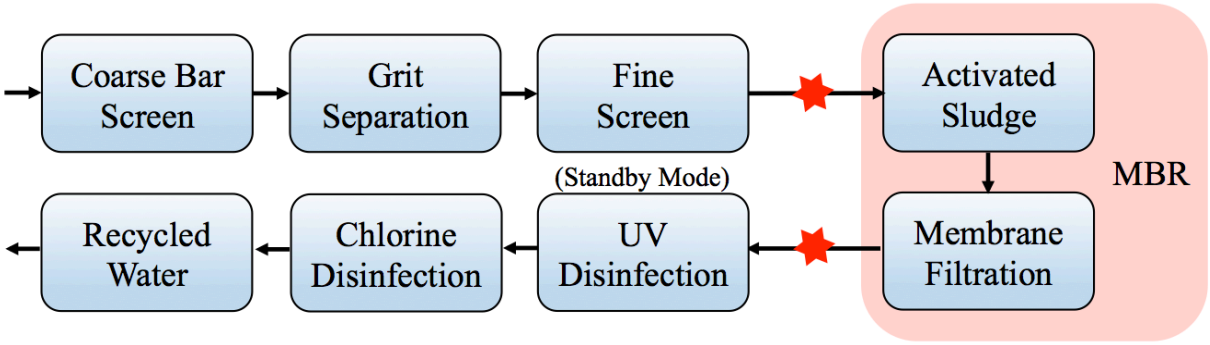

Figure S2. Flow diagram of the full-scale water reclamation facility and associated demonstration-scale potable reuse facility in California. As denoted by the red stars, samples were collected after (1) tertiary treatment at the full-scale water reclamation facility (i.e., demo feed), (2) ozonation, (3) biological activated carbon, (4) ultrafiltration, (5) reverse osmosis (as permeate), (6) reverse osmosis (as concentrate), and (7) the UV advanced oxidation process.

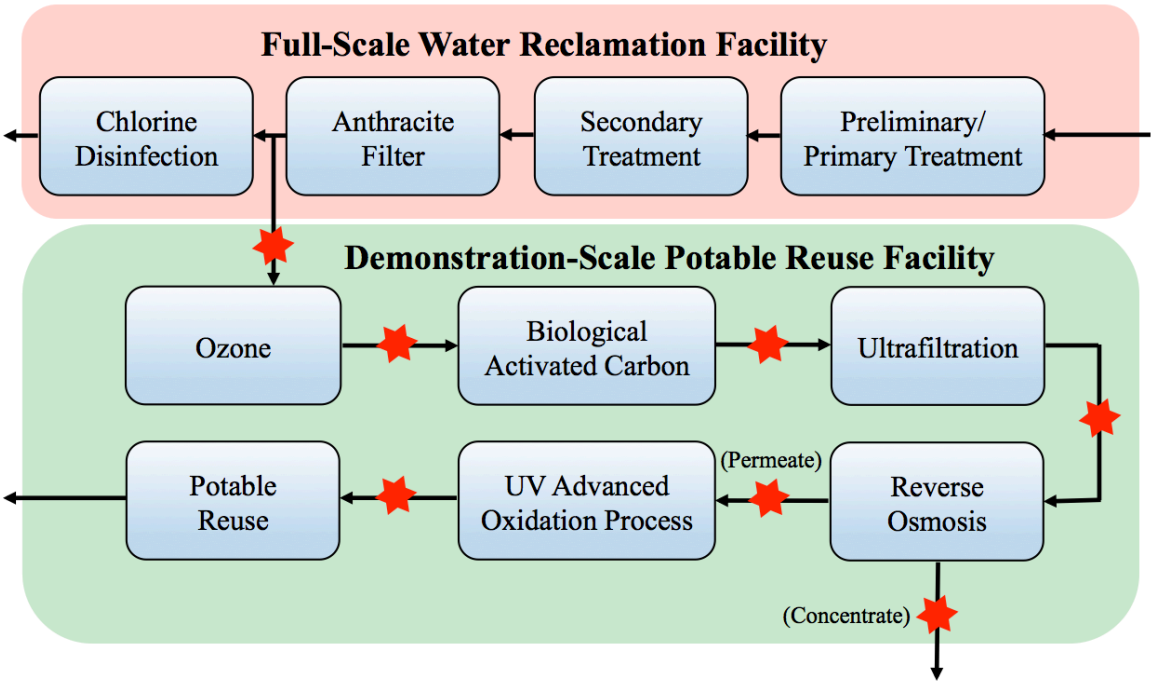

Figure S3. (Top) Gene block fragment serial dilutions used to develop the (bottom) standard curves for each assay. Data were omitted from the standard curves for inconsistent amplification, lack of amplification, or loss of linearity (i.e., plateauing). These standard curves were used to determine the limits of quantification (LoQs) for each qPCR assay (Table S4), as described in the main text. Similar standard curves were generated for independent qPCR runs.

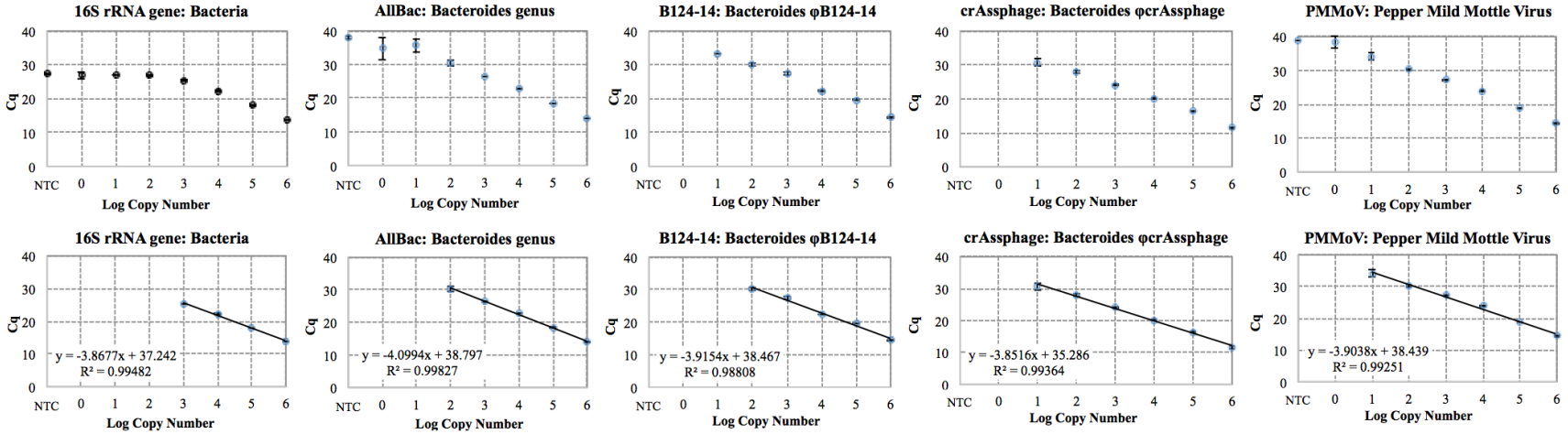

Figure S4. Correlation between the concentrations of (red) φB124-14 and (black) φcrAssphage with host *Bacteroides* in the MBR feed collected in (top) April of 2018, (middle) June of 2018, and (bottom) all samples combined. One outlier was omitted from the φcrAssphage dataset for April.

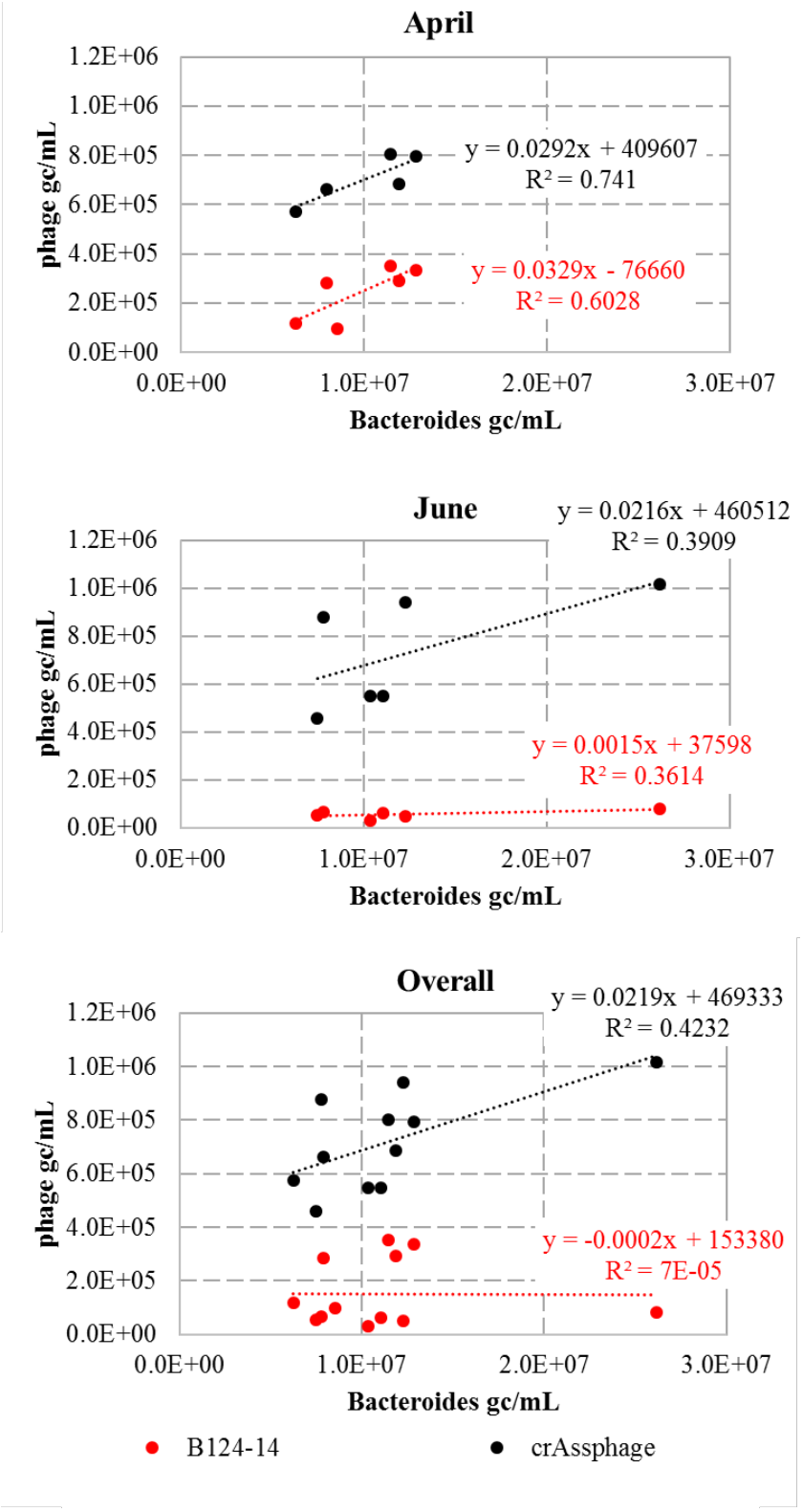

Table S1. List of manufacturer specifications and operational parameters for each treatment process at the MBR and FAT facilities.

Facility	Process	Specifications/Operational Parameters
MBR	MBR	Solids retention time = 8-10 days Nitrification/partial denitrification Model = ZeeWeed 500D Material = Polyvinylidene difluoride (PVDF) Pore size = 0.04 μm[1] Module surface area = 34 m^2 Flow configuration = Outside-in
FAT	Ozone	Manufacturer = Wedeco O_3/TOC = 0.93 CT = 1.5 mg-min/L
	BAC	GAC = Calgon Carbon F300 Condition = Exhausted toward TOC Bed depth = 2 m EBCT = 15 min
	UF	Model = Toray HFU-2020 Material = Polyvinylidene difluoride (PVDF) Pore size = 0.015 μm / MWCO = 150 kDa Module surface area = 72 m^2 Flow configuration = Outside-in Flux > 85 L/m^2-h Recovery > 95%
	RO	Two-stage configuration: Membrane Model = Hydranautics ESPA2 LD Salt rejection = 99.6% Three-stage configuration: Membrane Model = Toray TML20-400 Salt rejection = 99.7% Recovery = 75-80%
	UV AOP	Model = Trojan UVPhox (low pressure UV) System power = 18.0 kW Electrical energy = 0.079 kWh/m^3 Hydrogen peroxide dose = 3 mg/L

[1]No molecular weight cutoff (MWCO) listed

Table S2. Summary of sample collection details for the full-scale MBR facility.

Sample Location[a]	Collection Date	Collection Time	Number of Replicates	Procedure
MBR feed	April 24, 2018	~9:30 am	2	Each sample was collected directly into a 10-L Nalgene carboy at the facility's designated collection ports.
MBR filtrate	April 24, 2018	~9:50 am	2	
MBR feed	June 05, 2018	~8:15 am	2	
MBR filtrate	June 05, 2018	~8:35 am	2	

[a]See Figure S1 for depiction of sample locations

Table S3. Summary of sample collection details for the demonstration-scale FAT facility.

Sample Location[a]	Collection Date	Collection Time	Number of Replicates	Procedure
Demo Feed	July 10, 2018	9 am – 12 pm	2	Each sample was collected directly into a 10-L Nalgene carboy at the facility's designated collection ports.
Ozone Effluent			2	
BAC Effluent			2	
UF Filtrate			2	
RO Permeate			2	
RO Concentrate			2	
UV AOP Effluent			2	

[a]See Figure S2 for depiction of sample locations

Table S4. Summary of limits of quantification (LoQs; gc/µL of template) for the qPCR assays in the current study. LoQs were calculated using two different approaches: (1) the U.S. Environmental Protection Agency approach with 99% confidence or (2) a standard t test approach with 95% confidence. Bold font indicates the LoQs that were actually selected for the current study.

qPCR Assay	LoQ[a] (gc/µL)	LoQ[b] (gc/µL)
16S rRNA gene	**643**	157
AllBac	719	**175**
B124-14	305	**75**
crAssphage	11	**3**
PMMoV	30	**7**

[a]MDL = ts (99% confidence)

[b]MDL = ts/$\sqrt{N}$ (95% confidence)

Table S5. Human pathogen and coliphage occurrence at the MBR study site as part of a previous virus monitoring study. Adapted from Erdal and Vorheis (2015).

Virus in MBR Feed	**Units**	**Normal Condition 1**	**Normal Condition 2**	**Cleaning w/ Hypochlorite**	**Cleaning w/ Citric Acid**	**Increased Flux**
Adenovirus	gc/mL	6.7E+02	5.9E-01	3.8E+04	2.0E+00	3.4E+02
Norovirus GI	gc/mL	8.1E+00	<1.0e-03	1.0E+03	<1.0e-03	1.3E+04
Norovirus GII	gc/mL	2.6E+01	<1.0e-03	2.0E+02	<1.0e-03	1.3E+01
MS2 Coliphage	PFU/mL	1.9E+01	4.4E+01	2.4E+01	1.8E+01	2.1E+01
Somatic Coliphage	PFU/mL	1.6E+01	6.2E+01	1.9E+01	1.5E+01	2.3E+01
Virus in Filtrate	**Units**	**Normal Condition**	**Normal Condition**	**Cleaning w/ Hypochlorite**	**Cleaning w/ Citric Acid**	**Increased Flux**
Adenovirus	gc/mL	<5.0e-03	<1.0e-03	1.0e-03	<1.0e-03	<1.0e-03
Norovirus GI	gc/mL	<5.0e-03	<1.0e-03	<1.0e-03	<1.0e-03	<1.0e-03
Norovirus GII	gc/mL	<5.0e-03	<1.0e-03	<1.0e-03	<1.0e-03	<1.0e-03
MS2 Coliphage	PFU/mL	<1.0e-02	<1.0e-02	5.0e-02	2.1e-01	4.0e-02
Somatic Coliphage	PFU/mL	<1.0e-02	<1.0e-02	3.0e-02	7.0e-02	<1.0e-02
Log Removal Values	**Units**	**Normal Condition**	**Normal Condition**	**Cleaning w/ Hypochlorite**	**Cleaning w/ Citric Acid**	**Increased Flux**
Adenovirus	Log_{10}	>5.1	>2.8	7.6	>3.3	>5.5
Norovirus GI	Log_{10}	>3.2	N/A	>6.0	N/A	>7.1
Norovirus GII	Log_{10}	>3.7	N/A	>5.3	N/A	>4.1
MS2 Coliphage	Log_{10}	>3.3	>3.6	2.7	1.9	2.7
Somatic Coliphage	Log_{10}	>3.2	>3.8	2.8	2.3	>3.4