1 Supplementary Information - Reduction of nutrients, microbes, and personal care products in
2 domestic wastewater by a benchtop electrocoagulation unit

3 E.M. Symonds[a,*], M.M. Cook[a], S.M. McQuaig[b], R.M. Ulrich[a], R.O. Schenck[a], J.O. Lukasik[c],
4 E.S. Van Vleet[a], M. Breitbart[a]

5 [a] *University of South Florida, College of Marine Science, 140 7th Avenue South, St. Petersburg,*
6 *Florida, USA*
7 [b] *St. Petersburg College, 2465 Drew Street, Clearwater, Florida, USA*
8 [c]*BCS Laboratories, Inc., 4609-A NW 6th Street, Gainesville, Florida, USA*
9
10
11 * *Corresponding author. esymonds@mail.usf.edu. +1 (727) 553-3357*

12 **Methods**

13 ***Bacterial cultures and spores***

14 *Enterococcus faecalis* ATCC-29212™ and *Escherichia coli* strain C600, grown

15 separately overnight in Nutrient Broth (Difco™, Franklin Lakes, NJ, USA), were added to

16 domestic wastewater samples to yield an approximate final concentration of 10^6 colony forming

17 units (cfu)/ml prior to EC treatment. *Bacillus subtilis* spores were also added to the

18 aforementioned samples to reach an approximate final concentration 10^5 cfu/ml. The *B.subtilis*

19 spores were cultivated one week prior to the experiment from a pure broth culture of *B.subtilis*

20 ATCC-19659™ per Standard E2197-11 of ASTM International [1]. Briefly, the *B.subtilis* culture

21 was inoculated into five liters of 1:10 diluted Columbia Broth (Neogen Inc., Lansing, MI, USA)

22 supplemented with 0.1 mM MnSO4•4H2O. The culture was examined microscopically to verify

23 that >95% of the cells were in the endospore state. The suspension was then heated to 75°C

24 while agitating for 15 min in a water bath to kill vegetative cells and immediately cooled in an

25 ice bath. Spores were enumerated by diluting in phosphate-buffered water (Weber Scientific,

26 Hamilton, NJ, USA), spread plating onto Tryptic Soy Agar (TSA, Neogen, MI) and incubating at

27 36.5°C for 24 hours.

28

29 ***Viral cultures***

30 Domestic wastewater samples were augmented with pure cultures of human JC

31 polyomavirus (HPyV) ATCC-VR-1583™ and pepper mild mottle virus (PMMoV; provided by

32 Scott Adkins, USDA) to reach an approximate final concentration of 10^6 virus qPCR targets/ml

33 and 10^8 virus qPCR targets/ml, respectively. A purified culture of male-specific (F+) MS2

34 bacteriophages (ATCC-15597-B1™) was also added to reach approximate final concentrations

35 of 1.01×10^4 plaque forming units (pfu)/ml. The purified MS2 bacteriophage culture was prepared
36 following the US EPA method 1602 double-agar layer (DAL) protocol for spiking suspensions
37 with MS2 bacteriophages [2]. Minor modifications were made to recover the MS2 bacteriophage
38 culture. Unlike the EPA method 1602 DAL protocol, 10 ml of tryptic soy broth was added to
39 DAL petri plates and plates were incubated at room temperature for 1 hr. The MS2
40 bacteriophage culture was subsequently recovered using serological pipettes, filtered through a
41 0.22-μm EMD Millipore Sterivex filter (EMD Millipore, Billerica, MA, USA), quantified to
42 have a concentration of 4.4×10^6 pfu/ml, and stored at 4°C prior to the experiment.
43

44 ***Molecular analysis of human polyomavirus and pepper mild mottle virus***

45 Quantitative PCR (qPCR) and reverse transcription (RT)-qPCR were executed with
46 minor modifications for the analysis of HPyV [3] and PMMoV [4], respectively. Recombinant
47 plasmids, containing either the HPyVs qPCR-target DNA or the PMMoV RT-qPCR-target, were
48 diluted over 5 orders of magnitude to final concentrations ranging from 10^2 to 10^6 targets per μl
49 (e.g. five-point dilution series). Five microliters of each serial dilution were used as target in the
50 HPyV standard curve reactions. HPyV qPCR reactions were prepared by combining 25 μl
51 TaqMan® Environmental Master Mix 2.0 no UNG (Life Technologies, Grand Island, NY,
52 USA), 0.5 μM of each primer (SM2: 5'-AGT CTT TAG GGT CTT CTA CCT TT-3' and P6: 5'-
53 GGT GCC AAC CTA TGG AAC AG-3'), 0.125 μM of the labeled probe (KGJ3: 5'-(FAM)-
54 TCA TCA CTG GCA AAC AT-(MGBNFQ)-3'), 5 μl of template DNA, and nuclease-free water
55 to achieve a final volume of 50 μl [3]. PMMoV qPCR reactions were prepared by combining 12.5
56 μl Taqman Environmental Mastermix 2.0 no UNG, 0.4 μM of each primer (F: 5'-GAG TGG
57 TTT GAC CTT AAC GTT TGA-3' and R: 5'-TTG TCG GTT GCA ATG CAA GT-3'), 125 nM

probe (5'-(FAM)- CCT ACC GAA GCA AAT G -(TAMRA)-3'), 2 µl of template cDNA, and nuclease-free water to achieve a final volume of 25 µl [4].

For both HPyV and PMMoV analyses, the virus-target concentration of each sample was analyzed in duplicate alongside a 1:10 dilution of sample template (to check for possible PCR inhibition, which was identified when the Cq value of the diluted sample was less than the undiluted sample), all process and extraction controls, no-template controls, and a duplicate standard dilution series with an ABI7500 Real Time PCR system (Life Technologies, Grand Island, NY, USA), according to previously published protocols [3,4]. For HPyV analysis, the qPCR temperature profile was 95°C for 10 min followed by 40 cycles of 95°C for 15 sec, 55°C for 20 sec, and extension at 60°C for 60 sec. The PMMoV qPCR temperature profile was 95°C for 10 min followed by 40 cycles of 95°C for 30 sec, 53°C for 60 sec, and extension at 60°C for 60 sec. All standard curves had regression coefficients >0.97 and qPCR efficiencies between 90 and 110% [5]. Aside from the DNA of one process control showing inhibition for the HPyV assay, no PCR inhibition was observed.

Molecular analysis of Enterococcus

Enterococcus IC-NASBA (Internal control nucleic acid sequence based amplification). An IC-NASBA assay, targeting a 136-bp region of the large subunit ribosomal RNA gene (23s rRNA) of *Enterococcus* spp. related to water quality, was used to determine concentrations of enterococci in augmented wastewater samples before and after EC treatment. The internal control (IC)-RNA was synthesized using *in vitro* transcript generation oligonucleotides (F: 5'-AAT TCT AAT ACG ACT CAC TAT AGG GAG AGA CCC GAA ACC ATG TGA TCT ACC CAT GTC CAG GTT GAA GGT GCG GTA AAA CGC ACT GGA GGA CCG AAC CCA

81 CGT ACG T-3' and R: 5'-TAT CTC CAA GTT CGT TTG GAA TTT CAT TGT CAC CAT
82 AAG CAG CCA CCC GCA CTT TTC AAC GTA CGT GGG TTC GGT CCT-3'), as previously
83 described [6], and was used to identify amplification inhibition. For each IC-NASBA reaction, the
84 NucliSENS EasyQ® Basic Kit (bio-Mérieux, Durham, NC, USA) was used according to
85 manufacturer's instructions with the following final concentrations: 80 mM KCl (optimized, data
86 not shown), 0.4 mM of each primer (F: 5'-GAC CCG AAA CCA TGT GAT CTA-3' and R: 5'-
87 AAT TCT AAT ACG ACT CAC TAT AGG GAG AAT ATC TCC AAG TTC GTT TGG A-3'),
88 and 0.1 mM of each molecular beacon (Enterococcus: 5'-[6-FAM]-CGA TCG GAT GAG GTG
89 TGG GTA GCG GAC GAT CG-[DABCYL]-3' and IC-RNA: 5'-[6-ROX]-CAT GCG TGG
90 CTG CTT ATG GTG ACA ATC GCA TG-[DABCYL]-3'). Before the addition of 2.5 µl
91 enzyme mix to create a total reaction volume of 10 µl, 2.5 µl of RNA template was added to 5 µl
92 of reagent mixture (primers, beacons, and 1×10^7 IC-RNA copies) and incubated for 2 min at
93 65°C. Using a NucliSENS EasyQ® analyzer (bio-Mérieux, Durham, NC, USA), NASBA
94 amplification and fluorescence detection occurred at 41°C for 90 min. To quantify enterococci
95 concentrations, *E. faecalis* 29212™ (ATCC, Manassas, VA, USA) whole-cell RNA extracts
96 were serially-diluted from 10^5 to 10^2 cells (4-points) and analyzed in triplicate. The TTP ratio
97 method was used to determine unknown enterococci concentrations from the standard dilutions
98 with a regression coefficient of $\geq$0.95 [6]. No amplification inhibition was observed.
99 ***Enterococcus qPCR.*** An abbreviated version of U.S.EPA Method 1611 was executed, in
100 which only the sections regarding the base TaqMan® (Life Technologies®, Carlsbad, CA) qPCR
101 *Enterococcus* assay were used [7]. Briefly, each TaqMan® qPCR *Enterococcus* reaction had a
102 total volume of 25 µl and contained 1 µM forward primer (5'-GAG AAA TTC CAA ACG AAC
103 TTG-3'), 1 µM reverse primer (5'-CAG TGC TCT ACC TCC ATC ATT-3'), 0.08 µM

104 TaqMan® probe ([6-FAM]-5'-TGG TTC TCT CCG AAA TAG CTT TAG GGC TA-3'-

105 [TAMRA]), 12.5 µl TaqMan® Universal master mix, 2.5 µl bovine serum albumin (2 mg/ml

106 stock), and 2 µl DNA template. Each sample was analyzed in triplicate with an Applied

107 Biosystems® 7500 Real-Time PCR System (Life Technologies®, Carlsbad, CA, USA)

108 following the previously published thermal profile (50°C for 2 min, 95°C for 10 min, 40x (95°C

109 for 15 sec followed by 60°C for 1 min)) [7]. The standard dilution series, derived from *E. faecalis*

110 29212™ DNA extracts, contained four points ranging from 10^2 to 10^5 targets per reaction, and

111 was simultaneously analyzed in triplicate alongside samples and no-template controls. The

112 concentrations of *Enterococcus* spp. were calculated based upon the regression analysis of the

113 standard dilution series, which had a regression coefficient of 0.998 and 101% efficiency. All no-

114 template controls were negative. The absence of PCR inhibition was assumed given the lack of

115 inhibition observed during IC-NASBA analyses and the comparable *Enterococcus* spp.

116 concentrations obtained from both methods.

117 **References**

118

119 1 American Society for Testing and Materials International. ASTM Standard E2197:
120 Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal,
121 Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical
122 Germicides. (ASTM International, West Conshohocken, PA, 2011).

123

124 2 U.S. Environmental Protection Agency. Method 1602: Male-specific (F+) and somatic
125 coliphage in water by single agar layer (SAL) procedure. (Publication No. EPA 821-R-
126 01-029, Office of Research and Development, Washington, D.C., 2001).

127

128 3 McQuaig, S. M., Scott, T. M., Lukasik, J. O., Paul, J. H. & Harwood, V. J. Quantification
129 of human polyomaviruses JC virus and BK virus by TaqMan quantitative PCR and
130 comparison to other water quality indicators in water and fecal samples. *Appl Environ*
131 *Microbiol* 75, 3379-3388 (2009).

132

133 4 Rosario, K., Symonds, E. M., Sinigalliano, C., Stewart, J. & Breitbart, M. Pepper mild
134 mottle virus as an indicator of fecal pollution. *Appl Environ Microbiol* 75, 7261-7267
135 (2009).

136

137 5 Bustin, S. A. *et al.* The MIQE guidelines: Minimum information for publication of
138 quantitative real-time PCR experiments. *Clin Chem* 55, 611-622 (2009).

139

140 6 Ulrich, R. M. *et al.* Detection and quantification of *Karenia mikimotoi* using real-time
141 nucleic acid sequence-based amplification with internal control RNA (IC-NASBA).
142 *Harmful Algae* 9, 116-122 (2010).

143

144 7 U.S. Environmental Protection Agency. Method 1611: Enterococci in water by Taqman®
145 quantitative polymerase chain reaction (qPCR) assay. (Publication No. EPA-821-R-12-
146 008, Office of Water, Washington, DC., 2012).