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Comparative effectiveness of membrane bioreactors, conventional secondary treatment, and chlorine and UV disinfection to remove microorganisms from municipal wastewaters

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ABSTRACT

Log removals of bacterial indicators, coliphage, and enteric viruses were studied in three membrane bioreactor (MBR) activated-sludge and two conventional secondary activated-sludge municipal wastewater treatment plants during three recreational seasons (May–Oct.) when disinfection of effluents is required. In total, 73 regular samples were collected from key locations throughout treatment processes: post-preliminary, post-MBR, post-secondary, post-tertiary, and post-disinfection (UV or chlorine). Out of 19 post-preliminary samples, adenovirus by quantitative polymerase chain reaction (qPCR) was detected in all 19, enterovirus by quantitative reverse transcription polymerase chain reaction (qRT-PCR) was detected in 15, and norovirus GI by qRT-PCR was detected in 11. Norovirus GII and Hepatitis A virus were not detected in any samples, and rotavirus was detected in one sample but could not be quantified. Although culturable viruses were found in 12 out of 19 post-preliminary samples, they were not detected in any post-secondary, post-MBR, post-ultraviolet, or post-chlorine samples. Median log removals for all organisms were higher for MBR secondary treatment (3.02 to >6.73) than for conventional secondary (1.53–4.19) treatment. Ultraviolet disinfection after MBR treatment provided little additional log removal of any organism except for somatic coliphage (>2.18), whereas ultraviolet or chlorine disinfection after conventional secondary treatment provided significant log removals (above the analytical variability) of all bacterial indicators (1.18–3.89) and somatic and F-specific coliphage (0.71 and >2.98). Median log removals of adenovirus across disinfection were low in both MBR and conventional secondary plants (no removal detected and 0.24), and few removals of individual samples were near or above the analytical variability of 1.2 log genomic copies per liter. Based on qualitative examinations of plots showing reductions of organisms throughout treatment processes, somatic

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coliphage may best represent the removal of viruses across secondary treatment in both MBR and conventional secondary plants. F-specific coliphage and *Escherichia coli* may best represent the removal of viruses across the disinfection process in MBR facilities, but none of the indicators represented the removal of viruses across disinfection in conventional secondary plants.

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1. Introduction

Membrane bioreactors (MBRs) are a wastewater treatment technology in which membranes are submerged in the activated-sludge tank to perform the critical solids-separation process that the clarifiers and tertiary process units perform in conventional secondary treatment activated-sludge plants. The use of MBR technology is increasing in the United States because the quality of effluent is higher and plant capacity can be increased by retrofitting the technology into existing conventional secondary treatment activated-sludge units.

An important component of wastewater treatment is to minimize exposure of the public to bacterial, viral, and protozoan pathogens. Ultrafiltration (pore sizes 0.02–0.1 μm) and microfiltration (pore sizes 0.1 μm–0.4 μm) MBR treatments are both able to effectively remove protozoa (4–15 μm) and bacteria (0.5–3 μm) from wastewater (Lesjean et al., 2011). Viruses, which are considerably smaller (0.02–0.08 μm), in theory may be able to pass through membranes based on size exclusion alone. Human enteric viruses include enteroviruses, adenoviruses, noroviruses, rotaviruses, and Hepatitis A virus, and they cause a wide range of diseases and symptoms. In spite of the potential public health risk, wastewater effluents are seldom monitored for enteric viruses. Instead, bacterial indicators, such as *Escherichia coli* (*E. coli*) and fecal coliforms, are the required microbial measures of effluents for wastewater discharge permits in the United States.

Enteric virus removal by membranes in wastewater matrices has been investigated in the past. In a bench-scale study, ultrafiltration achieved complete rejection of culturable enterovirus, but microfiltration resulted in from 91 to >99% removal of enterovirus (Madaeni et al., 1995). In wastewater treatment plant pilot studies, MBR treatment removed different types of microorganisms, including enteric viruses in some studies, more effectively than did conventional secondary treatment (Ottoson et al., 2006a; Oota et al., 2005; Zhang and Farahbakhsh, 2007). A few studies done at full-scale wastewater treatment plants reported removal of viruses at MBR plants (da Silva et al., 2007; Kuo et al., 2010; and Simmons et al., 2011). None of the MBR full-scale studies included a component in which removal of viruses was compared before and after chlorine or ultraviolet (UV) disinfection. To obtain data on disinfection, one must look to studies of conventional plants. Fong et al. (2010) and Katayama et al. (2008) reported that virus concentrations were not significantly different between conventional secondary treatment and chlorinated effluents.

Indicator organism removals, especially the bacterial indicators required for wastewater permits, do not necessarily represent the removal of viruses. Harwood et al. (2005) did not find a strong correlation between indicator organism concentrations, including coliphage, and enteric viruses in disinfected effluents from conventional plants. They were, however, able to correctly predict virus presence in 72% of samples by using a suite of fecal-indicator organisms and discriminate analysis. Coliphages have long been suggested for use as a surrogate for the presence of enteric viruses in water and wastewater samples (Sobsey et al., 1995).

In this study, samples were collected at five wastewater treatment plants and analyzed for bacterial indicators, coliphage, and enteric viruses. The objectives were to (1) compare pre-disinfection log removals of enteric viruses in MBR systems to removals found for conventional secondary processes, (2) compare the additional log removal of enteric viruses achieved by disinfection of activated sludge wastewaters at conventional secondary plants with those achieved by disinfection of MBR-treated wastewaters, and (3) assess the relations between log removals of indicator bacteria and coliphage in wastewater and identify which indicator or indicators best represent the removal of enteric viruses. To the best of our knowledge, this is the first study to address these objectives at both MBR and conventional secondary municipal-scale plants using a large sample set. A companion report contains analytical, sampling, data analysis, and quality-assurance/quality-control procedures (Francy et al., 2011) and establishes procedures for qualifying uncertainties in virus measurements and interpreting quality-control results for all organisms. Details of the sampling sites, wastewater treatment processes, effectiveness of treatment processes, and application of results in the industry will be presented in a future companion report.

2. Materials and methods

2.1. Sampling sites and sample collection

The five wastewater treatment plants included in this study were the following:

Plants 1 and 4 – two medium-sized Kubota™ (Osaka, Japan) system microfiltration MBR plants with UV disinfection and an average daily flow of 12,900 m^3/d (3.4 million gallons per day (mgd)).

Plant 2 – one small-sized Kubota system microfiltration MBR plant with UV disinfection and an average daily flow of 950 m^3/d (0.25 mgd).

Plant 3 – one small-sized conventional secondary plant with tertiary treatment and UV disinfection and an average daily flow of 950 m^3/d (0.25 mgd).

Plant 5 – one medium-sized conventional secondary plant with chlorine disinfection and an average daily flow of 18,900 m^3/d (5 mgd).

The membranes used at the MBR plants have a maximum membrane pore size of 0.4 micron, are composed of chlorinated polyethylene, and were hydrophilically modified during manufacturing.

Twenty-three regular and three quality-control (QC) sampling trips were conducted during the recreational seasons (May 1 through October 31) of 2008–2010, with four to seven trips to each plant. A total of 73 regular samples were collected. In northern Ohio, USA, where these plants are located, disinfection of effluents is required during the recreational season. During each regular sampling trip, samples were collected at key points within the treatment stream: (1) before conventional secondary or MBR treatment (post-preliminary), (2) after conventional secondary or MBR treatment (post-secondary or post-MBR), (3) after tertiary treatment but before disinfection (post-tertiary, plant 3 only), and (4) after disinfection (post-disinfection). Tertiary treatment removes more solids than secondary treatment does, and plant 3 provides tertiary treatment through sand filtration. Compensation was made for detention time between sample points to ensure collection of the approximate same slug of water. Both filtration and fixed-interval grab sampling techniques were used.

For bacterial indicators and coliphage in all types of wastewater, 6-L grab samples were collected. For enteric viruses in post-preliminary wastewater, 4-L grab samples were collected. Grab samples were composited by collecting 1 L every 10 min for 40–60 min. For enteric viruses in all other types of wastewater (post-MBR, post-secondary, post-tertiary, and post-disinfection), filtration was done onsite by use of glass-wool fiber filters (Lambertini et al., 2008). Details of filtration procedures are described in Francy et al. (2011). Briefly, samples were filtered through glass-wool fiber filters connected to sterile inlet tubing and a peristaltic pump adjusted to achieve a flow rate of 2–3 L/min until the required volume was collected (or for 3.5 h, whichever came first) yielding about 150 L for post-secondary samples and 500 L for post-MBR, post-tertiary, and post-disinfection samples. If the pH of the wastewater was greater than 7.0, it was adjusted to pH 6.5 to 7.0 by injecting 0.5 N HCl through a second inlet line. Pumped wastewater was collected in a glass beaker every 30 min, and the pH was checked with a pH meter in accordance with standard USGS protocols (Wilde, variously dated). Post-preliminary grab samples were pH-adjusted and filtered within 24 h of collection at the U.S. Geological Survey (USGS) Ohio Water Microbiology Laboratory in Columbus, Ohio ("Ohio Laboratory").

2.2. *Indicator bacteria and coliphage analysis*

Analysis of samples for the bacterial indicators was done by preparing serial dilutions for plating by standard membrane filtration methods. These methods included modified mTEC agar (U.S. Environmental Protection Agency, 2006a) for *E. coli*, mEI agar for enterococci (U.S. Environmental Protection Agency, 2006b), and mFC agar for fecal coliforms (Britton and Greeson, 1989). Appropriate volumes were based on sample type and ranged from 0.0003 mL for post-preliminary samples to 250 mL for post-disinfection samples. Results from membrane filtration analyses were recorded as colony-forming units per 100 mL (CFU/100 mL).

Analyses of samples for F-specific and somatic coliphage were done by use of the single agar layer (SAL) procedure (U.S. Environmental Protection Agency, 2001a). Antibiotic-resistant host-culture strains *E. coli* CN-13 (resistant to nalidixic acid) and *E. coli* F-amp (resistant to streptomycin and ampicillin) were used as hosts for somatic and F-specific coliphage, respectively. Appropriate volumes ranged from 1, 0.1, and 0.01 mL for post-preliminary samples to 100 mL for all other samples. The quantity of coliphage in a sample is expressed as plaque forming units per 100 mL (PFU/100 mL).

2.3. *Virus elution and concentration*

At the Ohio Laboratory, viruses were eluted from glass-wool filters by use of a beef extract and glycine solution and concentrated by polyethylene glycol (PEG) precipitation as previously described (Lambertini et al., 2008; Francy et al., 2011). The final concentrated sample volume (FCSV) – 10 mL for post-preliminary samples or 5 mL (2008) or 6 mL (2009–2010) for other wastewater samples – was aliquoted into several centrifuge tubes for storage or analysis. One tube with 4–6 mL, depending on the sample type, was sent to the U.S. Department of Agriculture/U.S. Geological Survey Laboratory in Marshfield, Wisconsin, USA ("Wisconsin Laboratory") for enteric virus analysis.

2.4. *Enteric viruses by cell culture*

The cell culture method was done at the Wisconsin Laboratory, based on procedures described previously (Borchardt et al., 2004; U.S. Environmental Protection Agency, 2001b; Francy et al., 2011). For screening, 0.33 mL of FCSV was inoculated into three cell lines: buffalo green monkey kidney cells (BGMK), rhabdomyosarcoma (RD) cells, and Human Caucasian colon adenocarcinoma (Caco-2) cells. The cultures were observed for up to 14 days for cytopathic effects (CPE) to evaluate on which cell line the viruses were most prolific.

If a positive result was obtained through screening, then culturable enteric viruses were quantified by most-probable number (MPN) using the most prolific cell line. For MPN, 30 wells were inoculated with 0.04 mL of FCSV: 10 with undiluted FCSV, 10 with FCSV diluted 1:5 with sterile 0.15 M Na_2HPO_4, and 10 with FCSV diluted 1:25, for a total inoculum in the 30 wells of 0.496 mL. If, after 14 days, the initial dilutions did not result in the appropriate mix of virus-positive and virus-negative wells for calculating the MPN, a new set of 30 wells was inoculated with different FCSV dilutions and the incubation process was repeated for another 14 days. MPNs were calculated on the basis of CPE. To confirm that the CPE was produced by an enterovirus, the cell lysate from one positive was analyzed for enteroviruses by qRT-PCR.

2.5. DNA extraction and enteric viruses analysis by qPCR or qRT-PCR

At the Wisconsin Laboratory, viral nucleic acids were extracted from 280 μL of FCSV with a QIAamp® DNA Blood Mini Extraction Kit and AVL Buffer (Qiagen, Valencia, California, USA) to yield a suspension of 50 μL in 2008 and 65 μl in 2009–2010. The qPCR/qRT-PCR assays are described in Lambertini et al. (2008), and the primers and probes for enterovirus, adenovirus, norovirus, rotavirus, and Hepatitis A virus are listed in Francy et al. (2011). Assays for adenovirus groups C, D, and F were performed on all samples (Cromeans et al., 2005). In addition, assays for adenovirus A and B were performed on samples collected in 2009–2010 (Susan K. Spencer, USDA/USGS Wisconsin Laboratory, oral commun., 2009). Quantitative PCR was performed on a LightCycler® 480 System (Roche Diagnostics Corporation, Indianapolis, Indiana, USA) with LightCycler 480 Software Version 1.5.0. Hepatitis G virus (HGV) armored RNA (Asuragen, Inc., Austin, Texas, USA) was used as a control to measure PCR inhibition; sample extracts were diluted if HGV results indicated that inhibition occurred.

2.6. Calculations for virus concentrations

The standard curves for molecular detection of enteric viruses were created by using virus stocks treated with Benzonase™ (Novagen, Madison, Wisconsin, USA), as described previously (Lambertini et al., 2008). After stocks were extracted, the amount of virus RNA or DNA was measured by using RiboGreen™ or PicoGreen™ (Molecular Probes, Eugene, Oregon, USA), and the number of genomic copies (gc) was calculated. After quantification, viral stocks were serially diluted and seeded into an unseeded FCSV made by filtering a dechlorinated tapwater sample, eluting with beef extract, and precipitating with PEG. Each standard point was extracted in duplicate and then tested by qPCR or qRT-PCR in duplicate once a quarter. Performance characteristics for each standard for each assay were previously presented (Francy et al., 2011). With every qPCR or qRT-PCR run, a reference control (low copy standard from the standard curve) was run and compared to the results of the quarterly standard curve (Lambertini et al., 2008). Samples were quantified for viruses by using standard curves, adjusted for effective volumes (Francy et al., 2011), and reported as gc/L.

To support more robust data interpretations, assay limits of quantification (A_{LOQ}), assay limits of detection (A_{LOD}), and sample reporting limits (SRL) were determined for virus results. Calculations for these values were based on those defined in Rajal et al. (2007) but applied specifically for the present study (Francy et al., 2011). In the present study, the A_{LOQ} was the lowest concentration of virus genomes that remained within the linear range of quantification; this was set to 1 gc for consistency. The A_{LOQ} for cell culture screening was set as the lowest concentration of viruses that could be identified by observing CPE and was assumed to be 1 virus. For cell-culture MPN, the A_{LOQ} was 2.18 MPN/mL, as determined by placing 1 virus in standard MPN tables (U.S. Environmental Protection Agency, 2001b). In the present study, the A_{LOD} was the minimum virus concentration that could be measured and reported with confidence that the concentration was greater than zero. The A_{LOQ}s for molecular virus assays and for cell culture screening were used as the A_{LOD}s. For cell-culture MPN, the A_{LOD} was calculated as 1.08 MPN by multiplying the A_{LOQ} times the total inoculum in 30 wells (2.18 MPN/mL × 0.496 mL). The A_{LOD} values were estimated values because they were not created according to established method-detection-limit procedures; actual sensitivity will need to be established in the future. The A_{LODs} were divided by effective sample volumes to determine SRLs. Effective sample volumes were much less than original sample volumes because subsamples were continuously removed through processing and analytical steps (Francy et al., 2011). The SRLs were different for each sample and assay because effective sample volumes were different for each sample and assay. Non-detects were reported as <SRL. Codes were used to further qualify the virus data and identify uncertainties associated with a virus measurement: (1) *E*∼ indicated that the value was estimated because qPCR or qRT-PCR duplicates did not agree, (2) Eb indicated that the value was estimated because it was extrapolated at the low end of the standard curve, and (3) *M* indicated that material was present but not quantified.

2.7. Quality assurance and quality control

Quality-assurance and quality-control procedures were an integral part of this study and are described in detail in the companion report (Francy et al., 2011). Both laboratory and field quality-control (QC) samples were included.

Field QC samples used to support data analysis and interpretation included field blanks and concurrent replicates for all organisms (Francy et al., 2011). Field blanks were 300 mL of sterile buffered water for bacteria and coliphage or 10 L of sterile, dechlorinated tapwater for viruses, filtered and processed in the same manner as regular samples. The results for bacteria, coliphage, and enteric viruses were below detection for all field blanks, indicating that it was unlikely that samples were contaminated from equipment or processing procedures. Field concurrent replicates for bacteria, coliphage, and viruses in post-preliminary wastewater were collected by collecting two grab samples using identical techniques. The collection sequence of the two replicate samples was alternated and each sample was composited into a separate bottle. Field concurrent replicates for viruses in all other wastewater matrices were collected by concurrently filtering a second wastewater sample onsite with a second filtration apparatus. For bacterial indicators and coliphage, the results from concurrent replicates indicated that concentrations <100 CFU or PFU/L can differ between replicates by as much as 1 log, whereas higher concentrations can differ by as much as 0.3 log. The results for concurrent replicates for viruses indicated that log differences between replicates can be as great as 1.2 log gc/L in the present study, regardless of the concentration of virus. Relatively large differences in molecular results for viruses between replicate pairs were likely due to lack of accuracy for samples with small effective volumes. These values were used to quantify analytical variability when interpreting log removal data.

To estimate virus recoveries through all processing and analytical steps and aide in interpretations of virus results,

six seeded matrix controls were collected from representative wastewater matrices during the recreational season of 2011. Samples were seeded with Mahoney enterovirus (U.S. Environmental Protection Agency, Cincinnati, Ohio, USA) and adenovirus (University of North Carolina at Chapel Hill, USA) to achieve target seed amounts of 10^5 and 10^6 viruses, respectively, estimated by use of a RiboGreen assay. Two 4-L samples of post-preliminary wastewater were seeded in the laboratory and then filtered. For other wastewater samples, all but the last 20 L were filtered onsite (120 L for post-secondary and 480 L for post-MBR and post-disinfection). The 20-L remaining volume was collected, transported to the laboratory, seeded, and filtered using the same filter that was used onsite for that sample. Unseeded wastewater samples were analyzed in conjunction with a seeded matrix control to determine background concentrations of viruses. Virus elution and concentration was done as described above. Subsequent DNA extraction and analytical steps were done at the Ohio Laboratory. These steps differed somewhat from those described for the Wisconsin Laboratory and included the changes noted in the following paragraph.

The FCSVs were analyzed for enterovirus by use of qRT-PCR as described in Gregory et al. (2006) and for adenovirus by use of qPCR as described in Jothikumar et al. (2005). PCR inhibition was determined by using matrix spikes. An extra duplicate qPCR or qRT-PCR reaction was run for each test sample where the master mix was seeded with extracted positive control virus. The concentration of target virus in the test sample was then compared to the concentration of target virus in the no-template control that also used the same seeded master mix. Sample extracts were considered inhibited and were diluted if the seeded test sample was >2 Ct cycles higher than the seeded no-template control.

2.8. Data analysis

Log removals for all organisms were calculated from the equation $a = c - b$, as follows:

$$(a)\ \text{Log removal across conventional secondary or MBR treatment} = (c)\ \text{log10 concentration post-preliminary} - (b)\ \text{log10 concentration post-secondary or post-MBR} \quad (1)$$

$$(a)\ \text{Log removal across disinfection} = (c)\ \text{log10 concentration post-secondary or post-MBR} - (b)\ \text{log10 concentration post-disinfection} \quad (2)$$

Calculations for bacterial indicator and coliphage log removals were fairly straightforward. First, data were multiplied by 10 and reported as log10 CFU/L (instead of CFU/100 mL) for better comparisons with virus data. A decision tree was established for calculating and reporting log removals (Fig. 1). In some cases, the log removal was reported as no removal detected ("NRD"); this value was later estimated to be zero log removal in summary statistics. In other cases, the log removal was reported as "not calculated because data values were inconclusive." Additional decision trees were needed

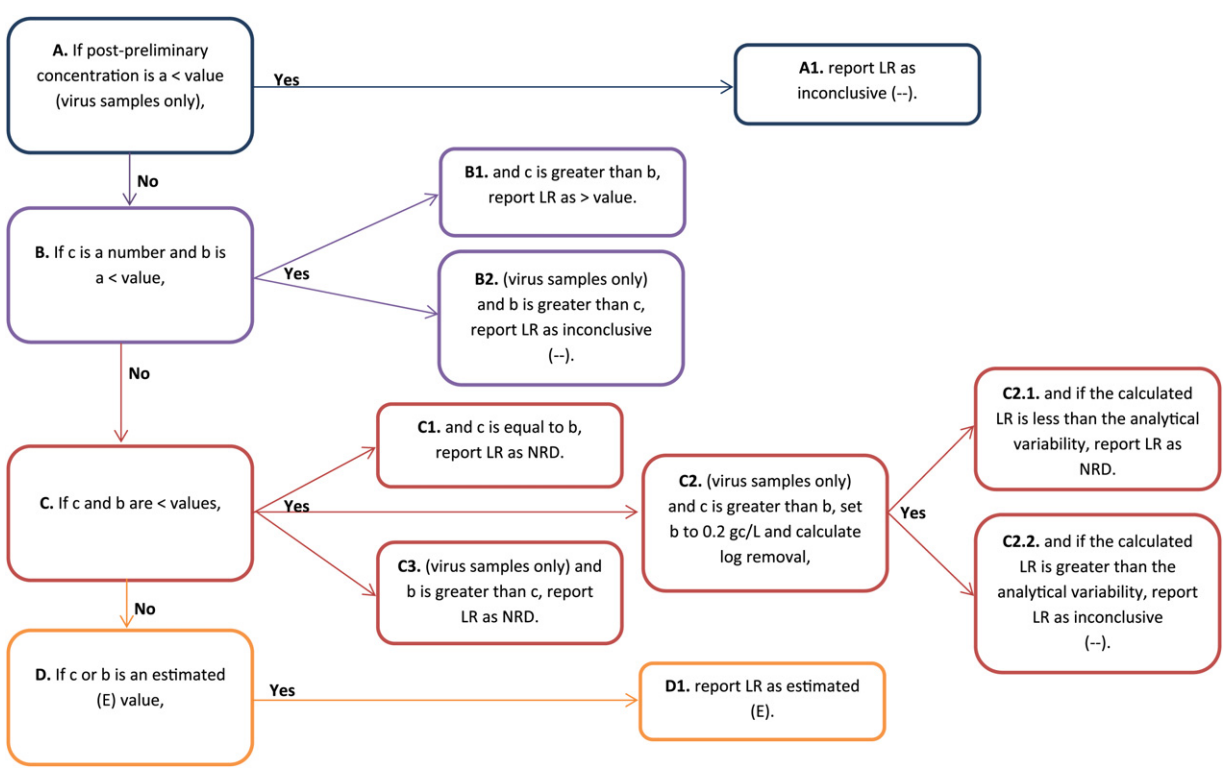

Fig. 1 – Log removal (LR) calculations and reporting rules (NRD, no removal was detected). Log removals were calculated as follows, with <values used in equations where appropriate: (*a*) $\log_{10}$ removal = (*c*) $\log_{10}$ concentration first treatment – (*b*) $\log_{10}$ concentration second treatment.

exclusively for virus data because calculations were complicated by multiple SRLs. Decision trees were established in an attempt to provide a balance between obtaining as much data as possible without making invalid assumptions.

The most complicated decision tree was followed for virus concentrations in which both *c* and *b* were <values and *c* was greater than *b* (Fig. 1, C.2). Two examples can be used for adenovirus by qPCR (Table S6 in supplementary materials, sample numbers 23 and 12) to illustrate the use of this section of the decision tree. For sample 23, adenovirus concentrations were <0.47 gc/L (−0.33 log gc/L) in post-MBR and <0.46 gc/L (−0.34 log gc/L) in post-disinfection samples. The post-disinfection concentration (*b* value) was then set to the lowest concentration of virus that was detected in any sample during this study (0.2 gc/L or −0.7 log gc/L). The calculation using Eq. (2) would then represent the highest log removal that could be detected as follows:

$$(-0.33\ \log\ \mathrm{gc/L}) - (-0.7\ \log\ \mathrm{gc/L}) = -0.37\ \log\ \mathrm{gc/L}$$

This result is less than the analytical variability (1.2 log gc/L), so the log removal was reported as NRD (Fig. 1, C.2.1). For sample 12, adenovirus concentrations were <43 gc/L (1.63 log gc/L) in post-secondary and <9.2 gc/L (0.96 log gc/L) in post-disinfection samples. Again, setting the *b* value to the lowest extreme, the highest log removal that could be detected would be calculated as follows:

$$(1.63\ \log\ \mathrm{gc/L}) - (-0.7\ \log\ \mathrm{gc/L}) = 2.33\ \log\ \mathrm{gc/L}$$

This result is greater than the analytical variability (1.2 log gc/L), so the log removal could range from no log removal to as great as 2.33 log removal using these values. The log removal was, therefore, reported as "not calculated because data values were inconclusive" (Fig. 1, C2.2).

Recoveries of viruses in seeded matrix controls were calculated from the following equation:

$$\text{Percent Recovery} = (\mathrm{Virus_R} - \mathrm{Virus_U})/\mathrm{Virus_S} * 100, \quad (3)$$

where $\mathrm{Virus_R}$ is the amount of virus, in gc, recovered in the eluate after seeding, filtration, and processing, adjusted for volume analyzed; $\mathrm{Virus_U}$ is the amount of virus, in gc, recovered in the eluate in unseeded samples after filtration and processing, adjusted for volume analyzed; and $\mathrm{Virus_S}$ is the original seed amount of virus, in gc.

3. Results

3.1. *Quantification of bacterial indicators, coliphage, and enteric viruses in wastewater*

Concentrations of bacterial indicators, coliphage, and enteric viruses are listed by plant for each sample in supplementary materials (Tables S1–S9). Data from Plant 2 were not included because the plant was not operating properly and corrective actions were not completed until after the sampling period (Francy et al., 2011).

Plots showing the distribution of concentrations of adenovirus, enterovirus, and norovirus GI by qPCR or qRT-PCR in wastewater samples are presented in Figs. 2–4. Detection percentages are presented in Table 1. Norovirus GII and Hepatitis A virus are not included because they were not detected in any samples. Similarly, rotavirus data are not presented because rotavirus was detected in only one sample but could not be quantified. For each organism, MBR and conventional secondary plants are presented separately to facilitate comparisons of concentrations and detections at different points in the treatment process by plant type. The plots also can be used to compare ranges of SRLs for non-detects (shown as open circles) to the reported concentrations (shown as solid circles). Viruses that were present but not quantified (M qualifier) are depicted with an open triangle with a dashed line extending to the *x* axis to signify the greater uncertainty associated with this reported concentration. Estimated values are marked with an "*E*" on the plots.

Adenovirus was detected in all 19 post-preliminary samples from both MBR and conventional secondary plants (Table 1), with concentrations ranging from 220 to 180,000 gc/L (Fig. 2, Table S6). Adenovirus was detected in 75% of conventional post-secondary samples but only 45% of post-MBR samples. Concentrations of adenovirus tended to be higher in post-secondary (<M1.7 to 120 gc/L) than post-MBR samples (0.32–19 gc/L). Adenovirus was detected in 75% of post-chlorine samples but in only 36% and 25% percent of post-ultraviolet disinfection samples for MBR and conventional secondary facilities, respectively. Most SRLs for the non-detects were on the low end of the range of detected sample concentrations.

Enterovirus by qRT-PCR was detected in the majority of post-preliminary samples (Table 1). Concentrations in post-preliminary samples ranged from 240 to 290,000 gc/L (Fig. 3, Table S7). SRLs for non-detects were high for post-preliminary samples, ranging from <550 to <5600 gc/L. For post-secondary, post-MBR, and post-UV samples, enterovirus was quantified in only one sample (post-MBR, 5.3 gc/L) and was detected but not quantified in four samples. Enterovirus was not detected in any of the four post-chlorine disinfection samples. For all wastewater types, SRLs for non-detects were in the same range or higher than the detected sample concentrations.

Norovirus GI by qRT-PCR was detected in over half of the post-preliminary samples (Table 1). Concentrations ranged from 49 to 18,000 gc/L (Fig. 4, Table S8). Detection concentrations of norovirus GI in post-preliminary samples were in the same range as SRLs for non-detects. There were only three detections of norovirus GI in post-secondary and post-MBR samples and all were *E* values. Norovirus GI was not detected in any post-UV samples and was detected in two post-chlorine samples.

Culturable viruses were detected in over half of post-preliminary samples (Table 1), at concentrations ranging from 3.9 to 163 MPN viruses/L (Table S9). Culturable viruses were not detected in any post-secondary, post-MBR, post-ultraviolet, or post-chlorine samples. Approximate SRLs for non-detects for each type of wastewater were <8 MPN viruses/L for post-preliminary, <0.15 for post-secondary, and <0.04 for post-MBR, post-UV, and post-chlorine samples (Table S9).

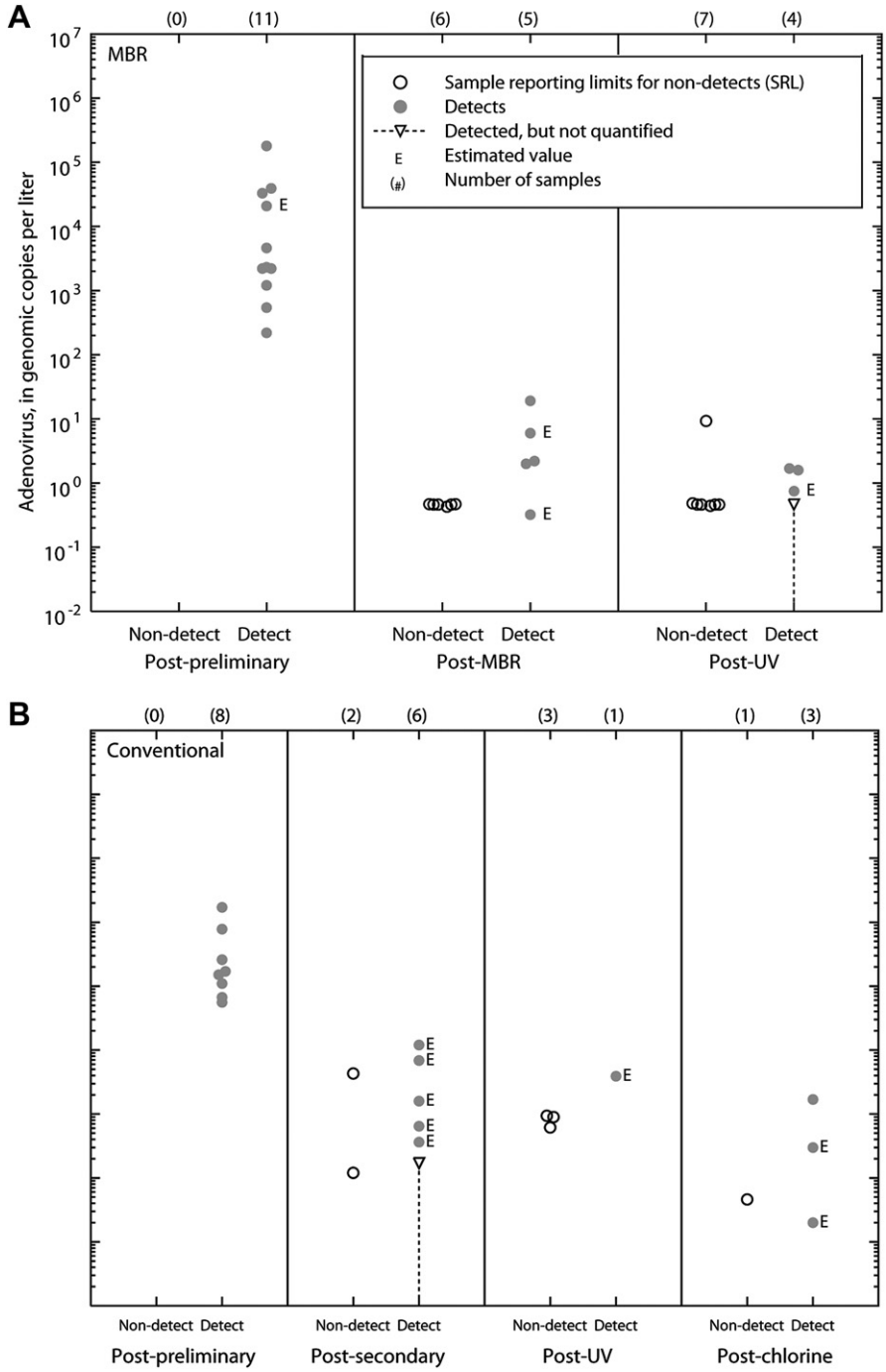

Fig. 2 – Concentrations of adenovirus in wastewater samples throughout treatment processes in MBR and conventional plants.

3.2. *Log removals of microorganisms in wastewater*

Log removals of bacterial indicators, coliphage, and enteric viruses across the different treatment processes are listed by plant for each sample in supplementary materials (Tables S1–S9). Summary statistics for log removals are presented in Table 2.

Median log removals for all organisms were higher for MBR than for conventional secondary treatment. Ultraviolet disinfection after conventional secondary treatment provided greater than 3–4 log removals for bacterial indicators and somatic coliphage but lower removals for F-specific coliphage (median = >1.17) and adenovirus (median = 0.24). Ultraviolet disinfection after MBR treatment typically provided little additional removal of any organism except for somatic coliphage (median = >2.18). Chlorine disinfection after conventional secondary treatment provided median log removals of bacterial indicators, coliphage, and adenovirus ranging from 0.71 to 2.57.

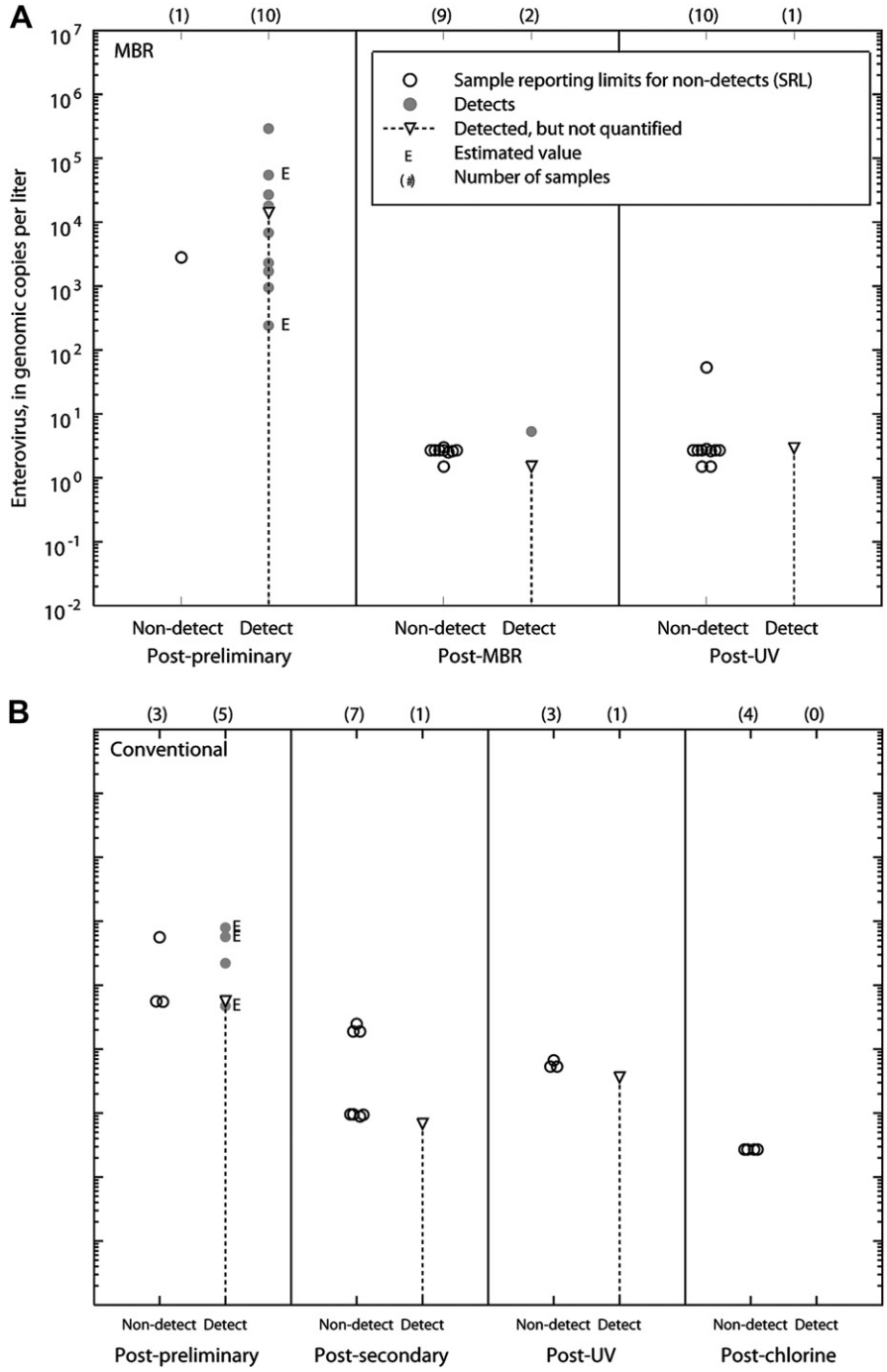

Fig. 3 – Concentrations of enterovirus in wastewater samples throughout treatment processes in MBR and conventional plants.

Many of the results on log removals of enteric viruses from disinfection after conventional secondary treatment were inconclusive, owing to the occurrence of non-detects with multiple reporting limits in both steps of the treatment process.

3.3. *Relations between removal of microbial indicators and viruses*

Plots were made to identify and compare concentration patterns of indicators and viruses throughout the treatment process in MBR plants and conventional secondary activated-sludge plants (Fig. 5). Slopes of the solid lines connecting median values were used as an analog for the change in concentration from one treatment to the next and provided a qualitative comparison of reductions among different organisms. The *E. coli* concentrations were used to represent all bacterial indicators because the slopes and patterns for *E. coli*, fecal coliform, and enterococci concentrations were very similar. Enterovirus and norovirus GI were not shown because of too few detections.

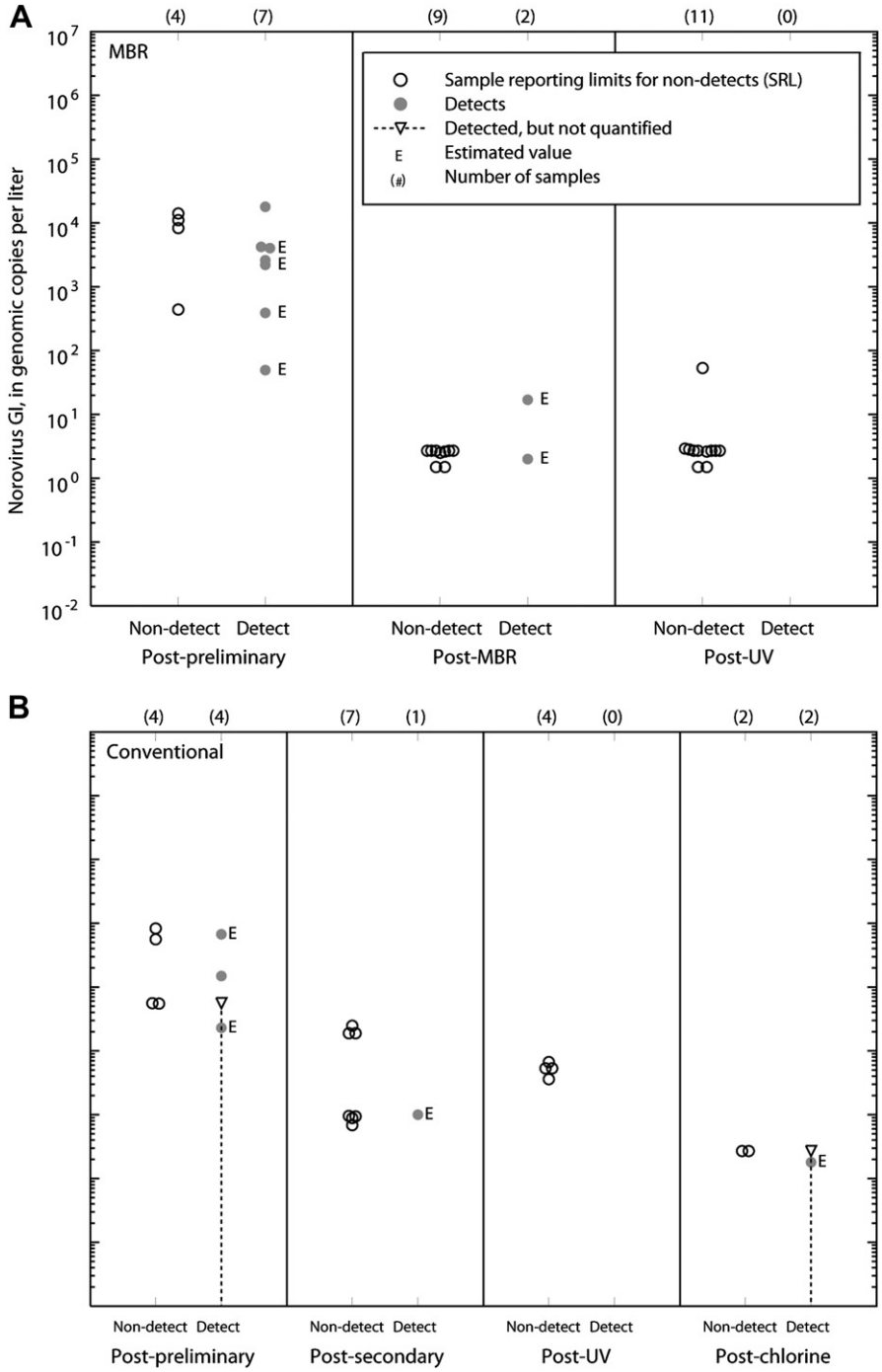

Fig. 4 – Concentrations of norovirus GI in wastewater samples throughout treatment processes in MBR and conventional plants.

For MBR plants, *E. coli* and F-specific coliphage concentrations show similar concentration patterns and slopes throughout treatment processes. Somatic coliphage, adenovirus, and culturable virus slopes were similar from post-preliminary to post-MBR samples, but slopes were different from post-MBR to post-disinfection for somatic coliphage. For conventional secondary plants from post-preliminary to post-secondary, the slopes were similar for *E. coli*, somatic coliphage, adenovirus, and culturable viruses but were different for F-specific coliphage. For conventional secondary plants, none of the indicators followed the same pattern as adenovirus and culturable virus concentrations from post-secondary to post-disinfection. For both MBR and conventional secondary plants, the slope was flat or nearly flat from post-MBR or post-secondary to post-disinfection samples for adenovirus. Note that culturable viruses for conventional secondary plants have different SRLs for post-secondary and post-

Table 1 – Detections of viruses by qPCR and qRT-PCR and cell culture in wastewater samples, 2008–2010.

Type of sample	Detections, including detected but not quantified (%)				
	Number of samples	Adenovirus by qPCR	Enterovirus by qRT-PCR	Norovirus GI by qRT-PCR	Culturable virus
Membrane bioreactor wastewater treatment plants					
Post-preliminary	11	100	91	64	73
Post-MBR	11	45	18	18	0
Post-ultraviolet disinfection	11	36	9	0	0
Conventional secondary activated-sludge wastewater treatment plants					
Post-preliminary	8	100	63	50	50
Post-secondary	8	75	12	12	0
Post-ultraviolet disinfection	4	25	25	0	0
Post-chlorine disinfection	4	75	0	50	0

disinfection samples, but no viruses were detected in either matrix.

3.4. *Virus recovery*

Using six seeded matrix controls, recoveries through all filtration, processing, and molecular analytical steps ranged from 0.09 to 5.13% for adenovirus and were less than 1% for enterovirus (Table 3). Seed amounts were 10^5 to 10^6, with volumes filtered ranging from 4 L for post-preliminary samples to 480 L for other types of wastewater samples. Seeded matrix controls determine recoveries of organisms through all processing and analytical steps. They do not distinguish between the effects of the matrix on recoveries from the effects from filtration, processing, and analysis.

4. Discussion

To our knowledge, this is the first study to compare concentrations and log removals of viruses in full-scale MBR and conventional secondary activated-sludge wastewater treatment plants, as well as to include these comparisons through disinfection processes. We evaluated indicator and virus concentrations and log removals in four wastewater treatment plants – two MBR activated-sludge plants, one conventional secondary activated-sludge plant with tertiary treatment and ultraviolet disinfection, and one conventional secondary activated-sludge plant with chlorine disinfection. Much effort was expended to identify sample reporting limits (SRLs) and quantify data as accurately as possible. We provided codes to qualify the data and to identify uncertainties associated with virus measurements – steps that we believe are important to aid in data interpretations but are seldom included with virus data. These efforts and the quality-control data used to help interpret results are presented in a companion report (Francy et al., 2011).

Adenovirus by qPCR was found in 100% of post-preliminary samples, 75% of conventional post-secondary samples, and 45% of post-MBR samples. Ultraviolet disinfection resulted in lower percentages of adenovirus detections (36% and 25%) than chlorine disinfection (75%). Kuo et al. (2010) found adenovirus by qPCR in all 32 samples collected from raw sewage, primary sedimentation effluent, MBR influent, and MBR effluent in an MBR ultrafiltration plant. Katayama et al. (2008) found adenovirus by qPCR in 100% of influent, 99% of secondary treated, and 100% of chlorinated effluent samples from conventional plants.

In the present study, concentrations of adenovirus ranged from 220 to 180,000 gc/L in post-preliminary samples, from <0.46 to 19 gc/L in post-MBR samples, from <M1.7 to E120 gc/L in post-secondary samples, and from 0.2 to 39 gc/L in post-disinfection samples (Table S6). These concentrations were lower than those found in other studies (Katayama et al., 2008; Kuo et al., 2010); however, it is difficult to compare our findings with other full-scale studies with respect to concentrations of viruses by qPCR. Wastewater types, sampling methods, assays used, and quantification methods can differ between studies and lead to different concentration results. Secondly, virus recoveries in different systems and in different water matrixes can vary and are seldom quantified. In our study, using a limited number of wastewater samples (6 samples), we found that recoveries of adenoviruses by qPCR and enterovirus by qRT-PCR in a wastewater matrix ranged from 0.09 to 5.13%. Other researchers found low recoveries of viruses in river water (Fong et al., 2010) and wastewater (Bofill-Mas et al., 2006). There are no published results on recoveries of viruses in wastewater by glass-wool filtration, the method used in this study. The limited recovery data from the present study indicate that actual virus concentrations may be higher than those presented.

Enterovirus and norovirus GI by qRT-PCR were found in the majority of post-preliminary samples in the present study, but norovirus GII was not found in any samples. In post-MBR, post-secondary, and post-ultraviolet disinfection samples, detection percentages for enterovirus and norovirus GI ranged from 0 to 25%. Simmons et al. (2011) did not find norovirus GI in any samples and found norovirus GII in 20 out of 32 different wastewater samples. Finding one norovirus genotype over the other may be due to the seasonal profiles of the genotypes (Katayama et al., 2008) and differences in study location (Said et al., 2008). In the present study, the sample reporting limits (SRLs) for non-detects of enterovirus and norovirus GI were in the same range or higher than the detected sample concentrations, adding uncertainty to the measured concentrations.

Table 2 – Summary statistics for log removals of microbial indicators and enteric viruses in different wastewater treatments (from previous step in the treatment process) at four treatment plants, 2008–2010.

Organism	Secondary				Membrane bioreactor				Ultraviolet after membrane bioreactor				Ultraviolet after secondary				Chlorine after secondary			
	Median	Min	Max	*n*	Median	Min	Max	*n*	Median	Min	Max	*n*	Median	Min	Max	*n*	Median	Min	Max	*n*
E. coli (CFU/L)	3.04	2.28	3.84	8	>6.11	5.37	>6.85	11	>0	NRD[a]	>0.9	11	3.82	2.63	4.38	4	2.57	2.30	3.15	4
Enterococci (CFU/L)	3.07	2.21	3.79	8	6.26	4.82	7.49	11	>0	−0.30	2.20	11	3.38	3.00	>3.94	4	1.18	0.98	1.57	4
Fecal coliforms (CFU/L)	2.89	1.96	3.37	8	>6.73	5.34	7.23	11	>0.30	NRD	>1.79	11	3.89	3.40	4.76	4	2.34	2.11	3.26	4
F-specific coliphage (PFU/L)	4.19	3.09	5.26	8	5.13	>4.58	>6.00	11	>0	<−0.30	>1.15	11	>1.17	>0.48	>1.72	4	0.71	0.51	>1.52	4
Somatic coliphage (PFU/L)	2.51	1.83	3.43	8	3.24	2.67	4.04	11	>2.18	>0.90	>2.96	11	>2.98	>0.95	>3.20	4	1.68	1.22	2.08	4
Adenovirus (gc/L)	2.61	0.92	>3.15	8	3.67	2.38	>4.86	11	NRD	−0.37	>1.60	11	0.24	NRD	>1.11	3	0.81	−0.42	>0.89	4
Enterovirus (gc/L)	1.53	>1.06	>3.07	4	3.40	>2.20	4.74	9	NRD	NRD	>0.26	9	–[b]	NRD	NRD	1	–	–	–	0
Norovirus GI (gc/L)	>1.43	>1.38	2.18	3	3.02	>1.51	3.32	7	NRD	NRD	>0.80	6	–	–	–	0	–	0.74	0.74	1
Culturable viruses (MPN/L)	>1.77	>1.61	>2.76	4	>2.52	>1.99	>3.61	8	NRD	NRD	NRD	8	–	–	–	0	–	–	–	0

a NRD, No removal detected because of two < values and considered a zero value.
b –, Not calculated because data values were inconclusive.

We detected culturable viruses in more than half of post-preliminary samples at concentrations ranging from 3.9 to 163 MPN viruses/L. Culturable viruses, however, were not found in any post-MBR, post-secondary, or post-disinfection samples. Viral nucleic acids found by qPCR or qRT-PCR can come from both infective and non-infective viruses and may also include free RNA or DNA. The results for culturable viruses reflect infective viruses only, so are more representative of actual health risk. Other investigators detected culturable viruses in effluents from conventional secondary plants (Fleischer et al., 2000; Harwood et al., 2005; Sedmak et al., 2005), but we are not aware of any full-scale studies that investigated culturable viruses in MBR plants.

Log removal data can be used to eliminate some of the biases that result from difficulties in recovering and accurately quantifying viruses in wastewater samples. Because of the way they are calculated, log removals are relative to each other. The data from the present study indicate that median log removals for all organisms were higher across MBR treatment than for conventional secondary treatment. It was no surprise that MBR technology removed bacterial indicators more efficiently than conventional secondary treatment. MBR treatment was also more efficient in removing F-specific and somatic coliphage than conventional secondary treatment. For somatic coliphage, however, this removal was not complete because concentrations of 2–3 log PFU/L often remained in post-MBR samples. This may be because F-specific coliphage tend to adsorb to solid surfaces (membrane surfaces and particulate matter) more than somatic coliphage, resulting in higher removals of F-specific coliphage (Zhang and Farahbakhsh, 2007; Gantzer et al., 2001). Coliphage may also be removed or inactivated by functions of an active microbial community on the filter, such as grazing or biologically produced proteases (Elliott et al., 2011). The reasons for the differences in F-specific and somatic coliphage removal rates by adsorption or microbial activities have not been investigated.

In the present study, log removals of adenovirus were higher for MBR (median = 3.67 gc/L) than for conventional secondary treatment (median = 2.61 gc/L). In another study in an ultrafiltration plant (Kuo et al., 2010), removals of adenovirus from MBR influent to MBR effluent were comparable to those in the present study (average = 5.0 log viruses/L). Although log removals for enterovirus and norovirus GI by qRT-PCR were also higher for MBR than conventional secondary treatment in the present study, these results may have been artifacts of higher SRLs for conventional secondary samples. In the present study, median log removals by MBR were 3.4 for enterovirus and 3.02 for norovirus. Simmons et al. (2011) found average removals for enterovirus and norovirus of 5.1 and 3.9 log viruses/L, respectively, in the same MBR plant investigated by Kuo et al. (2010).

More novel in the present study was the comparison of log removals from disinfection in MBR to disinfection in conventional secondary treatment plants. Analytical variability can be used to facilitate these comparisons. One has more confidence that log removals greater than the analytical variability are real, whereas log removals less than the

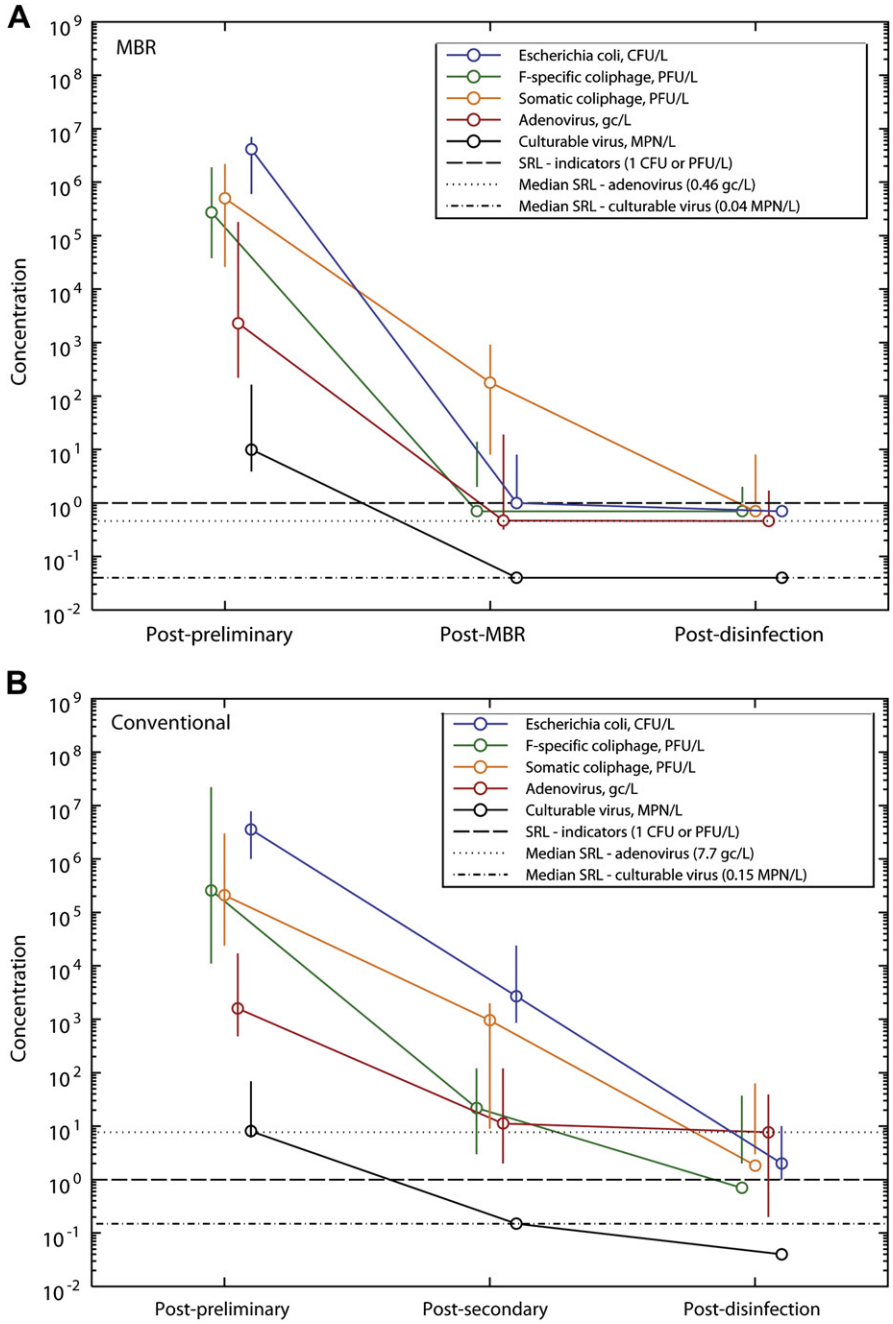

Fig. 5 – Concentration patterns of indicators and viruses and sample reporting limits (SRL) throughout treatment processes in MBR and conventional plants. The open circles indicate the median value, and the vertical lines are the range of detected values.

analytical variability may just be due to artifacts from the analytical variability. In this manner, we found that ultraviolet disinfection after MBR provided little additional removal of any organism except for somatic coliphage (median = >2.18 PFU/L, Table 2). Providing confidence that removals from ultraviolet disinfection after MBR were small for bacterial indicators and F-specific coliphage, the log removals by MBR were greater than the analytical variability for only 3 out of 44 individual sample results (samples 1 and 11, Tables S1–S4). (The analytical variability was found to be 1.0 log unit for <100 CFU or PFU/L and 0.3 log unit for >100 CFU or PFU/L.) In contrast, ultraviolet or chlorine disinfection after conventional secondary treatment resulted in 26 out of 32 log removals for bacterial indicators and F-specific coliphage that were greater than analytical variability. Log removals of adenovirus from disinfection were low in both MBR and conventional secondary treatment plants. Out of 18 values reported for log removals of adenovirus from ultraviolet or chlorine disinfection, only three (samples 6,8, and 9, Table S6) were below or at the analytical variability (1.2 log gc/L) and 15

Table 3 – Seed amounts, volumes filtered, and recoveries of adenovirus and enterovirus in seeded matrix controls, August 2011.

	Volume		Background (gc)	Recovered (gc)	Recovery (%)
	Seeded (gc)	Filtered (L)			
Adenovirus					
Plant 1 post-preliminary	440,000	4	2300	2700	0.09
Plant 1 post-MBR		480	330	1400	0.24
Plant 1 post-disinfection		480	180	760	0.13
Plant 5 post-preliminary	390,000	4	3000	23,000	5.13
Plant 5 post-secondary		120	500	4200	0.95
Plant 5 post-disinfection		480	890	3100	0.57
Enterovirus					
Plant 1 post-preliminary	810,000	4	BD[a]	4900	0.60
Plant 1 post-MBR		480	BD	4700	0.58
Plant 1 post-disinfection		480	BD	6200	0.77
Plant 5 post-preliminary	1,100,000	4	BD	1700	0.15
Plant 5 post-secondary		120	BD	M[b]	–[c]
Plant 5 post-disinfection		480	BD	M	–

a BD, Below detection.
b M, Material present but not quantified.
c –, Not calculated because data values were inconclusive.

were below the analytical variability. Log removals of enterovirus and norovirus GI from disinfection were less than the analytical variability or no removal was detected (NRD) in any sample.

The final objective of the study was to compare log removals of indicator bacteria and coliphage in wastewater and identify which indicator(s) best represented the removal of enteric viruses. Because of multiple sample reporting limits and the uncertainties associated with many of the virus measurements, a statistical comparison of log removals of indicators and viruses was contraindicated. Instead, plots of *E. coli* (representing all bacterial indicators), coliphage, adenovirus, and culturable viruses were used to facilitate comparisons (Fig. 5). These plots showed different ranges and patterns of concentrations throughout treatment processes in MBR as compared to conventional secondary treatment plants. From post-preliminary to post-MBR, somatic coliphage concentration reductions best represented reductions in virus concentrations (as evidenced by the slope of the line). For post-preliminary to post-secondary in conventional secondary plants, both *E. coli* and somatic coliphage concentration reductions represented reductions in viruses. From post-MBR to post-disinfection, both *E. coli* and F-specific coliphage showed a similar pattern to those of the viruses. From post-secondary to post-disinfection in conventional secondary plants, however, none of the indicators followed the same patterns as adenovirus and culturable virus concentrations. Other researchers found similar results. In a study at four conventional wastewater treatment plants in Sweden (Ottoson et al., 2006a), removal rates of somatic coliphage correlated moderately with removal of enterovirus and norovirus but not with any other indicators. At an MBR pilot plant, removals of indicators were strongly correlated to each other but only weakly correlated to enterovirus removal (Ottoson et al., 2006b).

This study focused on quantifying log removals of fecal indicators and viruses throughout wastewater treatment plant processes in MBR and conventional secondary activated-sludge treatment plants. With a large number of samples, we were able to draw conclusions based on summary statistics and qualitative comparisons among datasets. Problems with accurately quantifying viruses with molecular methods precluded statistical comparisons of log removals. More work needs to be done to improve and define virus recoveries in wastewater with different filtration systems, establish assay limits of detection for virus assays, and quantify uncertainties associated with virus measurements.

5. Conclusions

- Median log removals for all organisms (*E. coli*, fecal coliforms, enterococci, F-specific coliphage, somatic coliphage, adenovirus by qPCR, enterovirus and norovirus G1 by qRT-PCR, and culturable viruses) were higher across secondary municipal wastewater treatment for MBR plants than for conventional secondary plants.
- Although found in 12 out of 19 post-preliminary samples, culturable viruses were not detected in any post-secondary, post-MBR, post-UV, or post-chlorine samples.
- Ultraviolet disinfection after MBR secondary treatment provided little additional removal of any organism except for somatic coliphage.
- Ultraviolet or chlorine disinfection after conventional secondary treatment provided significant log removals (above the analytical variability) of all bacterial indicators and somatic and F-specific coliphage.
- Log removals of adenovirus from disinfection were low in both MBR and conventional secondary plants, and few removals were above the analytical variability.
- Somatic coliphage may best represent the removal of viruses in both MBR and conventional secondary treatment plants from post-preliminary to post-MBR or post-secondary samples.

- F-specific coliphage and *E. coli* may best represent the removal of viruses from post-MBR to post-disinfection samples. None of indicators represented the removal of viruses from post-secondary to post-disinfection samples in conventional secondary plants.
- *E. coli*, the indicator used to assess the microbial quality of wastewater, followed similar patterns to viruses in two processes: (1) from post-MBR to post-disinfection in MBR plants and (2) from post-preliminary to post-secondary in conventional secondary plants.
- More work needs to be done to improve and define virus recoveries in wastewater with different filtration systems, establish assay limits of detection for virus assays, and quantify uncertainties associated with virus measurements.

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Appendix A. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.watres.2012.04.044.

REFERENCES

Bofill-Mas, S., Albinana-Gimenez, N., Clemente-Casares, P., Hundesa, A., Rodruguez-Manzano, J., Allard, A., Calvo, M., Girones, R., 2006. Quantification and stability of human adenoviruses and polyomavirus JCPyV in wastewater matrices. Applied and Environmental Microbiology 72 (12), 7894–7896.

Borchardt, M.A., Haas, N.L., Hunt, R.J., 2004. Vulnerability of drinking-water wells in La Crosse, Wisconsin, to enteric-virus contamination from surface water contributions. Applied and Environmental Microbiology 70 (10), 5937–5946.

Britton, L.J., Greeson, P.E. (Eds.), 1989. Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples. Techniques of Water-Resources Investigations. U.S. Geological Survey, Reston, VA, pp. 37–40. book 5, chap. A4.

Cromeans, T., Narayanan, J., Jung, K., Ko, G., Wait, D., Sobsey, M.D., 2005. Development of Molecular Methods to Detect Infectious Viruses in Water. Report No. 90995F. American Water Works Association Research Foundation, Denver, CO.

da Silva, A.K., Le Saux, J.-C., Parnaudeau, S., Pommepuy, M., Elimelech, M., Le Guyader, F.S., 2007. Evaluation of removal of noroviruses during wastewater treatment using real-time reverse transcription-PCR: different behaviors of genogroups I and II. Applied and Environmental Microbiology 73 (24), 7891–7897.

Elliott, M.A., DiGiano, F.A., Sobsey, M.D., 2011. Virus attenuation by microbial mechanisms during the idle time of a household slow sand filter. Water Research 45, 4092–4102.

Fleischer, J., Schlafmann, K., Otchwemah, R., Botzenhart, K., 2000. Elimination of enteroviruses, other enteric viruses, F-specific coliphages, somatic coliphages and *E. coli* in four sewage treatment plants of southern Germany. Journal of Water Supply 49 (3), 127–138.

Fong, T.-T., Phanikumar, M.S., Xagoraraki, I., Rose, J.B., 2010. Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan River. Applied and Environmental Microbiology 76 (3), 715–723.

Francy, D.S., Stelzer, E.A., Bushon, R.N., Brady, A.M.G., Mailot, B.E., Spencer, S.K., Borchardt, M.A., Elber, A.G., Riddell, K.R., Gellner, T.M., 2011. Quantifying Viruses and Bacteria in Wastewater – Results, Interpretation Methods, and Quality Control. Scientific Investigations Report 2011–5150. U.S. Geological Survey, Reston, VA.

Gantzer, C., Gillerman, L., Kuznetsov, M., Oron, G., 2001. Adsorption and survival of faecal coliforms, somatic coliphages and F-specific RNA phages in soil irrigated with wastewater. Water Science and Technology 43 (12), 117–124.

Gregory, J.B., Litaker, R.W., Noble, R.T., 2006. Rapid one-step quantitative reverse transciptase PCR assay with competitive internal positive control for detection of enteroviruses in environmental samples. Applied and Environmental Microbiology 72 (6), 3960–3967.

Harwood, V.J., Levine, A.D., Scott, T.M., Chivukula, C., Lukasik, J., Farrah, S.R., Rose, J.B., 2005. Validity of the indicator organisms paradigm for pathogen reduction in reclaimed water and public health protection. Applied and Environmental Microbiology 71 (6), 3163–3170.

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D., Erdman, D.D., 2005. Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. Applied and Environmental Microbiology 71 (6), 3131–3136.

Katayama, H., Haramoto, E., Oguma, K., Yamashita, H., Tajima, A., Nakajima, H., Ohgaki, S., 2008. One-year monthly quantitative survey of noroviruses, enteroviruses, and adenoviruses in wastewater collected from six plants in Japan. Water Research 42, 1441–1448.

Kuo, D.H.-W., Simmons, F.J., Blair, S., Hart, E., Rose, J.B., Xagoraraki, I., 2010. Assessment of human adenovirus removal in a full-scale membrane bioreactor treating municipal wastewater. Water Research 44, 1520–1530.

Lambertini, E., Spencer, S.K., Bertz, P.D., Loge, F.J., Kieke, B.A., Borchardt, M.A., 2008. Concentration of enteroviruses, adenoviruses, and noroviruses from drinking water by use of glass wool filters. Applied and Environmental Microbiology 74 (10), 2990–2996.

Lesjean, B., Tazi-Pain, A., Thaure, D., Moeslang, H., Buisson, H., 2011. Ten persistent myths and the realities of membrane bioreactor technology for municipal applications. Water Science and Technology 63 (1), 32–39.

Madaeni, S.S., Fane, A.G., Grohmann, G.S., 1995. Virus removal from water and wastewater using membranes. Journal of Membrane Science 102, 65–75.

Oota, S., Murakami, T., Takemura, K., Noto, K., 2005. Evaluation of MBR effluent characteristics for reuse purposes. Water Science and Technology 51 (6–7), 441–446.

Ottoson, J., Hansen, A., Westrell, T., Johansen, K., Norder, H., Stenström, T.A., 2006a. Removal of noro- and enteroviruses, *Giardia* cysts, *Cryptosporidium* oocysts, and fecal indicators at four secondary wastewater treatment plants in Sweden. Water Environment Research 78 (8), 828–834.

Ottoson, J., Hansen, A., Björlenius, B., Norder, H., Stenström, T.A., 2006b. Removal of viruses, parasitic protozoa and microbial indicators in conventional and membrane processes in a wastewater pilot plant. Water Research 40, 1449–1457.

Rajal, V.B., McSwain, B.S., Thompson, D.E., Leutenegger, C.M., Wuertz, S., 2007. Molecular quantitative analysis of human viruses in California stormwater. Water Research 41, 4287–4298.

Said, M.A., Perl, T.M., Sears, C.L., 2008. Gastrointestinal flu: norovirus in health care and long-term care facilities. Clinical Infectious Diseases 47 (9), 1202–1208.

Sedmak, G., Bina, D., MacDonald, J., Couillard, L., 2005. Nine-year study of the occurrence of culturable viruses in source water for two drinking water treatment plants and the influent and effluent of a wastewater treatment plant in Milwaukee, Wisconsin. Applied and Environmental Microbiology 71 (2), 1042–1050.

Simmons, F.J., Kuo, D.H.-W., Xagoraraki, I., 2011. Removal of human enteric viruses by a full-scale membrane bioreactor during municipal wastewater processing. Water Research 45, 2739–2750.

Sobsey, M.D., Amanti, A., Handzel, T., 1995. Detection and occurrence of coliphage indicator viruses in water. In: Proceedings of the Water Quality Technology Conference, American Water Works Association, November 1995, New Orleans, LA, pp. 2087–2097.

U.S. Environmental Protection Agency, 2001a. Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure. EPA 821-R-01-029,. Office of Water, Washington DC (accessed October 2011) at: http://www.epa.gov/nerlcwww/1602ap01.pdf.

U.S. Environmental Protection Agency, 2001b. USEPA Manual of Methods for Virology – Chapter 15. EPA 600/4-84/013. Office of Research and Development, Washington DC (accessed October 2011) at: www.epa.gov/nerlcwww/chapt15.pdf.

U.S. Environmental Protection Agency, 2006a. Method 1603 – *Escherichia coli* in Water by Membrane Filtration Using Modified Membrane-Thermotolerant *Escherichia coli* Agar. EPA 821-R–06–011, Washington, DC. (accessed October 2011) at: http://www.epa.gov/waterscience/methods/method/biological/.

U.S. Environmental Protection Agency, 2006b. Method 1600 – Enterococci in Water by Membrane Filtration Using Membrane-Enterococcus Indoxyl-b-D-Glucoside Agar (mEI). EPA–821-R–06–009, Washington, DC. (accessed October 2011) at: http://www.epa.gov/waterscience/methods/method/biological/.

Wilde, F.D., ed., variously dated, Field measurements. Techniques of Water-Resources Investigations, book 9, chap. A6, sec. 6.4 and 6.7. U.S. Geological Survey, Reston, VA. (accessed October 2011) at: http://pubs.water.usgs.gov/twri9A6/.

Zhang, K., Farahbakhsh, K., 2007. Removal of native coliphages and coliform bacteria from municipal wastewater by various wastewater treatment processes: implications to water reuse. Water Research 41, 2816–2824.