Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2017, 51, 11918-11927

pubs.acs.org/est

Reduction of Human Norovirus GI, GII, and Surrogates by Peracetic Acid and Monochloramine in Municipal Secondary Wastewater Effluent

Nathan Dunkin,[†] ShihChi Weng,[‡] Caroline G. Coulter,[†] Joseph G. Jacangelo,[†,‡,§] and Kellogg J. Schwab*[,†,‡]

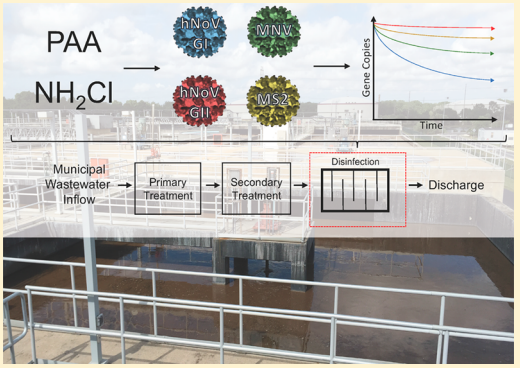
[†]Department of Environmental Health and Engineering, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland 21205, United States

[‡]JHU/MWH-Stantec Alliance, Johns Hopkins University, Baltimore, Maryland 21205, United States

[§]MWH-Stantec, Pasadena, California 91101, United States

Supporting Information

ABSTRACT: The objective of this study was to characterize human norovirus (hNoV) GI and GII reductions during disinfection by peracetic acid (PAA) and monochloramine in secondary wastewater (WW) and phosphate buffer (PB) as assessed by reverse transcription-qPCR (RT-qPCR). Infectivity and RT-qPCR reductions are also presented for surrogate viruses murine norovirus (MNV) and bacteriophage MS2 under identical experimental conditions to aid in interpretation of hNoV molecular data. In WW, RT-qPCR reductions were less than 0.5 $\log_{10}$ for all viruses at concentration−time (CT) values up to 450 mg-min/L except for hNoV GI, where 1 $\log_{10}$ reduction was observed at CT values of less than 50 mg-min/L for monochloramine and 200 mg-min/L for PAA. In PB, hNoV GI and MNV exhibited comparable resistance to PAA and monochloramine with CT values for 2 $\log_{10}$ RT-qPCR reduction between 300 and 360 mg-min/L. Less than 1 $\log_{10}$ reduction was observed for MS2 and hNoV GII in PB at CT values for both disinfectants up to 450 mg-min/L. Our results indicate that hNoVs exhibit genogroup dependent resistance and that disinfection practices targeting hNoV GII will result in equivalent or greater reductions for hNoV GI. These data provide valuable comparisons between hNoV and surrogate molecular signals that can begin the process of informing regulators and engineers on WW treatment plant design and operational practices necessary to inactivate hNoVs.



INTRODUCTION

Human noroviruses (hNoVs) are now acknowledged as the number one cause of acute gastroenteritis outbreaks and sporadic cases globally across all age groups.[1] In the United States, hNoVs are estimated to cause more than 21 million illnesses and 71 000 hospitalizations annually.[2] hNoVs are transmitted through the fecal−oral route, and waterborne transmission is known to be an important exposure pathway.[3,4]

Drinking water treatment plants (DWTPs) and wastewater treatment plants (WWTPs) serve as important barriers in limiting human exposure to viral pathogens, but there are differing reports of hNoV resistance in water. It is known that hNoVs can persist in water for long periods of time,[5] remaining infectious for at least 60 days,[6] and are stable to changes in water temperature.[7,8] Early research investigating the susceptibility of hNoVs to free available chlorine suggested that the viruses were highly resistant to chlorination after observing a hNoV suspension in water cause illness in human volunteers after a free chlorine dose of 3.75 mg/L for 30 min.[9] Other studies likewise concluded that hNoVs were resistant to chlorine after detecting hNoV RNA subsequent to exposure at very high free chlorine doses (i.e., 250−7000 mg/L).[10−12] However, others later postulated that hNoVs were much more sensitive to free chlorine than previously reported after observing a 3 $\log_{10}$ reduction of hNoV RNA by reverse transcription-polymerase chain reaction (RT-PCR) after concentration−time (CT) values of less than 15 mg-min/mL.[13,14] The investigators hypothesized that their findings differed from previous research due to variations in virus preparation that could have led to excess oxidant demand, viral aggregation, or viral association with particulate matter in prior studies. In short, the limited and differing results for hNoV disinfection in water suggests that there is a need for studies that utilize thorough hNoV purification prior to viral seeding and that employ relevant doses and contact times for water treatment processes.

Received: June 8, 2017
Revised: September 5, 2017
Accepted: September 14, 2017
Published: September 14, 2017

DOI: 10.1021/acs.est.7b02954
Environ. Sci. Technol. 2017, 51, 11918−11927

Furthermore, there is a paucity of hNoV inactivation data in applied water matrices despite the fact that previous work has shown that viral inactivation rates can vary between natural waters and laboratory grade water under similar conditions.[15−17] One such applied water matrix is municipal wastewater (WW), where disinfection is critical to controlling release of pathogens such as hNoVs into receiving waters. The most common method for disinfection of WW is chlorination, chiefly due to its low cost, efficacy against many types of microorganisms, and acceptance among regulatory agencies. Wastewater plants that do not fully nitrify are often constricted to chloramines for disinfection, which are formed when free chlorine reacts with available ammonia that is often present in WW. While monochloramine is known to be less virucidal than free chlorine,[18] it also produces fewer disinfection byproducts (DBPs) such as trihalomethanes and haloacetic acids.[19,20]

Alternatives to chlorine-based disinfection of WW are growing in popularity due in part to security concerns with gaseous chlorine and DBP toxicity concerns after effluent discharge into the environment.[21] This has led to a renewed interest in peracetic acid (PAA) as a disinfectant for WW in the United States. PAA is recognized as an efficient organic bactericide with applications in the food-processing, beverage, and pharmaceutical industries.[22] DBPs formed by PAA are mostly carboxylic acids that have less mutagenicity, carcinogenicity, and genotoxicity than halogenated DBPs produced from chlorination,[23,24] and PAA usually requires no post-treatment neutralization. Additionally, laboratory studies have demonstrated that PAA has comparable virucidal efficacy to monochloramine[25] and is able to reduce *Escherichia coli* and fecal coliform levels to meet permit regulations in WW effluent.[26,27] There remains, however, limited data in the literature on PAA or monochloramine disinfection efficacy against hNoVs in water or wastewater.

One challenge associated with addressing these data gaps is the lack of an easily employed cell culture model for hNoVs. Despite recent advancements,[28] the lack of a readily suitable hNoV culturing method for environmental research has necessitated the use of human volunteer studies to determine hNoV persistence and survival. However, the prohibitive costs and regulatory hurdles associated with such studies have led researchers to rely primarily on either hNoV reverse-transcription quantitative RT-PCR (RT-qPCR) signals or infectivity of cultivable surrogate viruses such as murine norovirus (MNV)[29] as estimates of hNoV infectivity. However, RT-qPCR signals are known to consistently underestimate reductions of surrogate virus titer even with RT-qPCR pretreatment designed to reduce spurious nonencapsulated nucleic acid (e.g., RNase, intercalating dyes),[30,31] and the relevance of surrogate virus infectivity measurements to hNoV risk continues to be debated.[32] Thus, it is important for hNoV treatability studies to include both surrogate infectivity and RT-qPCR data to aid in interpretation of hNoV RT-qPCR results generated under similar conditions.

The objective of this study was, therefore, to characterize hNoV GI and GII reductions during disinfection by PAA and monochloramine in secondary WW and phosphate buffer (PB) as assessed by RT-qPCR and to compare the results with infectivity[25] and RT-qPCR reductions for MNV and bacteriophage MS2 under identical experimental conditions to aid in interpretation of hNoV molecular data. This work contributes to our limited understanding of hNoV susceptibility to disinfection and provides some of the first hNoV inactivation data in an applied water matrix.

EXPERIMENTAL SECTION

Water Matrices. A 0.01 M phosphate buffer (PB) solution (pH 7) and municipal secondary treated effluent (nonchlorinated) from a water resource recovery facility at the Metro Wastewater Reclamation District (Denver, CO) were used in this study. One WW sample was used for the experiments in this study in order to minimize the impact of differences in water quality between WW samples on disinfection outcomes. Water quality analysis of secondary effluent (Table S1) was performed at JHU following overnight shipping at 4 °C using methods as previously described.[33]

Viruses. Deidentified stool samples were collected from two hNoV infected patients at an affiliated hospital under protocol approved by the Johns Hopkins Institutional Review Board. Stool samples were immediately transferred to 4 °C upon collection. The genogroup and genotype for the two noroviruses were identified as GI.3 and GII.2 by sequencing a portion of the C region of ORF2 according to the methods described by Kroneman and colleagues (data not shown).[34]

hNoV positive stool samples were diluted to 10% w/v in Dulbecco's phosphate buffered saline (DPBS) and subsequently sonicated (Branson B2510-DTH, Danbury, CT) at 40 kHz for 3 min. Each stool sample was then mixed with an equal volume of Vertrel XF (DuPont, Wilmington, DE) and subsequently homogenized (OMNI international, Inc., Marietta, GA) at 20 000 rpm for 3 min on ice. The emulsified mixtures were centrifuged for 15 min at 4000*g* and 4 °C, and the recovered supernatants were then filtered through 0.45 μm pore-size low-protein-binding membrane filters (Millex PVDF, Millipore, Billerica, MA). Following 0.45 μm filtration, hNoV filtrate was concentrated and further purified by ultracentrifugation on a sucrose cushion,[35] performed as previously described.[25,36] For surrogate viruses, MNV and MS2 stocks were propagated and purified as previously described.[25]

Experimental Procedure. All viral inactivation experiments were conducted at 20 °C and ambient pH for WW and pH 7.0 for PB. Sterile, chlorine demand-free 125 mL Erlenmeyer flasks were obtained following the protocol of Cromeans and colleagues[18] and contained an initial volume of 95 mL of water matrix, 5 mL of disinfectant, and 1 mL of virus cocktail for the disinfection experiments. Viruses employed in this study were hNoV GI.3, hNoV GII.2, MNV, and MS2. Sucrose cushion purified and concentrated hNoVs stocks were seeded to 0.0001% final stool concentration (corresponding to approximately 5×10^3 RT-qPCR units/10 μL for hNoV GI and 1×10^5 RT-qPCR units/10 μL for hNoV GII) in the experimental water matrices to minimize impacts of stool organics on disinfection experiments. Surrogate virus concentrations in the final volume were approximately 1×10^5 PFU/mL. Flasks were continuously mixed throughout the experiment. After the water matrix and viruses were added to the flask, 5 mL of disinfectant (PAA or NH_2Cl, prepared as previously described[25]), adjusted to achieve the final desired concentration, was added. Four PAA doses (0.7, 1.5, 5, and 10 mg/L) and four NH_2Cl doses (0.5, 1, 5, 15 mg/L) were selected for evaluation. hNoVs were not evaluated at low WW disinfectant doses (i.e., 0.7 and 0.5 mg/L). All experiments were performed in duplicate except for 5 mg/L doses which were performed individually due to hNoV stock limitations. Additionally, hNoVs were not assayed at the lowest disinfectant

DOI: 10.1021/acs.est.7b02954
Environ. Sci. Technol. 2017, 51, 11918−11927

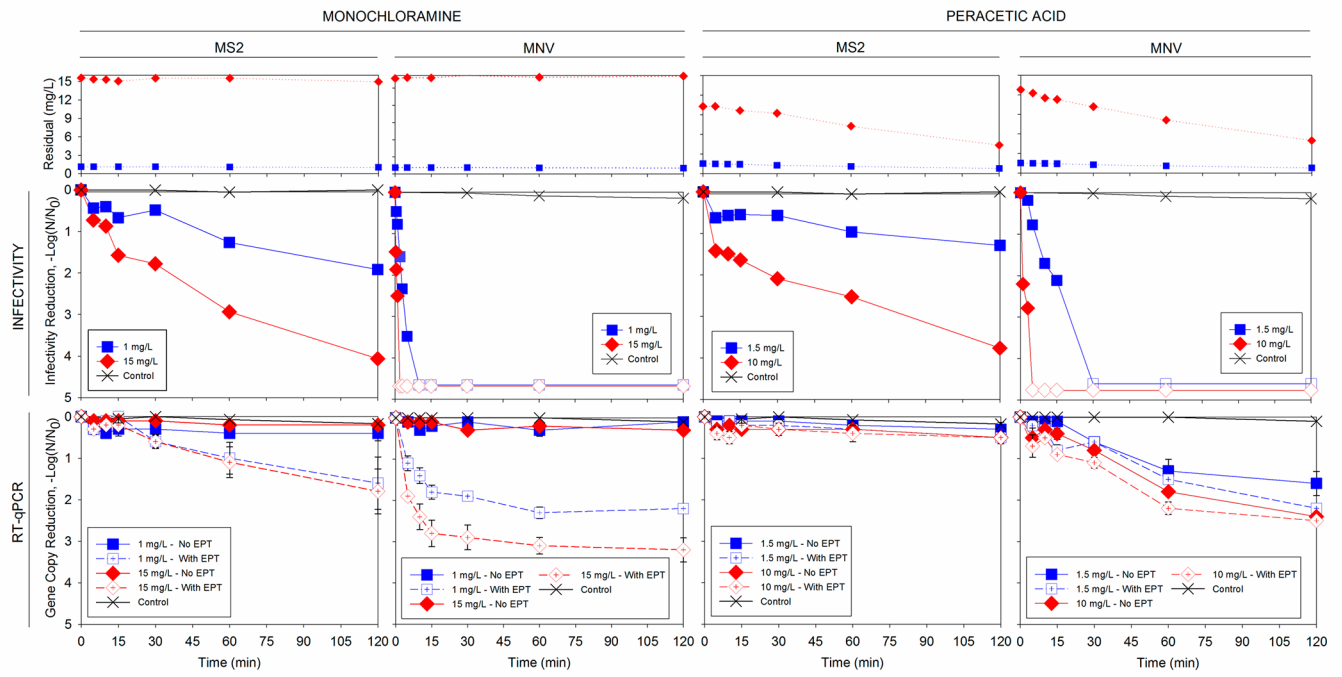


Figure 1. Infectivity and gene copy reduction of MS2 and MNV by NH_2Cl and PAA in 0.01 M phosphate buffer. RT-qPCR was performed both with and without enzymatic pretreatment (EPT) with RNase. Hollow symbols with no shading or crosses represent viral concentrations below the sensitivity limit of the assay. Error bars represent standard error of replicates. Infectivity data cited from Dunkin et al.[25]

doses due to stock limitations and nucleic acid persistence. For 0.01 M PB experiments, two PAA doses (1.5 and 10 mg/L) and two NH_2Cl doses (1 and 15 mg/L) were selected for evaluation for all viruses, and all experiments were performed in duplicate. Disinfectant residuals were measured at each sampling time point using methods previously described.[25] At each sampling time point, 4 mL was removed and immediately quenched with sodium thiosulfate (600 μg/mL) for viral analysis. For experiments with PAA, the quenching mixture also included catalase (2.5 mg/L) to ensure quenching of residual hydrogen peroxide. Quenched samples were then portioned and stored at −80 °C.

Molecular Analysis. Molecular analysis was performed using RT-qPCR. Two sets of analyses were performed on each experimental sample: one analysis with enzymatic pretreatment (EPT) by RNase ONE Ribonuclease (Promega, Madison, WI) and one without EPT. EPT was conducted to evaluate the impact of exogenous, nonencapsulated viral RNA on RT-qPCR results. To perform EPT, experimental samples (including time zero samples) were treated with RNase ONE Ribonuclease at a concentration of 1 unit per 10 μL of sample. RNase reaction buffer (1× final concentration) was added, and the mixture was incubated at 37 °C for 15 min. Following RNase treatment, nucleic acid was extracted from virions in the experimental samples using the MagNA Pure LC 2.0 (Roche, Indianapolis, IN) instrument with the Total Nucleic Acid High Performance Isolation Kit (Roche, Indianapolis, IN). Input volumes of 200 μL of sample and elution volumes of 100 μL were used. Dilution series on MNV and MS2 stocks with known titers were included as controls in nucleic acid extraction runs.

Following RNA extraction, RT-qPCR was performed using the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). Primers and probes were used for hNoV GI, hNoV GII, MNV, and MS2 as previously described,[5,37,38] and all primer and probes sets were tested to ensure no cross-reactivity with other targets in this study (data not shown). RT-qPCR protocols were followed as previously described[5,38] with slight modifications. Briefly, the QuantiTect Probe RT-PCR Kit (Qiagen, Germantown, MD) was used with the following thermocycling conditions for all targets: 50 °C for 30 min, 95 °C for 15 min, and 45 cycles at 95 °C for 15 s and 60 °C for 60 s. Cycle threshold (C_t) data corresponding to viral RNA concentrations were obtained. Well-characterized and archived hNoV GI, hNoV GII, MNV, and MS2 RNA were used as RT-qPCR controls.

Kinetic Modeling and Statistical Analyses. Disinfectant decay rate constants (k') for each experiment were calculated by regressing measured residuals using the least-squares method. First-order kinetics were assumed according to the equation:

$$C = C_0 \times e^{-k't} \quad (1)$$

where k' = first-order disinfectant decay rate constant, C = observed disinfectant residual (mg/L), C_0 = initial disinfectant dose (mg/L), and t = time from start of experiment to time of sample (min).

Viral reductions were determined by calculating the negative $\log_{10}$ of the ratio of remaining organisms to initial organisms (N/N_0) at sample times and fit by a series of well-validated inactivation models derived from the generalized inactivation rate:

$$r_d = \frac{dN}{dt} = -kC^n mN^x t^{m-1} \quad (2)$$

where r_d = inactivation rate, n, m, and x = dimensionless model parameters, k = inactivation rate constant, N = viral density (PFU or gene copies/mL), C = disinfectant concentration (mg/L), and t = contact time (min).

To account for the effect of disinfect decay throughout an experiment, eq 1 can be substituted into eq 2 and the resulting

DOI: 10.1021/acs.est.7b02954
Environ. Sci. Technol. 2017, 51, 11918−11927

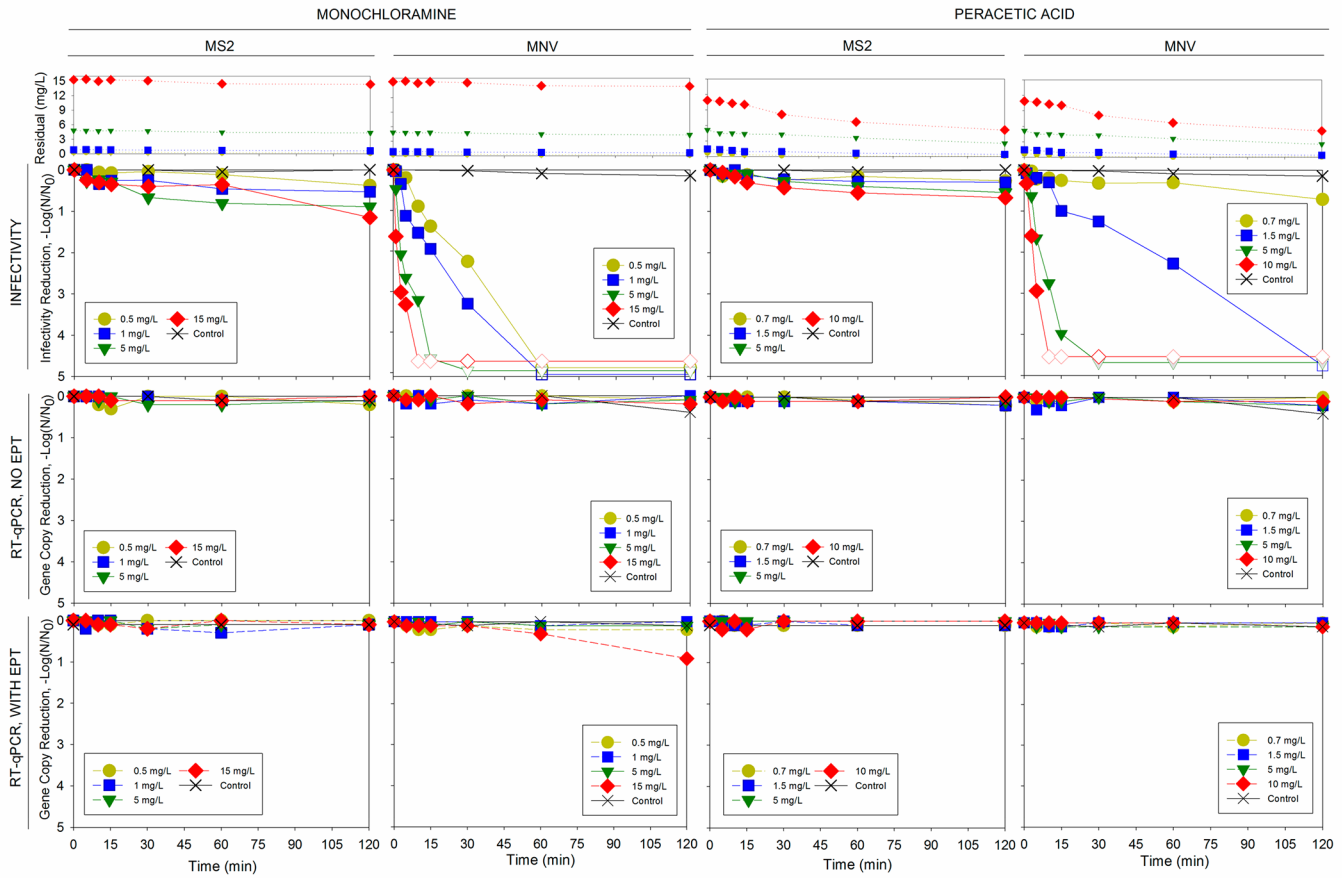


Figure 2. Infectivity and gene copy reduction of MS2 and MNV by NH_2Cl and PAA in municipal secondary wastewater effluent. RT-qPCR was performed both with and without enzymatic pretreatment (EPT) with RNase. Hollow symbols with no shading represent viral concentrations below the sensitivity limit of the assay. Error bars represent standard error of replicates. Infectivity data cited from Dunkin et al.[25]

expression can be integrated to derive the specialized inactivation models employed in this study, which included the Chick-Watson,[39]

$$\log\left(\frac{N}{N_0}\right) = -\frac{k}{k'n}(C_0{}^n - C_t{}^n) \tag{3}$$

the Incomplete Gamma Function (IGF) Hom,[40]

$$\log\left(\frac{N}{N_0}\right) = -\frac{kmC_0{}^n}{(k'n)^m} \times \gamma(m, nk't) \tag{4}$$

the Power Law,[41]

$$\log\left(\frac{N}{N_0}\right) = -\frac{\log\left[1 + (x-1) \times \frac{k}{k'n}(C_0{}^n - C_t{}^n) \times N_0{}^{x-1}\right]}{(x-1)} \tag{5}$$

and the Hom-Power Law,[42]

$$\log\left(\frac{N}{N_0}\right) = -\frac{\log\left[1 + (x-1) \times \frac{kmC_0{}^n}{(k'n)^m} \times \gamma(m, nk't) \times N_0{}^{x-1}\right]}{(x-1)} \tag{6}$$

These models were fit to experimental data using the least-squares method. Model fit was assessed by comparing the residual sum of squared errors (SSE). Partial F-tests were performed to assess significance of additional parameters in more highly parametrized models (α = 0.05). Model errors were checked for normality using the Shapiro-Wilk statistical test. Modeling and statistical analysis were performed in Microsoft Excel and R (www.r-project.org).

RESULTS AND DISCUSSION

Kinetic Modeling of Virus Inactivation Data. Temporal profiles of MS2 and MNV gene copy reductions by monochloramine and PAA are shown in Figure 1 for experiments in PB and in Figure 2 for experiments in WW. Temporal profiles for gene copy reductions of hNoV GI and GII by monochloramine and PAA in both PB and WW are shown in Figure 3. All RT-qPCR measurements were performed twice: once with EPT using RNase One prior to RT-qPCR and once without EPT. Surrogate infectivity reductions in the same wastewater generated in our previous publication[25] are displayed alongside surrogate gene copy reductions in order to aid in interpretation of hNoV RT-qPCR results.

Inactivation models that consider disinfectant demand were employed in this study and fit to viral gene copy reduction data. Data were segmented by virus, disinfectant type, and sample RT-qPCR pretreatment (i.e., with or without EPT), and the best-fit model for each scenario in 0.01 M PB is presented in Table S2 with associated parameters. Modeling results for nonselected models along with Shapiro-Wilk test statistics for all model residuals are provided in Tables S3, S4, S5, and S6. Modeling analysis focused on experimental data in PB due to the relative lack of gene copy reductions observed in WW (Figures 2 and 3).

The IGF Hom model provided the best fit to the viral gene copy reduction data for 11 of the 16 virus-disinfectant-sample treatment scenarios, with the Hom-Power Law as the best-fit

DOI: 10.1021/acs.est.7b02954
Environ. Sci. Technol. 2017, 51, 11918−11927

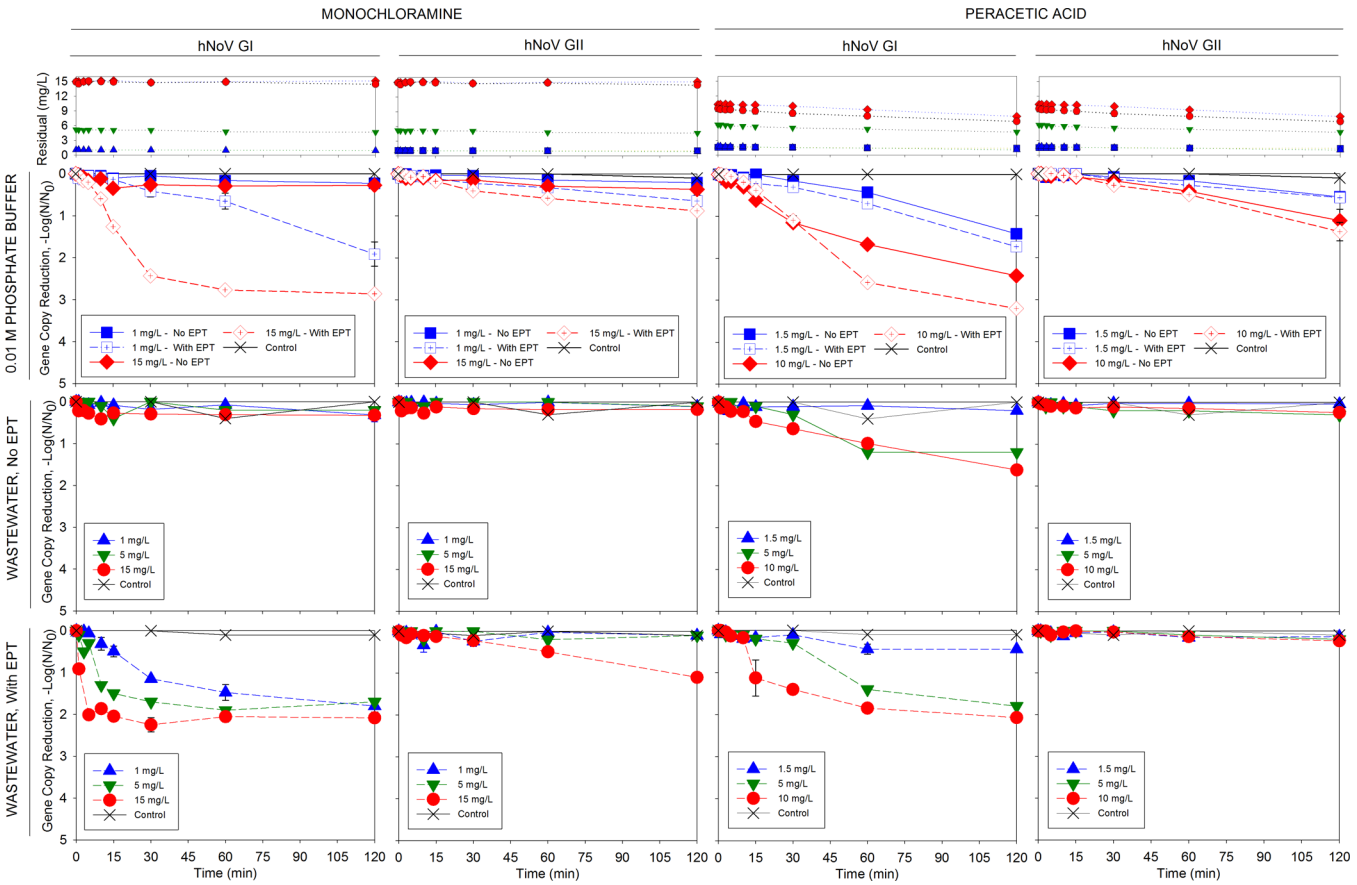


Figure 3. Infectivity and gene copy reduction of human norovirus (hNoV) GI and GII by NH_2Cl and PAA in municipal secondary wastewater effluent and 0.01 M phosphate buffer. RT-qPCR was performed both with and without enzymatic pretreatment (EPT) with RNase. All molecular measurements were above the sensitivity limit of the assay. Error bars represent standard error of replicates.

model for the remaining five scenarios. The IGF Hom's ability to account for virus tailing in experimental data likely contributed to the best fit of the data using this model. Furthermore, the Hom-Power Law contains a fourth parameter which, though statistically significant after evaluation by a partial F-test (α = 0.05), likely contributed to its effectiveness.

Model fits for all scenarios are shown in Figure 4, where observed gene copy reduction is plotted versus model-predicted gene copy reduction. Figure 4 demonstrates that selected models were able to effectively represent experimental data.

Effect of RT-qPCR Enzymatic Pretreatment on Molecular Results. Molecular methods such as RT-qPCR are commonly used for detection of enteric viruses in water, especially when no easily employed cell culture model exists for the given target (e.g., hNoVs). However, RT-qPCR signals have been shown to consistently underestimate reductions of viral infectivity for cultivable viruses,[30,31] the gold standard measurement for information relating to human health. As a result, researchers have investigated various methods to distinguish between infectious and noninfectious viruses using RT-qPCR.[43] Two sample preparation methods have been proposed: pretreatment with dyes such as ethidium monoazide (EMA) or propidium monoazide (PMA)[44−47] and pretreatment with RNase.[8,12,48] Both methods aim to allow RT-qPCR signals to more closely approximate infectivity by neutralizing exposed RNA or RNA within a damaged capsid prior to amplification. However, the effectiveness of intercalating dyes for ssRNA viruses (such as hNoVs) likely depend on secondary structures

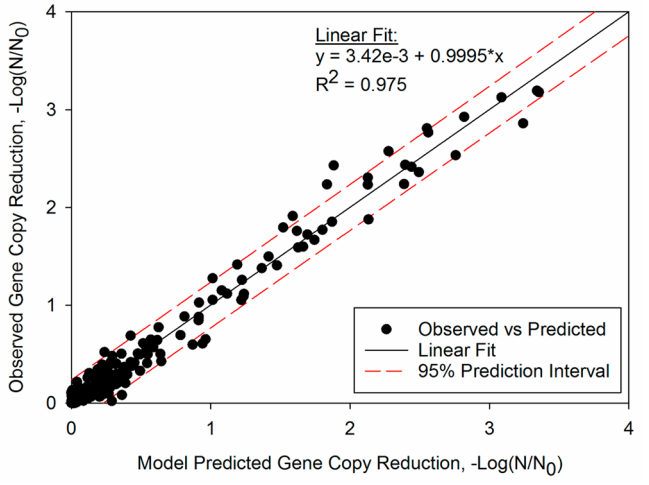


Figure 4. Observed gene copy reduction versus model predicted gene copy reduction for human norovirus GI, human norovirus GII, MNV, and MS2 in 0.01 M phosphate buffer.

of the RNA.[43,49] As a result, the efficacy of EMA and PMA may vary depending on the amplified region of the viral genome. To avoid these complications, RNase pretreatment was selected for this study. Additionally, samples were analyzed both with and without EPT in order to assess the impact of RT-qPCR pretreatment across disinfectant, water, and virus types.

DOI: 10.1021/acs.est.7b02954
Environ. Sci. Technol. 2017, 51, 11918−11927

Table 1. Model Predicted NH_2Cl and PAA CT Values Required for 1-, 2-, and 3-log_{10} Reductions of MS2, MNV, Human Norovirus GI, and Human Norovirus GII as Measured by RT-qPCR, EPT RT-qPCR, and Surrogate Infectivity in 0.01 M Phosphate Buffer[a]

		CT value (mg-min/L)									
		MS2			MNV			hNoV GI		hNoV GII	
reduction	disinfectant	infectivity[b]	EPT RT-qPCR	RT-qPCR	infectivity[b]	EPT RT-qPCR	RT-qPCR	EPT RT-qPCR	RT-qPCR	EPT RT-qPCR	RT-qPCR
1-log_{10}	NH_2Cl	92	474	N.O.	3	18	N.O.	123	N.O.	1103	6677
	PAA	59	N.O.	N.O.	9	128	201	177	313	551	620
2-log_{10}	NH_2Cl	317	1075	N.O.	7	308	N.O.	356	N.O.	2675	N.O.
	PAA	220	N.O.	N.O.	28	354	433	342	695	873	952
3-log_{10}	NH_2Cl	655	1720	N.O.	11	4520	N.O.	971	N.O.	4399	N.O.
	PAA	609	N.O.	N.O.	73	576	627	621	2311	1126	1205

[a]N.O.: Specified log_{10} viral molecular reductions were not observed during model simulation due to disinfectant residuals decaying to zero. PAA: peracetic acid; MNV: murine norovirus; hNoV: human norovirus; EPT: enzymatic pretreatment with RNase ONE prior to RT-qPCR. [b]Infectivity CT values were developed through application of best-fit models presented in Dunkin et al.[25]

Results in Figures 1 and 3 demonstrate that the impact of EPT was greater for viruses treated with monochloramine than with PAA in 0.01 M PB. After 120 min of contact time, model predicted MS2 gene copy reductions in PB (derived from the application of the model providing the best fit to experimental data) with and without EPT at a high monochloramine dose of 15 mg/L were 1.8 and 0.3 log_{10}, respectively. However, predicted MS2 reductions after 120 min by PAA in PB at a high dose of 10 mg/L were 0.5 log_{10} regardless of EPT. For MNV, a 15 mg/L monochloramine dose resulted in predicted gene copy reductions in PB of 3.1 and 0.2 log_{10} with and without EPT, respectively, while a 10 mg/L PAA dose resulted in reductions of 2.7 log_{10} with EPT and 2.4 log_{10} without EPT. Similar trends were observed for hNoVs in PB in Figure 3. Predicted reductions of hNoV GI after 120 min at a monochloramine dose of 15 mg/L were 3.3 and 0.3 log_{10} with and without EPT, respectively, while at a high dose of 10 mg/L PAA predicted reductions of hNoV GI were 3.3 and 2.1 log_{10} with and without EPT, respectively. Taken together, these results demonstrate that there are greater differences in molecular signals between RT-qPCR pretreated and nonpretreated water samples disinfected with monochloramine than for PAA.

These data indicate that monochloramine and PAA may inactivate viruses through different mechanisms. The relative persistence of unprotected viral RNA in the presence of monochloramine (even at CT values of up to 1800 mg-min/L in PB, calculated from Figure 1) may indicate that monochloramine acts primarily on viral capsids as opposed to viral genomes. This would be consistent with prior work that has shown capsid protein modifications induced by monochloramine to be important for inactivation of human adenovirus-2.[50] Additionally, it is interesting to note that potential loss of capsid integrity, persistence of viral RNA, and subsequent reduction of RT-qPCR signal by EPT was observed across all four viruses in this study (MS2 and MNV in Figure 1, hNoV GI and GII in Figure 3) and across two water matrices of differing complexities. While it is known in the literature that closely related viruses can exhibit very different inactivation kinetics to a single disinfectant (and thus, it is hypothesized, inactivation mechanisms may be unique to disinfectant−virus pairs),[51,52] our data suggest that there may be consistency in the mode of action by monochloramine on capsids as opposed to genomes for the four viruses employed in this study.

The susceptibility of unprotected viral RNA exposed after capsid damage during disinfection by PAA, however, suggests that PAA may act on both viral capsids and genomes. Researchers have hypothesized that PAA functions in a similar way to other peroxides by disrupting sulfhydryl (−SH) and disulfide (S−S) bonds in microbial proteins,[53] and others have suggested that oxidants in general tend to target capsids as opposed to genomes.[54] The fact that EPT failed to significantly reduce RT-qPCR signals for all viruses treated with PAA in this study, though, suggests that further work investigating the effect of PAA on viral genomes is warranted in order to more fully elucidate PAA inactivation mechanisms.

The impact of EPT was also moderated by the complexity of the WW matrix. In Figure 1, 4 log_{10} infectivity reduction of MNV by monochloramine in PB was associated with a 1−2 log_{10} reduction by EPT RT-qPCR. However, in WW, there was no reduction of MNV by EPT RT-qPCR even when approximately 5 log_{10} of infectivity reduction was observed. This same trend was observed for PAA, where no molecular reductions of MNV were observed in WW (Figure 2). These data suggest that complex environmental waters may moderate the relationship between infectivity and EPT RT-qPCR observed in cleaner laboratory water.

Reduction of Human Norovirus GI and GII versus Surrogates. It is commonly accepted that enteric viruses, and especially hNoVs, are persistent in water and other environmental media.[55] However, estimating human health risks from hNoVs in water and the subsequent effectiveness of engineering interventions to reduce this risk is complicated by the difficulty of culturing these viruses in vitro. The prohibitive costs and regulatory hurdles associated with human volunteer studies have led researchers to study the behavior and control of hNoV in water and wastewater through the use of cultivable surrogate viruses such as feline calicivirus, MNV and, more recently, Tulane virus.[5,56] However, the relevance of surrogate virus infectivity measurements to hNoVs has been questioned.[32] Additionally, viral reductions measured by RT-qPCR signals are thought to consistently underestimate infectivity reductions,[30] and attempts to modify molecular assays to measure capsid integrity[43] have not been accepted as universally effective. While recent progress in culturing hNoVs[28] has given hope that these difficulties will eventually be overcome, current best practice in assessing environmental persistence and resistance of hNoVs when human volunteer studies are not feasible

DOI: 10.1021/acs.est.7b02954
Environ. Sci. Technol. 2017, 51, 11918−11927

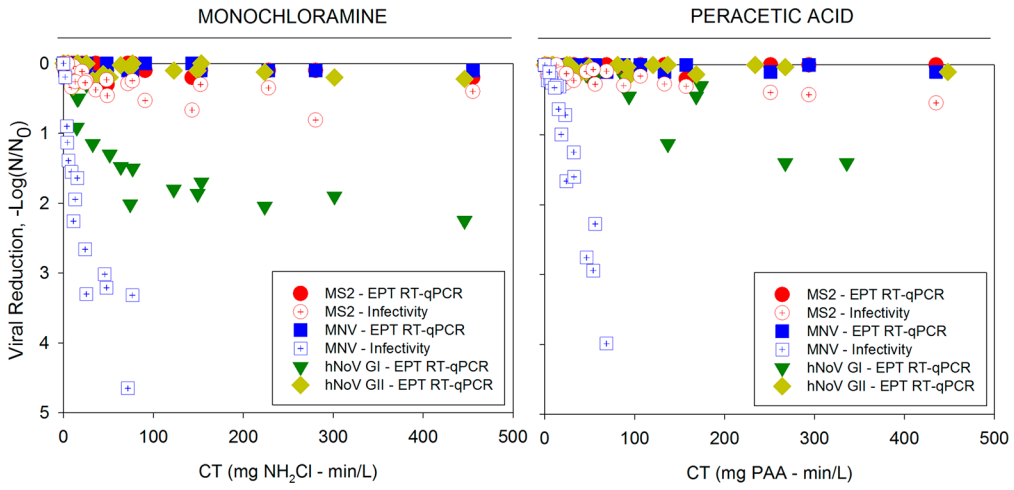


Figure 5. Comparison of integrated NH_2Cl and PAA CT values for reduction of MS2, MNV, human norovirus (hNoV) GI, and hNoV GII in municipal secondary wastewater effluent by RT-qPCR with enzymatic pretreatment (EPT) using RNase ONE. Infectivity data cited from Dunkin et al.[25]

remains to interpret hNoV RT-qPCR signals in light of associated surrogate infectivity and RT-qPCR measurements.

In order to comparatively investigate hNoV GI and GII viral reductions assessed by RT-qPCR (Figure 3) with surrogate RT-qPCR and infectivity reductions in 0.01 M PB (Figure 1), CT values for 1, 2, and 3 $\log_{10}$ viral reductions were developed using best-fit models. The results are shown in Table 1. Human norovirus GII was observed to be most resistant to monochloramine as measured by EPT RT-qPCR, with CT values for 1 and 2 $\log_{10}$ gene copy reductions of 1103 and 2675 mg-min/L, respectively. MS2 was also very resistant to monochloramine using the same detection method, with CT values of 474 and 1075 mg-min/L for 1 and 2 $\log_{10}$ reduction, respectively. MNV was most sensitive to monochloramine with a CT value of 18 mg-min/L for 1 $\log_{10}$ reduction by EPT RT-qPCR and 308 mg-min/L for 2 $\log_{10}$ reduction. Monochloramine CT values for hNoV GI were 123 mg-min/L for 1 $\log_{10}$ reduction and 356 mg-min/L for 2 $\log_{10}$ reduction.

MS2 and hNoV GII were also very resistant to PAA, though MS2 was more so. Predicted PAA CT values for 1 $\log_{10}$ (or greater) MS2 reduction by EPT RT-qPCR could not be derived due to disinfectant residual decay to zero during model simulation. CT values for 1 and 2 $\log_{10}$ EPT RT-qPCR reduction of hNoV GII by PAA were 551 and 873 mg-min/L, respectively. Similar to what was observed with monochloramine, MNV and hNoV GI were more susceptible than MS2 and hNoV GII. PAA CT values for 1 and 2 $\log_{10}$ reductions by EPT RT-qPCR were 128 and 354 mg-min/L for MNV and 177 and 342 for hNoV GI, respectively.

Modeling analysis was focused on experimental data in PB due to the relative lack of gene copy reductions observed in WW (Figures 2 and 3). However, comparison of hNoV and surrogate reductions in WW is shown in an integrated CT plot for monochloramine and PAA in Figure 5. While resistance to inactivation was generally greater in WW relative to PB as measured by both surrogate infectivity and molecular signals for all viruses, trends in comparative susceptibilities between the four viruses were similar. MS2 and hNoV GII exhibited nearly zero reduction by monochloramine and PAA as measured by EPT RT-qPCR at CT values up to 500 mg-min/L. Infectivity reductions for MS2 were also minimal in WW with less than 1 $\log_{10}$ reduction observed at CT values up to 500 mg-min/L for both disinfectants.[25] MNV likewise exhibited very little reduction by EPT RT-qPCR, though 4 $\log_{10}$ infectivity reduction was achieved at CT values under 100 mg-min/L for both disinfectants.[25] Interestingly, comparable reductions were observed for hNoV GI by EPT RT-qPCR in WW as in PB for both monochloramine and PAA. In short, MS2 and hNoV GII exhibited the most resistance to inactivation by monochloramine and PAA in both PB and WW while MNV and hNoV GI were more susceptible to both disinfectants in both matrices.

A recent helpful review conducted by Knight and colleagues on the persistence of hNoV compared to surrogates concluded that hNoVs are more resistant to inactivation than commonly used cultivable surrogate viruses.[30] However, our data suggests that the relationship between hNoV reduction and that of cultivable surrogates may vary by norovirus genogroup. We observed EPT RT-qPCR signals for hNoV GII to be as or more resistant to monochloramine and PAA than surrogate viruses, especially MNV. This is consistent with most studies that have compared the susceptibility of hNoV GII to free chlorine with that of cultivable surrogates.[10,12,14,57−59] However, we observed EPT RT-qPCR signals for hNoV GI to be as or less resistant to monochloramine and PAA than surrogate viruses, especially in WW. There are fewer data on hNoV GI responses to chemical oxidation relative to surrogates, but the limited evidence does suggest greater hNoV GI susceptibility. Shin and Sobsey reported that hNoV GI was less resistant to free chlorine than poliovirus-1 but comparable in resistance to MS2 by RT-qPCR.[13] Another study compared resistance of hNoV GI and feline calicivirus on berries and herbs to free chlorine but observed mixed results depending on the food surface.[57] In one study directly comparing hNoV GI and GII, Cromeans and colleagues observed hNoV GI to be more sensitive than hNoV GII to treatment with alcohols, chlorine, and high pressure.[60]

Complicating the comparison with existing data are the different study designs and large number of experimental variables that can affect experimental outcomes in hNoV persistence and inactivation studies. For example, disinfectant dose, exposure time, RT-qPCR amplicon size, PCR efficiency and inhibition, RT-qPCR pretreatment (e.g., RNase, intercalating dye, no pretreatment), experimental matrix, and viral preparation can all influence experimental results. One of the most important considerations when evaluating inactivation

DOI: 10.1021/acs.est.7b02954
Environ. Sci. Technol. 2017, 51, 11918−11927

data is that viruses are commonly analyzed in their original matrices: hNoVs in diluted fecal material and surrogate viruses in growth media. However, it is known that organic loading can impact virus survival during inactivation experiments.[30] In this study, we attempted to minimize impacts on experimental water matrices from fecal material or surrogate growth media by performing thorough purification for all viruses prior to seeding (density-based separation with ultracentrifugation for MNV, hNoV GI, and hNoV GII; ultrafiltration for MS2). As a result, all viral stocks together contributed only 0.2 mg/L as Cl_2 of chlorine demand to the final water matrices at the seeding concentrations employed in this study (data not shown).

In summary, RT-qPCR data indicate that hNoV GI and MNV are more susceptible to monochloramine and PAA than hNoV GII and MS2. As such, MNV infectivity data may be more representative of hNoV GI behavior than previously supposed.[30] However, the data also indicate that MNV inactivation data likely overestimate reductions for hNoV GII. These findings suggest that noroviruses exhibit genogroup dependent resistance to inactivation and highlight the importance of distinguishing between genogroups in hNoV persistence studies.

PAA versus Monochloramine Efficacy. Interest in PAA as a WW disinfectant has grown as utilities have searched for alternatives to chlorine in order to reduce halogenated DBP formation.[22] Additionally, there is limited information in the literature on the efficacy of PAA and monochloramine in reducing hNoVs. Therefore, one objective of this study was to compare the virucidal efficacy of these two disinfectants in treating hNoVs in secondary WW effluent.

From Figure 5, it is evident that neither disinfectant readily reduced hNoV GII in WW as measured by RT-qPCR. CT values approaching 500 mg-min/L for both disinfectants were unable to achieve even 0.5 $\log_{10}$ reduction. In PB, PAA was somewhat more effective in reducing hNoV GII than monochloramine, though neither disinfectant was very efficient. From Table 1, model predicted CT values for 1 $\log_{10}$ reduction of hNoV GII in PB by monochloramine and PAA as measured by EPT RT-qPCR were 1103 and 551 mg-min/L, respectively. For 2 $\log_{10}$ reduction, CT values were 2675 and 873 mg-min/L, respectively. Both disinfectants were more effective in treating hNoV GI. From Figure 5, monochloramine was able to achieve approximately 2 $\log_{10}$ reduction of hNoV GI in WW at CT values around 100 mg-min/L, while PAA achieved less than 1 $\log_{10}$ reduction at similar CT values. In PB, monochloramine and PAA exhibited similar effectiveness against hNoV GI. From Table 1, CT values for 1 $\log_{10}$ reduction of hNoV GI by monochloramine and PAA as measured by EPT RT-qPCR were 123 and 177 mg-min/L, respectively. For 2 $\log_{10}$ reduction, CT values were 356 and 342, respectively. In summary, monochloramine outperformed PAA in reducing hNoV GI in WW, though the differences in efficacy for the two disinfectants were less pronounced in PB. Additionally, PAA outperformed monochloramine in reducing hNoV GII in PB, though this effect was not observed in WW due to low efficacies for both disinfectants.

Implications for Wastewater Disinfection. Common operational CT values used in WWTP disinfection processes employing PAA or monochloramine to achieve a 3 $\log_{10}$ bacterial reduction are typically in the range of 30 to 60 mg-min/L.[22] At these CT values, minimal viral reductions for hNoV GII and surrogates were observed in WW as measured by RT-qPCR, while more reduction, up to 1.5 $\log_{10}$, was observed for hNoV GI. This indicates that wastewater treatment disinfection practices targeting hNoV GII will likely also result in equivalent or greater reductions for hNoV GI. It should be noted that RT-qPCR reductions are known to systematically underestimate infectivity,[30] suggesting that infectivity reductions for hNoV GI and GII were likely greater than observed molecular reductions. This observation is supported by the surrogate data presented under identical conditions in this study. While precise estimates of hNoV viability must wait until a suitable culturing method is developed, these data provide valuable comparisons between hNoV and surrogate molecular signals generated under identical conditions that can begin the process of informing regulators and engineers on WWTP design and operational practices. Once a culturing method is developed and the relationship between RT-qPCR and hNoV viability is established, then molecular technique will allow wastewater treatment staff to make a more rapid assessment and confirmation of the inactivation of the virus.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b02954.

Figure S1, dynamic light scattering results for MS2 bacteriophage stocks after Amicon filtration; Table S1, water quality parameters for secondary wastewater effluent sample; Table S2, summary of best fit model parameters for gene copy reductions of MS2, MNV, human norovirus GI, and human norovirus GII by PAA and NH_2Cl in 0.01 M phosphate buffer with and without enzymatic pretreatment with RNase; Table S3, summary of MS2 and MNV model parameters for each virus, disinfectant, and EPT scenario; Table S4, summary of MS2 and MNV model error statistics with Shapiro Wilk test for normality; Table S5, summary of human norovirus model parameters for each genogroup, disinfectant, and EPT scenario; Table S6, summary of human norovirus model error statistics with Shapiro Wilk test for normality (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: kschwab1@jhu.edu.

ORCID

Nathan Dunkin: 0000-0002-3786-051X

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was partially supported by the Metro Wastewater Reclamation District in Denver, CO (Jim McQuarrie, Edyta-Stec-Uddin, and Bradley Van Anderson). This work was also supported by the National Science Foundation Graduate Research Fellowship Program (award ID# DGE-1232825), the JHU/MWH Alliance, the Johns Hopkins Water Institute, and the Osprey Foundation of Maryland. Thanks are given to Dr. Robin Avery for her assistance in obtaining human norovirus samples.

DOI: 10.1021/acs.est.7b02954
Environ. Sci. Technol. 2017, 51, 11918−11927

REFERENCES

(1) Ahmed, S. M.; Hall, A. J.; Robinson, A. E.; Verhoef, L.; Premkumar, P.; Parashar, U. D.; Koopmans, M.; Lopman, B. A. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *Lancet Infect. Dis.* **2014**, *14* (8), 725−730.

(2) Lopman, B. A.; Hall, A. J.; Curns, A. T.; Parashar, U. D. Increasing rates of gastroenteritis hospital discharges in US adults and the contribution of norovirus, 1996−2007. *Clin. Infect. Dis.* **2011**, *52* (4), 466−74.

(3) Lopman, B. A.; Vinjé, J.; Glass, R. I. Noroviruses, Sapoviruses, and Astroviruses. In *Viral Infections of Humans*; Springer: New York, 2014; pp 479−499.

(4) Nenonen, N. P.; Hannoun, C.; Larsson, C. U.; Bergstrom, T. Marked genomic diversity of norovirus genogroup I strains in a waterborne outbreak. *Appl. Environ. Microbiol.* **2012**, *78* (6), 1846−52.

(5) Bae, J.; Schwab, K. J. Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. *Appl. Environ. Microbiol.* **2008**, *74* (2), 477−84.

(6) Seitz, S. R.; Leon, J. S.; Schwab, K. J.; Lyon, G. M.; Dowd, M.; McDaniels, M.; Abdulhafid, G.; Fernandez, M. L.; Lindesmith, L. C.; Baric, R. S.; Moe, C. L. Norovirus infectivity in humans and persistence in water. *Appl. Environ. Microbiol.* **2011**, *77* (19), 6884−8.

(7) Tuladhar, E.; Bouwknegt, M.; Zwietering, M.; Koopmans, M.; Duizer, E. Thermal stability of structurally different viruses with proven or potential relevance to food safety. *J. Appl. Microbiol.* **2012**, *112* (5), 1050−1057.

(8) Topping, J. R.; Schnerr, H.; Haines, J.; Scott, M.; Carter, M. J.; Willcocks, M. M.; Bellamy, K.; Brown, D. W.; Gray, J. J.; Gallimore, C. I.; Knight, A. I. Temperature inactivation of feline calicivirus vaccine strain FCV F-9 in comparison with human noroviruses using an RNA exposure assay and reverse transcribed quantitative real-time polymerase chain reaction-A novel method for predicting virus infectivity. *J. Virol. Methods* **2009**, *156* (1−2), 89−95.

(9) Keswick, B. H.; Satterwhite, T. K.; Johnson, P. C.; DuPont, H. L.; Secor, S. L.; Bitsura, J. A.; Gary, G. W.; Hoff, J. C. Inactivation of Norwalk virus in drinking water by chlorine. *Appl. Environ. Microbiol.* **1985**, *50* (2), 261−264.

(10) Duizer, E.; Bijkerk, P.; Rockx, B.; De Groot, A.; Twisk, F.; Koopmans, M. Inactivation of caliciviruses. *Appl. Environ. Microbiol.* **2004**, *70* (8), 4538−4543.

(11) Poschetto, L. F.; Ike, A.; Papp, T.; Mohn, U.; Böhm, R.; Marschang, R. E. Comparison of the sensitivities of noroviruses and feline calicivirus to chemical disinfection under field-like conditions. *Appl. Environ. Microbiol.* **2007**, *73* (17), 5494−5500.

(12) Nowak, P.; Topping, J.; Bellamy, K.; Fotheringham, V.; Gray, J.; Golding, J.; Wiseman, G.; Knight, A. Virolysis of feline calicivirus and human GII. 4 norovirus following chlorine exposure under standardized light soil disinfection conditions. *J. Food Prot.* **2011**, *74* (12), 2113−2118.

(13) Shin, G.-A.; Sobsey, M. D. Inactivation of norovirus by chlorine disinfection of water. *Water Res.* **2008**, *42* (17), 4562−4568.

(14) Kitajima, M.; Tohya, Y.; Matsubara, K.; Haramoto, E.; Utagawa, E.; Katayama, H. Chlorine inactivation of human norovirus, murine norovirus and poliovirus in drinking water. *Lett. Appl. Microbiol.* **2010**, *51* (1), 119−121.

(15) Haas, C. N.; Joffe, J.; Jacangelo, J. G.; Anmangandla, U.; Heath, M. Water quality and disinfection kinetics. *J. - Am. Water Works Assoc.* **1996**, *88* (3), 95−100.

(16) Thurston-Enriquez, J. A.; Haas, C. N.; Jacangelo, J.; Gerba, C. P. Chlorine inactivation of adenovirus type 40 and feline calicivirus. *Appl. Environ. Microbiol.* **2003**, *69* (7), 3979−85.

(17) Kahler, A. M.; Cromeans, T. L.; Roberts, J. M.; Hill, V. R. Source water quality effects on monochloramine inactivation of adenovirus, coxsackievirus, echovirus, and murine norovirus. *Water Res.* **2011**, *45* (4), 1745−1751.

(18) Cromeans, T. L.; Kahler, A. M.; Hill, V. R. Inactivation of adenoviruses, enteroviruses, and murine norovirus in water by free chlorine and monochloramine. *Appl. Environ. Microbiol.* **2010**, *76* (4), 1028−1033.

(19) Cowman, G. A.; Singer, P. C. Effect of bromide ion on haloacetic acid speciation resulting from chlorination and chloramination of aquatic humic substances. *Environ. Sci. Technol.* **1996**, *30* (1), 16−24.

(20) Krasner, S. W.; McGuire, M. J.; Jacangelo, J. G.; Patania, N. L.; Reagan, K. M.; Marco Aieta, E. The Occurrence of disinfection by-products in US drinking water. *J. - Am. Water Works Assoc.* **1989**, *81* (8), 41−53.

(21) Watson, K.; Shaw, G.; Leusch, F. D. L.; Knight, N. L. Chlorine disinfection by-products in wastewater effluent: Bioassay-based assessment of toxicological impact. *Water Res.* **2012**, *46* (18), 6069−6083.

(22) Kitis, M. Disinfection of wastewater with peracetic acid: a review. *Environ. Int.* **2004**, *30* (1), 47−55.

(23) Monarca, S.; Richardso, S. D.; Feretti, D.; Grottolo, M.; Thruston, A. D.; Zani, C.; Navazio, G.; Ragazzo, P.; Zerbini, I.; Alberti, A. Mutagenicity and disinfection by-products in surface drinking water disinfected with peracetic acid. *Environ. Toxicol. Chem.* **2002**, *21* (2), 309−318.

(24) Crebelli, R.; Conti, L.; Monarca, S.; Feretti, D.; Zerbini, I.; Zani, C.; Veschetti, E.; Cutilli, D.; Ottaviani, M. Genotoxicity of the disinfection by-products resulting from peracetic acid- or hypochlorite-disinfected sewage wastewater. *Water Res.* **2005**, *39* (6), 1105−1113.

(25) Dunkin, N.; Weng, S.; Schwab, K. J.; McQuarrie, J.; Bell, K.; Jacangelo, J. G. Comparative inactivation of murine norovirus and MS2 bacteriophage by peracetic acid and monochloramine in municipal secondary wastewater effluent. *Environ. Sci. Technol.* **2017**, *51* (5), 2972−2981.

(26) Rossi, S.; Antonelli, M.; Mezzanotte, V.; Nurizzo, C. Peracetic acid disinfection: A feasible alternative to wastewater chlorination. *Water Environ. Res.* **2007**, *79* (4), 341−350.

(27) Gehr, R.; Wagner, M.; Veerasubramanian, P.; Payment, P. Disinfection efficiency of peracetic acid, UV and ozone after enhanced primary treatment of municipal wastewater. *Water Res.* **2003**, *37* (19), 4573−4586.

(28) Ettayebi, K.; Crawford, S. E.; Murakami, K.; Broughman, J. R.; Karandikar, U.; Tenge, V. R.; Neill, F. H.; Blutt, S. E.; Zeng, X.-L.; Qu, L.; Kou, B.; Opekun, A. R.; Burrin, D.; Graham, D. Y.; Ramani, S.; Atmar, R. L.; Estes, M. K. Replication of human noroviruses in stem cell−derived human enteroids. *Science* **2016**, *353* (6306), 1387−1393.

(29) Wobus, C. E.; Karst, S. M.; Thackray, L. B.; Chang, K. O.; Sosnovtsev, S. V.; Belliot, G.; Krug, A.; Mackenzie, J. M.; Green, K. Y.; Virgin, H. W. Replication of norovirus in cell culture reveals a tropism for dendritic cells and macrophages. *PLoS Biol.* **2004**, *2* (12), e432.

(30) Knight, A.; Haines, J.; Stals, A.; Li, D.; Uyttendaele, M.; Knight, A.; Jaykus, L.-A. A systematic review of human norovirus survival reveals a greater persistence of human norovirus RT-qPCR signals compared to those of cultivable surrogate viruses. *Int. J. Food Microbiol.* **2016**, *216*, 40−49.

(31) Cook, N.; Knight, A.; Richards, G. P. Persistence and elimination of human norovirus in food and on food contact surfaces: a critical review. *J. Food Prot.* **2016**, *79* (7), 1273−1294.

(32) Richards, G. P. Critical review of norovirus surrogates in food safety research: rationale for considering volunteer studies. *Food Environ. Virol.* **2012**, *4* (1), 6−13.

(33) Weng, S.; Luo, Y.; Li, J.; Zhou, B.; Jacangelo, J. G.; Schwab, K. J. Assessment and speciation of chlorine demand in fresh-cut produce wash water. *Food Control* **2016**, *60*, 543−551.

(34) Kroneman, A.; Vega, E.; Vennema, H.; Vinjé, J.; White, P. A.; Hansman, G.; Green, K.; Martella, V.; Katayama, K.; Koopmans, M. Proposal for a unified norovirus nomenclature and genotyping. *Arch. Virol.* **2013**, *158* (10), 2059−2068.

(35) Hwang, S.; Alhatlani, B.; Arias, A.; Caddy, S. L.; Christodoulou, C.; Cunha, J. B.; Emmott, E.; Gonzalez-Hernandez, M.; Kolawole, A.; Lu, J.; Rippinger, C.; Sorgeloos, F.; Thorne, L.; Vashist, S.; Goodfellow, I.; Wobus, C. E. Murine norovirus: propagation,

DOI: 10.1021/acs.est.7b02954
Environ. Sci. Technol. 2017, 51, 11918−11927

quantification, and genetic manipulation. *Curr. Protoc Microbiol* **2014**, *33*, 15K.2.1−15K.2.61.
(36) Dunkin, N.; Weng, S.; Jacangelo, J. G.; Schwab, K. J. Minimizing bias in virally seeded water treatment studies: Evaluation of optimal bacteriophage and mammalian virus preparation methodologies. *Food Environ. Virol.* **2017**, DOI: 10.1007/s12560-017-9307-3.
(37) Kageyama, T.; Kojima, S.; Shinohara, M.; Uchida, K.; Fukushi, S.; Hoshino, F. B.; Takeda, N.; Katayama, K. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *J. Clin Microbiol* **2003**, *41* (4), 1548−1557.
(38) Gibson, K. E.; Opryszko, M. C.; Schissler, J. T.; Guo, Y.; Schwab, K. J. Evaluation of human enteric viruses in surface water and drinking water resources in southern Ghana. *Am. J. Trop. Med. Hyg.* **2011**, *84* (1), 20−29.
(39) Watson, H. E. A note on the variation of the rate of disinfection with change in the concentration of the disinfectant. *J. Hyg.* **1908**, *8* (04), 536−542.
(40) Haas, C. N.; Joffe, J. Disinfection under dynamic conditions: modification of Hom's model for decay. *Environ. Sci. Technol.* **1994**, *28* (7), 1367−9.
(41) Haas, C. N.; Joffe, J.; Anmangandla, U.; Hornberger, J.; Heath, M.; Glicker, J. *Development and Validation of Rational Design Methods of Disinfection*; AWWA: Denver, 1995.
(42) Anotai, J. *Effect of calcium ion on chemistry and disinfection efficiency of free chlorine at pH 10*. Ph.D. Dissertation, Drexel University, Philadelphia, PA, 1996.
(43) Knight, A.; Li, D.; Uyttendaele, M.; Jaykus, L. A. A critical review of methods for detecting human noroviruses and predicting their infectivity. *Crit. Rev. Microbiol.* **2013**, *39* (3), 295−309.
(44) Leifels, M.; Jurzik, L.; Wilhelm, M.; Hamza, I. A. Use of ethidium monoazide and propidium monoazide to determine viral infectivity upon inactivation by heat, UV-exposure and chlorine. *Int. J. Hyg. Environ. Health* **2015**, *218* (8), 686−693.
(45) Karim, M. R.; Fout, G. S.; Johnson, C. H.; White, K. M.; Parshionikar, S. U. Propidium monoazide reverse transcriptase PCR and RT-qPCR for detecting infectious enterovirus and norovirus. *J. Virol. Methods* **2015**, *219*, 51−61.
(46) Parshionikar, S.; Laseke, I.; Fout, G. S. Use of propidium monoazide in reverse transcriptase PCR to distinguish between infectious and noninfectious enteric viruses in water samples. *Appl. Environ. Microbiol.* **2010**, *76* (13), 4318−4326.
(47) Bertrand, I.; Schijven, J.; Sanchez, G.; Wyn-Jones, P.; Ottoson, J.; Morin, T.; Muscillo, M.; Verani, M.; Nasser, A.; de Roda Husman, A.; et al. The impact of temperature on the inactivation of enteric viruses in food and water: a review. *J. Appl. Microbiol.* **2012**, *112* (6), 1059−1074.
(48) Nuanualsuwan, S.; Cliver, D. O. Pretreatment to avoid positive RT-PCR results with inactivated viruses. *J. Virol. Methods* **2002**, *104* (2), 217−225.
(49) Prevost, B.; Goulet, M.; Lucas, F. S.; Joyeux, M.; Moulin, L.; Wurtzer, S. Viral persistence in surface and drinking water: Suitability of PCR pre-treatment with intercalating dyes. *Water Res.* **2016**, *91*, 68−76.
(50) Gall, A. M.; Shisler, J. L.; Mariñas, B. J. Inactivation kinetics and replication cycle inhibition of adenovirus by monochloramine. *Environ. Sci. Technol. Lett.* **2016**, *3* (4), 185−189.
(51) Sigstam, T.; Gannon, G.; Cascella, M.; Pecson, B. M.; Wigginton, K. R.; Kohn, T. Subtle differences in virus composition affect disinfection kinetics and mechanisms. *Appl. Environ. Microbiol.* **2013**, *79* (11), 3455−3467.
(52) Wigginton, K. R.; Pecson, B. M.; Sigstam, T. r.; Bosshard, F.; Kohn, T. Virus inactivation mechanisms: impact of disinfectants on virus function and structural integrity. *Environ. Sci. Technol.* **2012**, *46* (21), 12069−12078.
(53) Flores, M. J.; Lescano, M. R.; Brandi, R. J.; Cassano, A. E.; Labas, M. D. A novel approach to explain the inactivation mechanism of Escherichia coli employing a commercially available peracetic acid. *Water Sci. Technol.* **2014**, *69* (2), 358−363.
(54) Mayer, B. K.; Yang, Y.; Gerrity, D. W.; Abbaszadegan, M. The impact of capsid proteins on virus removal and inactivation during water treatment processes. *Microbiol. Insights* **2015**, 15.
(55) Kotwal, G.; Cannon, J. L. Environmental persistence and transfer of enteric viruses. *Curr. Opin. Virol.* **2014**, *4*, 37−43.
(56) Kniel, K. E. The makings of a good human norovirus surrogate. *Curr. Opin. Virol.* **2014**, *4*, 85−90.
(57) Butot, S.; Putallaz, T.; Sanchez, G. Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *Int. J. Food Microbiol.* **2008**, *126* (1), 30−35.
(58) Girard, M.; Ngazoa, S.; Mattison, K.; Jean, J. Attachment of noroviruses to stainless steel and their inactivation, using household disinfectants. *J. Food Prot.* **2010**, *73* (2), 400−404.
(59) Tung, G.; Macinga, D.; Arbogast, J.; Jaykus, L.-A. Efficacy of commonly used disinfectants for inactivation of human noroviruses and their surrogates. *J. Food Prot.* **2013**, *76* (7), 1210−1217.
(60) Cromeans, T.; Park, G. W.; Costantini, V.; Lee, D.; Wang, Q.; Farkas, T.; Lee, A.; Vinje, J. Comprehensive comparison of cultivable norovirus surrogates in response to different inactivation and disinfection treatments. *Appl. Environ. Microbiol.* **2014**, *80* (18), 5743−51.

DOI: 10.1021/acs.est.7b02954
Environ. Sci. Technol. 2017, 51, 11918−11927