Evaluation of virus reduction efficiency in wastewater treatment unit processes as a credit value in the multiple-barrier system for wastewater reclamation and reuse

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ABSTRACT

The virus reduction efficiency of each unit process is commonly determined based on the ratio of virus concentration in influent to that in effluent of a unit, but the virus concentration in wastewater has often fallen below the analytical quantification limit, which does not allow us to calculate the concentration ratio at each sampling event. In this study, left-censored datasets of norovirus (genogroup I and II), and adenovirus were used to calculate the virus reduction efficiency in unit processes of secondary biological treatment and chlorine disinfection. Virus concentration in influent, effluent from the secondary treatment, and chlorine-disinfected effluent of four municipal wastewater treatment plants were analyzed by a quantitative polymerase chain reaction (PCR) approach, and the probabilistic distributions of log reduction (LR) were estimated by a Bayesian estimation algorithm. The mean values of LR in the secondary treatment units ranged from 0.9 and 2.2, whereas those in the free chlorine disinfection units were from −0.1 and 0.5. The LR value in the secondary treatment was virus type and unit process dependent, which raised the importance for accumulating the data of virus LR values applicable to the multiple-barrier system, which is a global concept of microbial risk management in wastewater reclamation and reuse.

Key words | Bayesian estimation, left-censored data, log-normal distribution, paired and unpaired data, virus reduction efficiency, wastewater reclamation and reuse

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INTRODUCTION

Wastewater reclamation is one of the practical options to mitigate water stress, in which reclaimed wastewater is used for multiple purposes, including irrigation (Lubello *et al.* 2004), ground water recharge (Asano & Cotruvo 2004), recreational impoundment (Levine & Asano 2004), and drinking water source (Rodriguez *et al.* 2009). However, chemical and microbial constituents impose health risks on users of reclaimed wastewater and individuals who work in wastewater treatment (Toze 2006). Enteric viruses, such as human noroviruses, are major microbial constituents causing infection risks in wastewater reclamation, because these viruses are released to sewage with feces from symptomatic/asymptomatic individuals (Ozawa *et al.* 2007), and the reduction efficiency of these viruses from sewage is relatively lower than those of indicator microorganisms such as *Escherichia coli* (Ottoson *et al.* 2006).

To reduce the risks of waterborne disease outbreaks through reclaimed wastewater, it is critical to significantly reduce the virus quantity in reclaimed wastewater. The

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World Health Organization (WHO) guidelines stipulate that virus infection risks in wastewater reclamation should be managed by the concept of multiple-barrier system, in which a wastewater reclamation process is designed to achieve a target log reduction (LR) value by combining treatment unit processes with predetermined virus reduction efficiency (WHO 2006a). The target reduction efficiency is the sum of virus LR values in each unit process, which is determined not to exceed the additional tolerable burden of disease (10^{-6} disability adjusted life year per person per year ($DALY_{pppy}$)) in wastewater reclamation (Sano *et al.* 2016).

Under the multiple-barrier system concept, the virus reduction efficiency of wastewater treatment unit processes, such as secondary treatment and disinfection, has to be determined prior to the operation of the wastewater reclamation system. Commonly, the ratio of virus concentration in influent to that in effluent is regarded as the virus reduction efficiency, and this ratio is repeatedly analyzed to obtain the average efficiency of virus reduction (Ottoson *et al.* 2006; Sima *et al.* 2011; Frohnert *et al.* 2015; Schmitz *et al.* 2016). However, this practice of evaluating virus reduction efficiency is not always successful because the virus concentrations in influent and effluent often fall below the analytical quantification limit, which makes it impossible to calculate the virus concentration ratio at some sampling events. It is necessary to estimate the representative value of the virus reduction efficiency based on left-censored datasets, which include significant numbers of non-detects.

In this study, we evaluated the virus reduction efficiency in two treatment unit processes (secondary biological treatment and chlorine disinfection) of four municipal wastewater treatment plants (WWTPs) using observed left-censored datasets from the 1-year monthly quantitative survey data of norovirus genogroup I (NoV GI), norovirus genogroup II (NoV GII), and adenovirus (AdV). The posterior predictive distributions of virus concentration in influent and effluent were separately estimated using a Bayesian algorithm, and were used for calculating the probabilistic distribution of LR. Then, the applicability of the representative values of LR obtained in this study to the multiple-barrier system was discussed.

MATERIALS AND METHODS

Virus concentration datasets

The datasets of virus concentration acquired in our previous study (Katayama *et al.* 2008) were used in the present study. Briefly, wastewater samples of influent, secondary-effluent, and chlorine-disinfected effluent were collected monthly for a year from four municipal WWTPs. NoV GI, NoV GII, and AdV in the wastewater samples were quantified by the most probable number reverse transcription quantitative polymerase chain reaction (MPN-RT-qPCR) or MPN-qPCR assay. The decimally and serially diluted DNA/cDNA samples were applied to qPCR assay in triplicate for each sample, and in cases where some positive results were obtained among the most diluted series, further decimal dilution was done until all three tubes were virus-negative. Then, MPN was calculated from the positive/negative results of the qPCR assay. The virus number was given as PCR detection units (PDU)/mL for NoV GI, NoV GII, and AdV after adjustment of the volume used for detection.

Bayesian estimation of the distributions of virus concentration and LR

The extended Bayesian model reported in our previous studies (Kato *et al.* 2013, 2016) was employed to estimate the posterior predictive distributions of virus concentration in the wastewater samples. Virus concentration in wastewater from six municipal WWTPs were analyzed in our previous study (Katayama *et al.* 2008), but it was found that the datasets from two WWTPs did not comply with the requirement of number of datasets for the accurate estimation of contribution distribution (Ito *et al.* 2015). Thus, only the virus concentration datasets from four WWTPs were used in this study. Area of sewer coverage, number of residents in the covered area, and daily volume of influent in the four WWTPs are indicated in Table S1 (available with the online version of this paper).

To check the applicability of the extended Bayesian model to the datasets obtained in this study, the goodness of fit of the datasets for the normal, log-normal, and gamma distributions was tested using the Akaike information criterion (AIC) and Bayesian information criterion

(BIC) (Vrieze 2012). The AIC and BIC statistics are defined as follows:

$$AIC = -2(\log L - k) \quad (1)$$

and

$$BIC = -2\log L + k\log n, \quad (2)$$

where $\log L$ is the logarithmic maximum likelihood value, k is number of parameters, and n is the total number of data. The better fitting distribution to the virus density dataset was selected with the lowest AIC and BIC statistics. Since the extended Bayesian model assumes log-normality of the data, any datasets fitted to another distribution (normal or gamma distribution) were excluded from further analysis. The AIC and BIC values were calculated using R code, shown in the Supplementary information (available with the online version of this paper).

In the extended Bayesian model, a truncated log-normal distribution is adopted to interpret the data only above the quantification limit values as a conditional probability. The likelihood function is written as $p(X|\mu,\ \beta) = \prod_{i=1}^{n}(\varphi(\sqrt{\beta}\ (\theta_i - \mu))\ )^{1-y_i}\ \left((1-\varphi(\sqrt{\beta}(\theta_i-\mu)))TLN(x_i;\mu,\beta^{-1},\theta_i)\right)^{y_i}$. The virus concentration dataset X consists of n data pairs $X = \{(x_i,\ y_i)\}_{i=1}^{n}$, where x_i is the i-th sample and y_i is a Bernoulli variable based on quantification limit 10^{θ_i}; $y_i = 1$ if $x_i \geq 10^{\theta_i}$, and $y_i = 0$, otherwise. The two model parameters of mean μ and precision β are given with $\mu = \tilde{N}(0,\ 100)$ and $\beta = \widetilde{Gam}(0.01,\ 0.01)$ as a prior distribution (Paulo *et al.* 2005). The posterior predictive distribution of the virus concentration is obtained by $P_{pred}(x_{\log}|X) = \int N(x_{\log};\mu,\beta^{-1})p(\mu,\beta|X)d\mu d\beta$. Thereafter, the probabilistic distribution of virus LR is simply referred to as a log-ratio distribution between two corresponding distributions (Ito *et al.* 2015).

Representative LR value

For extracting percentiles of LR, random sampling of 10,000 values was performed based on the estimated probabilistic distribution of LR. Outliers in each set of 10,000 values were detected by using interquartile range (IQR) between first (25%tile) and third (75%tile) quartiles, in which any values at a greater distance from first or third quartiles than 1.5 times IQR were excluded as outliers. After the outlier exclusion, the percentiles were extracted from 0th to 100th percentile at a 1% interval (101 values in total). One-way analysis of variance (ANOVA) was then conducted to test the significant difference in the virus reduction efficiency among virus types or unit processes using the sets of extracted 101 values. The normality of the percentiles was checked by chi-square test before performing one-way ANOVA and Scheffe test. After one-way ANOVA, a Scheffe test (a multiple comparison test) was performed to compare the individual mean values of LR. These statistical analyses were performed using the Microsoft Excel statistics program version 2012 (Microsoft Corporation, SSRI, Tokyo, Japan).

RESULTS

Parameter estimation and prediction of NoV GI, NoV GII, and AdV concentrations

AIC and BIC statistics for three candidate probabilistic distributions (normal distribution, log-normal distribution, and gamma distribution) are indicated in Table S2 (available with the online version of this paper). The lower AIC and BIC statistics are given for the better fitting distribution to a dataset. All datasets except NoV GII in the chlorine-disinfected effluent in plant D were more closely fitted to the log-normal distribution (Table S2). Thus, the reduction efficiency of NoV GII by the chlorine disinfection in plant D was not calculated in the following step.

The logarithmic values of mean, standard deviation (SD), and 95% confidence interval of the concentrations of NoV GI, NoV GII, and AdV in influent, secondary-effluent, and chlorine-disinfected effluent were obtained from the estimated virus concentration distributions (Table S3, available with the online version of this paper). The mean concentration values of AdV in influent ranged from 2.0 to 2.8 $\log_{10}$ PDU/mL, and were higher than those of NoV GI (ranged from 1.2 to 1.7 $\log_{10}$ PDU/mL) and NoV GII (ranged from 1.3 to 2.0 $\log_{10}$ PDU/mL). The mean concentration values of all three viruses in the secondary effluent were reduced from those in the influent, where NoV GI was between −0.2 and 0.4 $\log_{10}$ PDU/mL, NoV GII was between −0.6 and 0.2 $\log_{10}$ PDU/mL, and AdV was

between 0.8 and 1.2 $\log_{10}$ PDU/mL. On the other hand, the reduction of these viruses during the chlorine disinfection unit processes was not recognizable. The maximum reduction (difference between mean values) was 0.4 $\log_{10}$ PDU/mL of NoV GI in plant C, but it is not clear at this stage of investigation whether this reduction is significantly larger than 0.0.

Comparison of virus LR values between virus types and plants

From these estimated distributions of virus concentration, a log-ratio distribution as a probabilistic distribution of LR was calculated for the secondary treatment (Figure 1(a)–1(c)) and the chlorine disinfection (Figure 2(a)–2(c)). To compare the virus reduction efficiency statistically, the percentile values from 0th to 100th were obtained at a 1% interval from 10,000 values randomly generated from the distributions of LR, and an ANOVA and the Scheffe test were performed (Figure 1(d)–1(f) and Figure 2(d)–2(f)). The normality of the extracted 101 percentiles from the distributions of LR was analyzed by chi-square test, which revealed that the extracted 101 percentiles were normally distributed (data not shown). Mean and SD values of each distribution are indicated in Table 1 (the secondary treatment) and Table 2 (the chlorine disinfection). ANOVA results show that there is a statistically significant ($p < 0.01$) difference between LR mean values in the secondary treatment of the four plants (Figure 1(d)–1(f)). The Scheffe test revealed that the LRs of NoV GI in plants A and B were lower than that in plant D ($p < 0.01$) (Figure 1(d)). NoV GII was reduced at a higher efficiency in plant D compared to plant B ($p < 0.01$) (Figure 1(e)). AdV was reduced in plant D more efficiently than plants A and B ($p < 0.01$) (Figure 1(f)). These results mean that the LR of test viruses is unit process dependent. The one-way ANOVA and

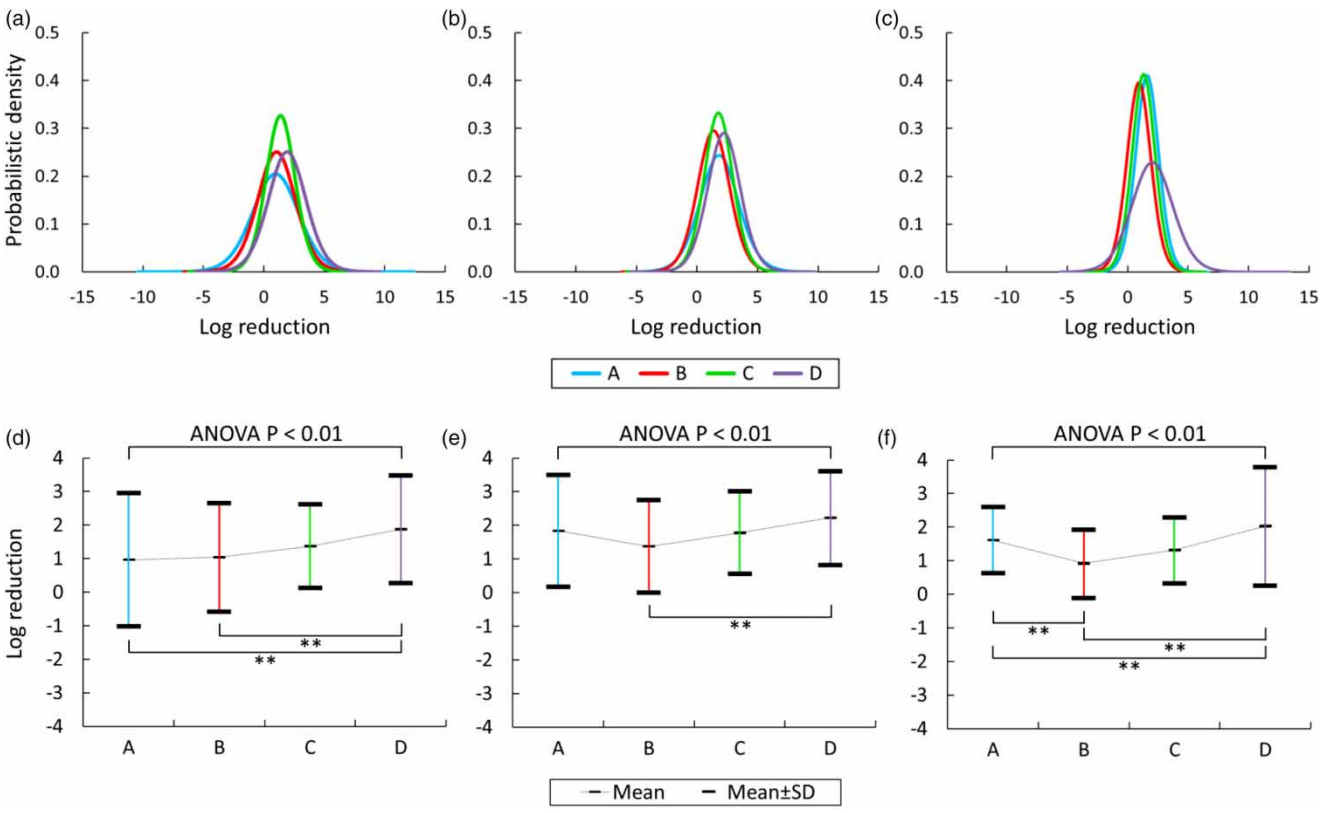

Figure 1 | Comparison of virus reduction efficiency among the secondary treatment unit processes of WWTPs A, B, C, and D: (a) LR distributions of NoV GI; (b) LR distributions of NoV GII; (c) LR distributions of AdV; (d) statistical tests (ANOVA and Scheffe test) of the difference in NoV GI reduction among the four plants; (e) statistical tests of the difference in NoV GII reduction among the four plants; (f) statistical tests of the difference in AdV reduction among the four plants. The Scheffe test at the significant levels of 0.05 (*) and 0.01 (**) was performed.

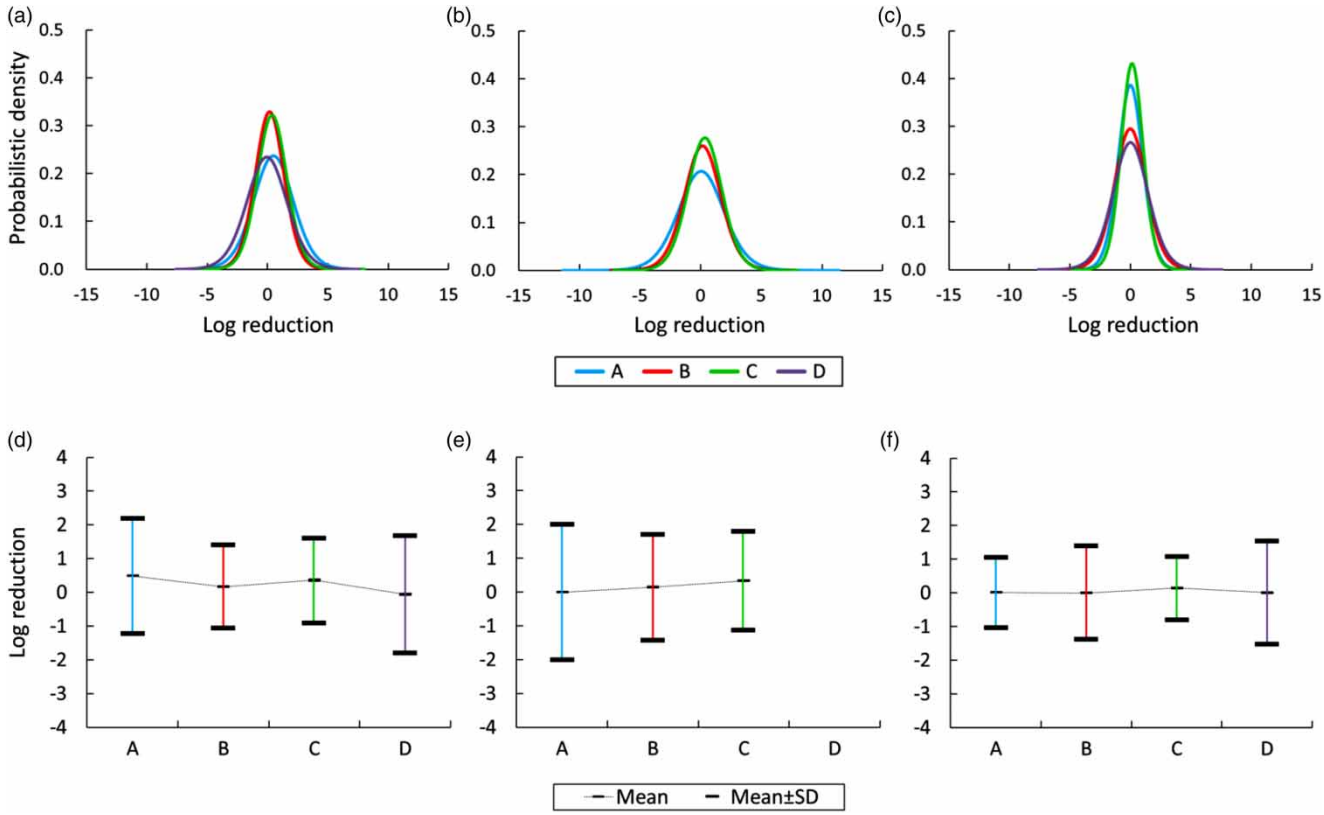

Figure 2 | Comparison of virus reduction efficiency among the chlorine disinfection unit processes of WWTPs A, B, C, and D: (a) LR distributions of NoV GI; (b) LR distributions of NoV GII; (c) LR distributions of AdV; (d) statistical tests (ANOVA and Scheffe test) of the difference in NoV GI reduction among the four plants; (e) statistical tests of the difference in NoV GII reduction among the four plants; (f) statistical tests of the difference in AdV reduction among the four plants. The Scheffe test at the significant levels of 0.05 (*) and 0.01 (**) was performed.

Table 1 | The mean and SD of LR of NoV GI, NoV GII, and AdV in secondary treatment of four WWTPs

Virus	Plant	Number of detects from the influent (positive rate)	Number of detects from the secondary effluent (positive rate)	Unpaired		Paired	
				Mean	SD	Mean	SD
NoV GI	A	11/12 (92%)	12/12 (100%)	1.0	2.0	–	–
	B	12/12 (100%)	12/12 (100%)	1.0	1.6	1.1	0.6
	C	12/12 (100%)	12/12 (100%)	1.4	1.2	1.4	0.6
	D	12/12 (100%)	11/12 (92%)	1.9	1.6	–	–
NoV GII	A	12/12 (100%)	10/12 (83%)	1.8	1.7	–	–
	B	12/12 (100%)	12/12 (100%)	1.4	1.4	1.4	0.5
	C	12/12 (100%)	12/12 (100%)	1.8	1.2	1.8	0.7
	D	12/12 (100%)	12/12 (100%)	2.2	1.4	2.2	0.8
AdV	A	12/12 (100%)	12/12 (100%)	1.6	1.0	1.6	0.6
	B	12/12 (100%)	12/12 (100%)	0.9	1.0	0.9	0.8
	C	12/12 (100%)	12/12 (100%)	1.3	1.0	1.3	0.6
	D	12/12 (100%)	11/12 (92%)	2.0	1.8	–	–

The mean and SD values were calculated from the 101 values of percentiles in 10,000 random values of virus log-reduction.

Table 2 | The mean and SD of LR of NoV GI, NoV GII, and AdV in the chlorine disinfection of four WWTPs

Virus	Plant	Number of detects from the influent (positive rate)	Number of detects from the chlorine-disinfected effluent (positive rate)	Unpaired		Paired	
				Mean	SD	Mean	SD
NoV GI	A	12/12 (100%)	12/12 (100%)	0.5	1.7	0.5	0.6
	B	12/12 (100%)	12/12 (100%)	0.2	1.2	0.2	0.5
	C	12/12 (100%)	12/12 (100%)	0.4	1.3	0.4	0.6
	D	11/12 (92%)	11/12 (92%)	−0.1	1.7	–	–
NoV GII	A	10/12 (83%)	10/12 (83%)	0.0	2.0	–	–
	B	12/12 (100%)	11/12 (91%)	0.1	1.6	–	–
	C	12/12 (100%)	12/12 (100%)	0.3	1.5	0.3	0.4
	D	12/12 (100%)	12/12 (100%)	–	–	0.3	0.4
AdV	A	12/12 (100%)	12/12 (100%)	0.0	1.1	0.0	0.4
	B	12/12 (100%)	12/12 (100%)	0.0	1.4	0.0	0.6
	C	12/12 (100%)	12/12 (100%)	0.1	0.9	0.1	0.8
	D	11/12 (92%)	12/12 (100%)	0.0	1.5	–	–

The mean and SD values were calculated from the 101 values of percentiles in 10,000 random values of virus reduction efficiency.

Scheffe test were also performed to test the significant difference in the LR mean values during the chlorine disinfection between WWTPs. No difference of LR mean values was observed for all test viruses in the chlorine disinfection unit process (Figure 2(d)–2(f)). This result is consistent with the qualitative recognition from Table S3, where almost no difference between virus concentrations in the secondary effluent and the chlorine-disinfected effluent was observed.

The one-way ANOVA and Scheffe test were then conducted to test the difference in the LR mean values among virus types during the secondary treatment (Figure 3). In

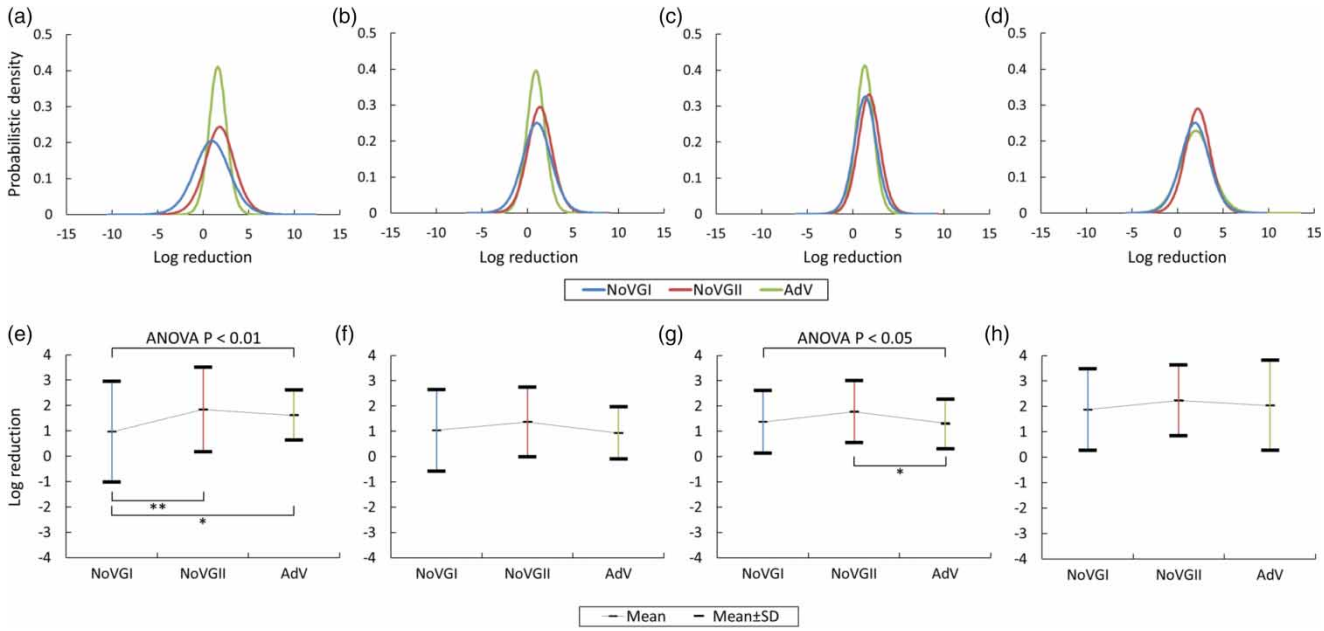

Figure 3 | Comparison of virus reduction efficiency in the secondary treatment unit process among the virus types: (a) LR distributions of NoV GI, NoV GII, and AdV in the secondary treatment unit process of plant A; (b) LR distributions of the viruses in the secondary treatment unit process of plant B; (c) LR distributions of the viruses in the secondary treatment unit process of plant C; (d) LR distributions of the viruses in the secondary treatment unit process of plant D; (e) statistical tests (ANOVA and Scheffe test) of the difference among virus types in plant A; (f) statistical tests of the difference among virus types in plant B; (g) statistical tests of the difference among virus types in plant C; (h) statistical tests of the difference among virus types in plant D. The Scheffe test at the significant levels of 0.05 (*) and 0.01 (**) was performed.

plant A, the LR mean value of NoV GI was significantly lower than those of NoV GII ($p < 0.01$) and AdV ($p < 0.05$) in the Scheffe test (Figure 3(e)). In plant C, the significant difference in the LR mean values was detected among virus types ($p < 0.05$) in the one-way ANOVA, and the LR mean value of NoV GII was higher than that of AdV ($p < 0.05$) in the Scheffe test (Figure 3(g)). Meanwhile, there was no significant difference in the LR mean values among virus types (Figure 3(f) and 3(h)) in plants B and D. The one-way ANOVA was also performed to compare the LR mean values of three tested viruses during the chlorine disinfection (Figure 4). There was no significant difference in the LR mean values among virus types in all four plants.

Output of virus LR values with paired or unpaired data

In the present study, the mean values of LR were calculated in such a way that the datasets of virus concentration in influent and effluent are separately used for estimating the probabilistic distribution (Tables 1 and 2, unpaired). On the other hand, it is possible to calculate the average value of the ratio of logarithmic virus concentration in influent and effluent when the positive rate is 100% for both influent and effluent (Tables 1 and 2, paired). Mean values were almost identical between unpaired and paired because the positive rate of the samples used in this study is relatively high (greater than 80%, Table S3). Meanwhile, SD values in unpaired datasets were larger than those in paired datasets. For example, the LR mean ± SD of NoV GI in plant B was 1.0 ± 1.6 $\log_{10}$ in the unpaired calculation, whereas 1.1 ± 0.6 $\log_{10}$ was obtained by the paired calculation. The larger SD values obtained from the unpaired datasets are attributable to the SD of virus concentration (Table S3).

DISCUSSION

In the present study, left-censored datasets of the concentration of enteric viruses (NoV GI, NoV GII, and AdV) in the influent and effluent of two unit processes (secondary treatment and chlorine disinfection processes) were used to separately estimate the probabilistic distributions of virus concentration in the influent and effluent, and then the probabilistic distributions of virus LR in each unit

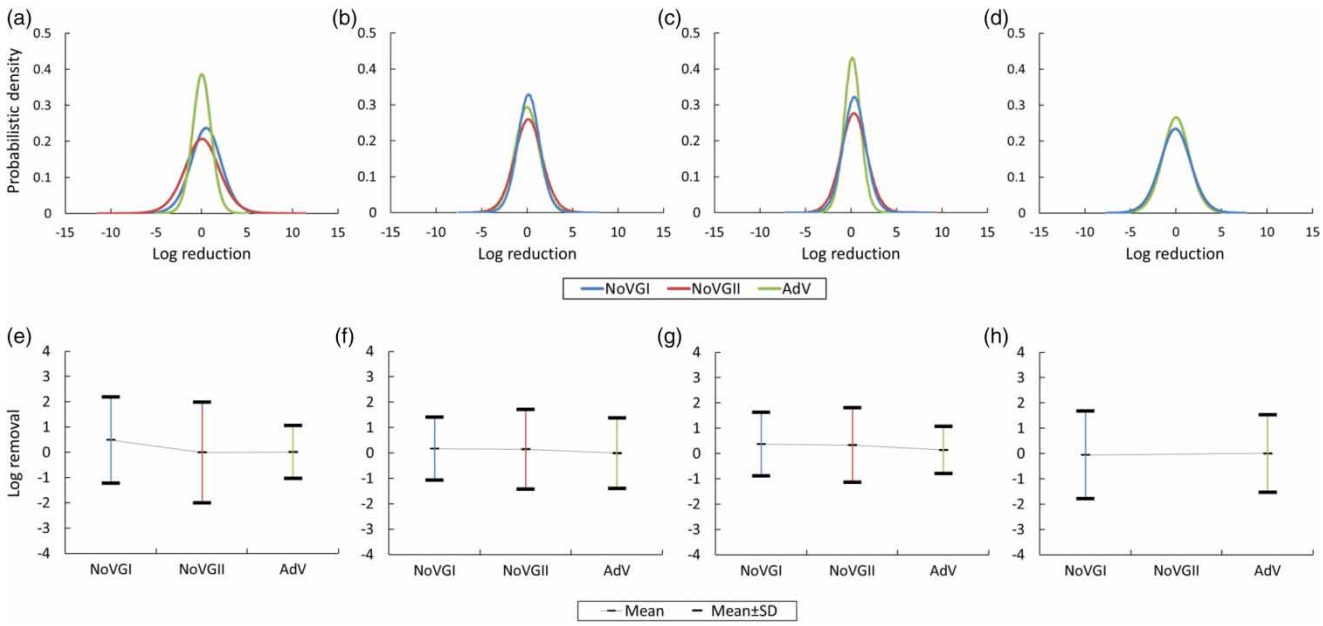

Figure 4 | Comparison of virus reduction efficiency in the chlorine disinfection unit process among the virus types: (a) LR distributions of NoV GI, NoV GII, and AdV in the chlorine disinfection unit process of plant A; (b) LR distributions of the viruses in the chlorine disinfection process of plant B; (c) LR distributions of the viruses in the chlorine disinfection unit process of plant C; (d) LR distributions of the viruses in the chlorine disinfection unit process of plant D; (e) statistical tests (ANOVA and Scheffe test) of the difference among virus types in plant A; (f) statistical tests of the difference among virus types in plant B; (g) statistical tests of the difference among virus types in plant C; (h) statistical tests of the difference among virus types in plant D. The Sheffe test at the significant levels of 0.05 (*) and 0.01 (**) was performed.

process of four municipal WWTPs were calculated. Percentile values of each estimated LR distribution were obtained and used to compare the virus LR values, which showed that the virus reduction efficiency in secondary treatment unit processes was virus type and unit process dependent.

Virus reduction in wastewater treatment unit processes is usually evaluated using paired (influent and effluent) datasets of the virus concentration (Ottoson *et al.* 2006; Sima *et al.* 2011; Dizer *et al.* 2015). The calculation of virus reduction efficiency using a paired dataset is based on an implicit assumption that a wastewater treatment unit is stably operated, and the variation in virus concentration in influent and effluent is small enough to detect the significant difference in mean values of virus concentration between inlet and outlet. However, it is very commonly observed that the virus concentration in effluent occasionally exceeds that in influent, which is caused by the large variation of virus concentration in wastewater samples (Katayama *et al.* 2008). The statistical approach proposed in this study, in which the datasets of virus concentration in influent and effluent are unpaired and separately used for estimating the probabilistic distribution, can circumvent the uncertainty issue in the quantification of virus concentration in wastewater. The 'unpaired' approach also makes sense from the viewpoint of wastewater sampling, because the true retention time of viruses in a unit reactor is never known, and the appropriate time interval between the sampling of influent and effluent cannot be determined (Rachmadi *et al.* 2016). The unpaired approach facilitates the design of a sampling plan because investigators do not need to take influent and effluent samples simultaneously, or can take these samples even in a different period separately, as long as the unit process is continuously operated without any problems. One issue to which we must pay attention in the unpaired calculation is the larger SD values of LR compared with those in the paired calculation (Tables 1 and 2). These calculated LR values will be used as LR credit values in quantitative microbial risk assessment (QMRA) (WHO 2006a), and thus a larger SD will give a broader interval in the risk assessment. Since a broader interval of virus infection risk allows us to address an unsafe situation (very low or no virus reduction) in wastewater treatment, a larger SD value obtained in the unpaired calculation is preferable to those in the paired calculation from the viewpoint of safer usage of reclaimed wastewater.

The one-way ANOVA showed that the virus reduction efficiency in the secondary treatment was dependent on the unit process (Figure 1). Operational conditions in the secondary treatment and chlorine disinfection, such as retention time, water temperature, and flow volume, are not identical between plants (Table S1), which explains the divergent virus reduction efficiency among plants. With the multiple-barrier system, water engineers have to determine the combination of unit processes for wastewater reclamation to exceed the target value of LR (WHO 2006a), which means that the average value of pathogen reduction efficiency in each unit process has to be determined in advance. Systematic review and meta-analysis approaches can be employed for this purpose (Xagoraraki *et al.* 2014; Pouillot *et al.* 2015). Since a variety of uncertainties in the unit operation (e.g., influent volume fluctuation, water temperature change, etc.) and configuration difference among units (e.g., reaction tank volume, mixture strength, etc.) have to be taken into account, a framework such as Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) is recommended for calculating the average value of pathogen reduction efficiency (Sano *et al.* 2016). Three parameters are required in PRISMA: mean, SD, and sample number (Moher *et al.* 2009). In this study, percentile values from the 0th to 100th at a 1% interval (101 values in total) were extracted from the 10,000 values generated from the estimated probabilistic distribution of LR, and mean and SD values were calculated (Tables 1 and 2). These representative values and the sample number (101) are available in the PRISMA framework.

The dependency of virus reduction efficiency on virus type in the secondary biological treatment raises one important issue about the selection of indicators for the pathogen reduction. Since the daily monitoring of pathogen reduction in a wastewater reclamation system is not practical because of the labor- and cost-intensive practice of pathogen quantification, the usage of indicator microorganisms, such as *Escherichia coli* and phages, for validating the significant reduction of pathogens in wastewater have been discussed (Harwood *et al.* 2005). The WHO and the Australian Academy of Technological Sciences and Engineering (ATSE) suggested selecting bacteriophages, especially somatic coliphages and F-specific bacteriophages, as viral process efficiency indicators (WHO 2006a; ATSE 2013). However,

the inconsistency of removal property between three virus types (NoV GI, NoV GII, and AdV) even within the identical biological treatment unit (Figure 3) makes it difficult to select one indicator microorganism that can represent the reduction of multiple types of pathogenic viruses. WHO guidelines also point out that there is a limitation of using a single indicator to show the whole microbiological risk (WHO 2006b). The development of appropriate methodology for validating virus reduction and disinfection performance in the daily operation of wastewater reclamation systems is a challenging issue (Sano *et al.* 2016).

Another important issue is the assumed statistical model for virus concentration in wastewater. The microorganisms in water have been considered as discrete particles, and the microbe concentration may follow a probabilistic distribution (Eisenhart & Wilson 1943). In this study, we assumed that virus concentration in wastewater is log-normally distributed (Kato *et al.* 2013). The log-normal distribution has been used for expressing microbe concentration in water (Tanaka *et al.* 1998; Haas *et al.* 1999), but the fitting test must be conducted before the estimation of virus concentration distribution Bayesian model. In this study, AIC and BIC were used because these are common statistics for selecting appropriate probabilistic distributions to datasets (Penny 2012; Vrieze 2012). All datasets except NoV GII concentration in the chlorine-disinfected effluent of plant D were better fitted to the log-normal distribution (Table S2). Another algorithm assuming other probabilistic distributions, such as a gamma distribution, should be prepared in future studies. One possible situation is that two different distributions may have to be used for the virus concentrations in the influent and effluent. It is not always possible to derive a ratio distribution between different probabilistic distributions mathematically. Future studies should construct a methodology for estimating the LR probabilistic distribution in the event that two distributions have to be used separately for the virus concentrations in the influent and effluent.

The multiple-barrier system concept has been employed not only in the WHO guidelines (WHO 2006a) but also in those of the United States Environmental Protection Agency (USEPA 2012) and the Natural Resource Management Ministerial Council of Australia (NRMMC 2006), which means that the multiple-barrier system has been accepted as a global concept for health risk management in wastewater reclamation (Sano *et al.* 2016). The proposed approach in this study is compatible with the multiple-barrier system, enabling evaluation of virus reduction efficiency of wastewater treatment unit processes even based on the left-censored dataset. The estimated distribution of LR gives all representative values (mean, SD, and sample number) required in the PRISM framework, which makes it possible to involve left-censored datasets of virus concentration in the influent and effluent of a unit process in the multiple-barrier system.

CONCLUSIONS

The LR values of enteric viruses in secondary biological treatment processes were calculated based on left-censored datasets. The virus reduction efficiency was dependent on virus type and unit process, which emphasizes the importance of data accumulation of enteric virus concentration in influent and effluent of a wastewater treatment unit process. The proposed approach in this study provides all the information required in meta-analysis for calculating the average value of virus LR, and is compatible with the multiple-barrier system for wastewater reclamation and reuse.

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