Title:

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

Short title:

Virus log removal as a credit value in multiple-barrier system for water reuse

Author names and affiliations:

Toshihiro Ito[a], Tsuyoshi Kato[b], Makoto Hasegawa[b], Hiroyuki Katayama[c], Satoshi Ishii[d], Satoshi Okabe[a], Daisuke Sano[a]*

[a]Division of Environmental Engineering, Faculty of Engineering, Hokkaido University, North 13, West 8, Kita-ku, Sapporo, Hokkaido 060-8628, Japan

[b]Department of Computer Science, Graduate School of Engineering, Gunma University, Tenjinmachi 1-5-1, Kiryu, Gunma 376-8515, Japan

[c]Department of Urban Engineering, School of Engineering, University of Tokyo, Bunkyo-ku, Tokyo, 190-8518, Japan

[d]Department of Soil, Water, and Climate, University of Minnesota, 258 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA

***Corresponding Author Address:**

Division of Environmental Engineering, Faculty of Engineering, Hokkaido University, North 13, West 8, Kita-ku, Sapporo, Hokakaido 060-8628, Japan.

Telephone/Fax: +81-11-706-7597.

E-mail: dsano@eng.hokudai.ac.jp

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, norovirus genogroup II and adenovirus were used to evaluate the virus reduction efficiency in unit processes of the secondary biological treatment and the chlorine disinfection. The virus concentration in influent, effluent from the secondary treatment and chlorine-disinfected effluent of four municipal wastewater treatment plants were analyzed by a quantitative PCR approach, and the probabilistic distributions of log reduction (LR) in each unit 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 of the data accumulation of virus reduction with respect to each unit process for acquiring representative LR values applicable to the multiple-barrier system, which is a global concept of microbial risk management in wastewater reclamation and reuse.

Keywords

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

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 the 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 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 number 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 one-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 qPCR (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 study (Kato et al. 2013; Kato et al., 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 resident in the covered area and daily volume of influent in the four WWTPs are indicated in Table S1.

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:

$$\mathrm{AIC} = -2(\log L - k) \qquad (1)$$

and

$$\mathrm{BIC} = -2\log L + k\log n, \qquad (2)$$

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.

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 $p(X|\mu,\beta) = \prod_{i=1}^{n}\left(\varphi\left(\sqrt{\beta}(\theta_i-\mu)\right)\right)^{1-y_i}\left(\left(1-\varphi\left(\sqrt{\beta}(\theta_i-\mu)\right)\right)TLN(x_i;\mu,\beta^{-1},\theta_i)\right)^{y_i}$. 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 $10^{\theta i}$; $y_i = 1$ 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 $\mu = \widetilde{N}(0,100)$ 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 analysis were performed using the Microsoft Excel statistics program version 2012 (Microsoft corporation, SSRI, Tokyo).

151

152 **Results**

153 **Parameter estimation and prediction of NoVGI, NoVGII and AdV concentrations**

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

160 The logarithmic values of mean, standard deviation (SD) and 95% confidence interval
161 of the concentrations of NoV GI, NoV GII and AdV in influent, secondary-effluent and chlorine-
162 disinfected effluent were obtained from the estimated virus concentration distributions (Table
163 S3). The mean concentration values of AdV in influent ranged from 2.0 to 2.8 $\log_{10}$ PDU/mL,
164 were higher than those of NoV GI (ranged from 1.2 to 1.7 $\log_{10}$ PDU/mL) and NoV GII (ranged
165 from 1.3 to 2.0 $\log_{10}$ PDU/mL). The mean concentration values of all three viruses in the
166 secondary effluent were reduced from those in the influent, where NoV GI was between -0.2 and
167 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
168 and 1.2 $\log_{10}$ PDU/mL. On the other hand, the reduction of these viruses during the chlorine
169 disinfection unit processes was not recognizable. The maximum reduction (difference between
170 mean values) was 0.4 $\log_{10}$ PDU/mL of NoV GI in the plant C, but it is not clear at this stage of
171 investigation whether this reduction is significantly larger than 0.0.

172

173 **Comparison of virus LR values between virus types and plants**

174 From these estimated distributions of virus concentration, a log-ratio distribution as a
175 probabilistic distribution of LR was calculated for the secondary treatment (Fig. 1(a)-(c)) and the
176 chlorine disinfection (Fig. 2(a)-(c)). To compare the virus reduction efficiency statistically, the

177 percentile values from 0th to 100th were obtained at a 1% interval from 10,000 values randomly
178 generated from the distributions of LR, and an ANOVA and the Scheffe test were performed (Fig.
179 1(d)-(f) and Fig. 2(d)-(f)). The normality of the extracted 101 percentiles from the distributions
180 of LR was analyzed by chi-square test, which revealed that the extracted 101 percentiles were
181 normally distributed (data now shown). Mean and SD values of each distribution are indicated in
182 Table 1 (the secondary treatment) and Table 2 (the chlorine disinfection). ANOVA results show
183 that there is a statistically significant ($p < 0.01$) difference between LR mean values in the
184 secondary treatment of the four plants (Fig. 1(d)-(f)). The Scheffe test revealed that the log
185 reductions of NoV GI in plants A and B were lower than that in plant D ($p < 0.01$) (Fig. 1(d)).
186 NoV GII was reduced at higher efficiency in plant D compared to plant B ($p < 0.01$) (Fig. 1(e)).
187 AdV was reduced in plant D more efficiently than plants A and B ($p < 0.01$) (Fig. 1(f)). These
188 results mean that the LR of test viruses is unit process-dependent. The one-way ANOVA and
189 Scheffe test were also performed to test the significant difference in the LR mean values during
190 the chlorine disinfection between WWTPs. No difference of LR mean values was observed for
191 all test viruses in the chlorine disinfection unit process (Fig. 2(d)-(f)). This result is consistent
192 with the qualitative recognition from Table S3, where almost no difference between virus
193 concentrations in the secondary effluent and the chlorine-disinfected effluent was observed.
194 The one-way ANOVA and Scheffe test were then conducted to test the difference in the
195 LR mean values among virus types during the secondary treatment (Fig. 3). In plant A, the LR
196 mean value of NoV GI was significantly lower than those of NoV GII ($p < 0.01$) and AdV ($p <$
197 0.05) in Scheffe test (Fig. 3(e)). In plant C, the significant difference in the LR mean values was
198 detected among virus types ($p < 0.05$) in the one-way ANOVA, and the LR mean value of NoV
199 GII was higher than that of AdV ($p < 0.05$) in Scheffe test (Fig. 3(g)). Meanwhile, there was no
200 significant difference in the LR mean values among virus types (Fig. 3(f) and (h)) in plants B
201 and D. The one-way ANOVA was also performed to compare the LR mean values of three tested
202 viruses during the chlorine disinfection (Fig. 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 (Table 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 (Table 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 (NoVGI, NoV GII and AdV) in the influent and effluent of two unit processes (the secondary treatment and the 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 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 (Table 1 and 2). These calculated LR values will be used as LR credit values in 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 (Fig. 1). Operational conditions in the secondary treatment and chlorine disinfection, such as retention time, water temperature and flow volume,

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

271 The dependency of virus reduction efficiency on virus type in the secondary biological
272 treatment raises one important issue about the selection of indicators for the pathogen reduction.
273 Since the daily monitoring of pathogen reduction in wastewater reclamation system is not
274 practical because of the labor- and cost-intensive practice of pathogen quantification, the usage
275 of indicator microorganisms, such as *Escherichia coli* and phages, for validating the significant
276 reduction of pathogens in wastewater have been discussed (Harwood et al. 2005). WHO and the
277 Australian Academy of Technological Sciences and Engineering (ATSE) suggested selecting
278 bacteriophages, especially somatic coliphages and F-specific bacteriophages, as viral process
279 efficiency indicators (WHO, 2006a; ATSE, 2013). However, the inconsistency of removal
280 property between three virus types (NoV GI, GII and AdV) even within the identical biological

treatment unit (Fig. 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 in the further study (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 and 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 (Haas et al., 1999; Tanaka et al., 1998), but the fitting test must be conducted before the 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) (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.

5. 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.

Acknowledgements

This work was supported by the Japan Society for the Promotion of Science Through Grant-in-Aid for Scientific Research (A) (26249075).

References

Asano T. & Cotruvo J. 2004 Groundwater recharge with reclaimed municipal wastewater: Health and regulatory considerations. *Water Research*, **38**(8), 1941-1951. doi:10.1016/j.watres.2004.01.023

ATSE (Australian Academy of Technological Sciences and Engineering). 2013 Drinking Water Through Recycling. The Benefits and Costs of Supplying Direct to the Distribution System. Available: http://www.australianwaterrecycling.com.au/projects/direct-potable-reuse-in-australia-a-discussion-paper [accessed 19 October 2015].

Dizer H., Brackmann B., Rahman M. A., Szewzyk R., Spenger C., Holzbecher E. & Lopez-Pila J. M. 2015 Virus removal vs. subsurface water velocity during slow sand filtration. *Journal of Water and Health*, **13**(2), 371-382. doi:10.2166/wh.2014.086.

Eisenhart C. & Wilson P. W. 1943 Statistical methods and control in bacteriology. *Microbiology and Molecular Biology Reviews*, **7**(2), 57-137.

Frohnert, A., Kreißel, K., Lipp, P., Dizer, H., Hambsch, B., Szewzyk, R. & Selinka, H. C. 2015 Removal of surrogate bacteriophages and enteric viruses from seeded environmental waters using a semi-technical ultrafiltration unit. *Food and Environmental Virology*, **7**(2), 173-182. doi:10.1007/s12560-015-9190-8

Haas C. N., Rose J. B. & Gerba C. P. 1999 Quantitative microbial risk assessment. John Wiley and Sons.

Harwood V. J., Levine A. D., Scott T. M., Chivukula V., Lukasik J., Farrah S. R. & Rose J. B. 2005 Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Applied and Environmental Microbiology*, 71(6), 3163. doi:10.1128/AEM.71.6.3163-3170.2005

Ito T., Kato T., Takagishi T., Okabe S. & Sano D. 2015 Bayesian modeling of virus removal efficiency in wastewater treatment processes. *Water Science and Technology*, 72(10), 1789-1795. doi:10.2166/wst.2015.402

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**(6-7), 1441-1448.

359 doi:10.1016/j.watres.2007.10.029
360 Kato, T., Kobayashi, A., Ito, T., Miura, T., Ishii, S., Okabe, S. & Sano, D. 2016 Estimation of
361 concentration ratio of indicator to pathogen-related gene in environmental water based on
362 left-censored data. *Journal of Water and Health*, **14**(1), 14-25. doi:10.2166/wh.2015.029
363 Kato T., Miura T., Okabe S., Sano D. 2013 Bayesian modeling of enteric virus density in
364 wastewater using left-censored data. *Food and Environmental Virology*, **5**(4), 185–193.
365 doi:10.1007/s12560-013-9125-1
366 Levine A. D. & Asano T. 2004 Recovering sustainable water from wastewater. *Environmental*
367 *Science and Technology*, **38**(11), 201A-208A. doi:10.1021/es040504n
368 Lubello C., Gori R., Nicese F.P. & Ferrini F. 2004 Municipal-treated wastewater reuse for plant
369 nurseries irrigation. *Water Research*, **38**(12), 2939-2947. doi:10/1016/j.watres.2004.03.037
370 Moher D., Liberati A., Tetzlaff J., Altman D. G. & Grp P. 2009 Preferred reporting items for
371 systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6:e1000097.
372 doi:10.7326/0003-4819-151-4-200908180-00135
373 NRMMC (Natural Resource Management Ministerial Council). 2006 National Water Quality
374 Management Strategy. Australian Guidelines for Water Recycling: Managing Health and
375 Environmental Risks (Phase 1). Available:
376 http://www.environment.gov.au/system/files/resources/044e7a7e-558a-4abf-b985-
377 2e831d8f36d1/files/water-recycling-guidelines-health-environmental-21.pdf [accessed 19
378 October 2015].
379 Rodriguez D., van Buynder P., Lugg R., Blair P., Devine B., Cook A. & Weinstein P. 2009
380 Indirect potable reuse: A sustainable water supply alternative. *International Journal of*
381 *Environmental Research and Public Health*, **6**(3), 1174-1209. doi:10.3390/ijerph6031174
382 Toze S. 2006. Water reuse and health risks - Real vs. perceived. *Desalination* **187**, 41–51.
383 doi:10.1016/j.desal.2005.04.066
384 Ottoson J., Hansen A., Björlenius B., Norder H. & Stenström T. A. 2006 Removal of viruses,

parasitic protozoa and microbial indicators in conventional and membrane processes in a wastewater pilot plant. *Water Research*, **40**(7), 1449–1457.

Ozawa K., Oka T., Takeda N. & Hansman G. S. 2007 Norovirus infections in symptomatic and asymptomatic food handlers in Japan. *Journal of Clinical Microbiology*, **45**(12), 3996-4005. doi:10.1128/JCM.01516-07

Paulo M. J., van der Voet H., Jansen M. J. W., ter Braak C. J. F. & van Klaveren J. D. 2005 Risk assessment of dietary exposure to pesticides using a Bayesian method. *Pest Management Science*, 61, 759–766.

Penny W. D. 2012 Comparing dynamic causal models using AIC, BIC and free energy. *Neuroimage*, **59**(1), 319-330.

Pouillot R., van Doren J. M., Woods J., Plante D., Smith M., Goblick G., Roberts C., Locas A., Hajen W., Stobo J., White J., Holtzman J., Buenaventura E., Burkhardt W., Catford A., Edwards R., DePaola A. & Calci K. R. 2015 Meta-analysis of the reduction of norovirus and male-specific coliphage concentrations in wastewater treatment plants. *Applied and Environmental Microbiology*, **81**(14), 4669-4681. doi:10.1128/AEM.00509-15

Rachmadi, A. T., Kitajima, M., Pepper, I. L. & Gerba, C. P. 2016 Enteric and indicator virus removal by surface flow wetlands. *Science of the Total Environment*, **542**, 976-982. doi:10.1016/j.scitotenv.2015.11.001

Sano D., Amarasiri M., Hata A., Watanabe T. & Katayama H. 2016 Risk management of viral infectious diseases in wasteawater reclamation and reuse: Review. *Environment International*, **91**, 220-229. doi:10.1016/j.envint.2016.03.001

Schmitz, B. W., Kitajima, M., Campillo, M. E., Gerba, C. & Pepper, I. L. 2016 Virus reduction during advanced bardenpho and conventional wastewater treatment processes. *Environmental Science and Technology*. doi:10.1021/acs.est.6b01384

Sima L. C., Schaeffer J., Le Saux J. C., Parnaudeau S., Elimelech M. & Le Guyader F. S. 2011 Calicivirus removal in a membrane bioreactor wastewater treatment plant. *Applied and*

411 *Environmental Microbiology*, **77**, 5170-5177. doi:10.1128/AEM.00583-11
412 Tanaka H., Asano T. Schroeder E. D. & Tchobanoglous G. 1998 Estimating the safety of
413 wastewater reclamation and reuse using enteric virus monitoring data. *Water Environment*
414 *Research*, **70**(1), 39-51. doi:10.2175/106143098X126874
415 USEPA (United States Environmental Protection Agency). 2012 Guidelines for Water Reuse.
416 Available: http://nepis.epa.gov/Adobe/PDF/P100FS7K.pdf [accessed 19 October 2015].
417 Vrieze S. I. 2012 Model selection and psychological theory: a discussion of the differences
418 between the Akaike information criterion (AIC) and the Bayesian information criterion
419 (BIC). *Psychological Methods*, **17**(2), 228-243. doi:10.1037/a0027127
420 WHO. 2006a WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater. Volume
421 1. Policy and Regulatory Aspects.
422 WHO, 2006b. WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater. Volume
423 4. Excreta and greywater use in agriculture.
424 Xagoraraki I., Yin Z. & Svambayev Z. 2014 Fate of viruses in water systems. *Journal of*
425 *Environmental Engineering*, **140**(7), 04014020-1-18. doi:10.1061/(ASCE)EE.1943-
426 7870.0000827
427

Figure legends

Figure 1. Comparison of virus reduction efficiency among the secondary treatment unit processes of wastewater treatment plant A, B, C and D. (a), Log reduction (LR) distributions of norovirus genogroup I (NoV GI); (b), LR distributions of norovirus genogroup II (NoV GII); (c) LR distributions of adenovirus (AdV); (d), Statistical tests (ANOVA and Scheffe test) of the difference in NoV GI reduction among 4 plants; (e), Statistical tests of the difference in NoV GII reduction among 4 plants; (f), Statistical tests of the difference in AdV reduction among 4 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 wastewater treatment plant A, B, C, and D. (a), Log reduction (LR) distributions of norovirus genogroup I (NoV GI); (b), LR distributions of norovirus genogroup II (NoV GII); (c) LR distributions of adenovirus (AdV); (d), Statistical tests (ANOVA and Scheffe test) of the difference in NoVGI reduction among 4 plants; (e), Statistical tests of the difference in NoV GII reduction among 4 plants; (f), Statistical tests of the difference in AdV reduction among 4 plants. The Scheffe test at the significant levels of 0.05 (*) and 0.01 (**) was performed.

Figure 3. Comparison of virus reduction efficiency in the secondary treatment unit process among the virus types. (a), Log reduction (LR) distributions of norovirus genogroup I (NoV GI), norovirus genogroup II (NoV GII) and adenovirus (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

454 types in plant C; (h), Statistical tests of the difference among virus types in plant D. The Scheffe
455 test at the significant levels of 0.05 (*) and 0.01 (**) was performed.
456
457 **Figure 4**. Comparison of virus reduction efficiency in the chlorine disinfection unit process
458 among the virus types. (a), Log reduction (LR) distributions of norovirus genogroup I (NoVGI),
459 norovirus genogroup II (NoVGII) and adenovirus (AdV) in the chlorine disinfection unit process
460 of plant A; (b), LR distributions of the viruses in the chlorine disinfection process of plant B; (c),
461 LR distributions of the viruses in the chlorine disinfection unit process of plant C; (d), LR
462 distributions of the viruses in the chlorine disinfection unit process of plant D; (e), Statistical
463 tests (ANOVA and Scheffe test) of the difference among virus types in plant A; (f), Statistical
464 tests of the difference among virus types in plant B; (g), Statistical tests of the difference among
465 virus types in plant C; (h), Statistical tests of the difference among virus types in plant D. The
466 Sheffe test at the significant levels of 0.05 (*) and 0.01 (**) was performed.
467

Table 1. The mean and standard deviation (SD) of log reduction of norovirus genogroup I (NoV GI), norovirus genogroup II (NoV GII) and adenovirus (AdV) in secondary treatment of four wastewater treatment plants. The mean and SD values were calculated from the 101 values of percetiles in 10,000 random values of virus log-reduction.

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	—	—

Table 2. The mean and standard deviation (SD) of log reduction of norovirus genogroup I (NoV GI), norovirus genogroup II (NoV GII) and adenovirus (AdV) in the chlorine disinfection of four wastewater treatment plants. The mean and SD values were calculated from the 101 values of percentiles in 10,000 random values of virus reduction efficiency.

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	—	—