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Review article

Risk management of viral infectious diseases in wastewater reclamation and reuse: Review

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A B S T R A C T

Inappropriate usage of reclaimed wastewater has caused outbreaks of viral infectious diseases worldwide. International and domestic guidelines for wastewater reuse stipulate that virus infection risks are to be regulated by the multiple-barrier system, in which a wastewater treatment process composed of sequential treatment units is designed based on the pre-determined virus removal efficiency of each unit. The objectives of this review were to calculate representative values of virus removal efficiency in wastewater treatment units based on published datasets, and to identify research topics that should be further addressed for improving implementation of the multiple-barrier system. The removal efficiencies of human noroviruses, rotaviruses and enteroviruses in membrane bioreactor (MBR) and conventional activated sludge (CAS) processes were obtained by a systematic review protocol and a meta-analysis approach. The log10 reduction (LR) of norovirus GII and enterovirus in MBR were 3.35 (95% confidence interval: 2.39, 4.30) and 2.71 (1.52, 3.89), respectively. The LR values of rotavirus, norovirus GI and GII in CAS processes were 0.87 (0.20, 1.53), 1.48 (0.96, 2.00) and 1.35 (0.52, 2.18), respectively. The systematic review process eliminated a substantial number of articles about virus removal in wastewater treatment because of the lack of information required for the meta-analysis. It is recommended that future publications should explicitly describe their treatment of left-censored datasets. Indicators, surrogates and methodologies appropriate for validating virus removal performance during daily operation of wastewater reclamation systems also need to be identified.

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1. Introduction

Enteric viruses have caused infectious diseases owing to fecally-contaminated irrigation and environmental water. For example, a large outbreak of norovirus occurred in eastern Germany in 2012, which was likely caused by imported frozen strawberries from China (Bernard et al., 2014). Multiple genotypes of norovirus were found...
from tested strawberries and gastroenteritis patients, which supported the speculation that imported strawberries were cultivated with untreated wastewater (Måde et al., 2013). Imported foodstuffs contaminated with pathogenic viruses originating from wastewater have also caused outbreaks in the other developed countries (Éthelberg et al., 2010; Pérez-Sautu et al., 2011; Terio et al., 2015), in particular when food products are imported from areas where water and sanitation systems are poorly installed and wastewater is directly used for irrigation. In the worst scenario, pathogens eradicated from developed countries can be imported from endemic areas via foodstuff, after which such neglected pathogens can cause outbreaks of infectious diseases in importing countries without any protective aids such as vaccination.

In the future, wastewater will have to be used as irrigation water in arid areas such as Australia, India, Pakistan, Vietnam, the northern part of China, and Middle Eastern countries including Israel (Hanjra et al., 2012). Wastewater is also becoming an important irrigation water source in the USA due to the heavy dependence of agricultural production on fossil waters from three major over-exploited aquifers (Marston et al., 2015). The National Science Foundation/Mathematics and Physical Sciences Directorate (NSF/MPS) (2014) reported that one opportunity for transformative research can be found in ensuring a sustainable water supply for agriculture, including the expansion of supply through wastewater recycling. In these settings, enteric viruses require special attention because they are excreted to wastewater in larger numbers by infected individuals (up to 10^{12} viruses per gram feces) compared to other enteric pathogens (Drexier et al., 2011; Kirby et al., 2014), and they are typically so highly infectious that less than 10 particles is often enough to cause an infection in susceptible individuals (Thebault et al., 2013; Kirby et al., 2015). Removal of these viruses by wastewater treatment processes is required if treated wastewater is reclaimed; however, enteric viruses are more resistant than pathogenic bacteria to several water disinfection treatments such as filtration, chlorination, UV irradiation, and ozonation, due to the physicochemical properties of viral particles (WHO, 2011). These features highlight enteric viruses as the most important health issue for the safe use of reclaimed water (ATSE, 2013).

In this review article, current guidelines for designing wastewater reclamation and reuse systems from the viewpoint of virus risk management are overviewed, and the efficiency of virus removal from wastewater by currently employed wastewater treatment units are shown by the results of meta-analysis. The regulations for validating the virus removal efficiency of wastewater treatment processes during daily operation are then described in a separate section. Finally, the research areas that should be addressed in the future for better application of wastewater reclamation and reuse are identified from the viewpoint of virus risk management.

2. Guidelines for designing wastewater reclamation and reuse systems from the viewpoint of virus risk management

The World Health Organization (WHO) published four volumes of guidelines for the safe use of wastewater, excreta, and greywater in 2006. Policy and regulatory aspects are compiled in volume 1, where WHO employs 10^{-6} disability adjusted life year loss per person per year (DALY loss ppyp) as a tolerable additional disease burden (WHO, 2006), which is equivalent to rotavirus disease and infection risks of approximately 10^{-4} and 10^{-3} per person per year, respectively (Mara, 2006). These values of tolerable additional disease burden can be used to calculate the acceptable level of pathogen concentration in water if the dose-response relationship is available for the pathogen of interest; however, the WHO suggests guideline users set performance targets for wastewater treatment processes in place of the acceptable pathogen concentrations in treated wastewater. The performance target is a value of removal efficiency expressed in common logarithm of the ratio of pathogen concentration in untreated wastewater to that in treated wastewater. For example, the WHO guideline describes that the health-based target (10^{-6} DALY loss ppyp) for viral pathogens can be achieved by a total virus reduction of 2–3 log_{10} in reclaimed wastewater for restricted irrigation (the irrigation of all crops except salad crops and vegetables that may be eaten uncooked), while a 6–7 log_{10} pathogen reduction is needed for unrestricted irrigation (including the irrigation of salad crops and vegetables that may be eaten uncooked) and localized irrigation (e.g., drip irrigation). The total pathogen reduction in log_{10} units is obtained by a combination of wastewater treatment including disinfection, natural die-off and health protection measures such as washing produce prior to consumption. The combination of wastewater treatment units is called the multiple-barrier system, consisting of multiple steps where the pathogen removal efficiency (pathogen removal credit) of each step has already been evaluated and determined as a credited log_{10} reduction (LR) value (Fig. 1). WHO (2006) recommends guideline users measure reduction/inactivation of adenoviruses, reoviruses, enteroviruses, and hepatitis A virus to validate the efficacy of each treatment when the treatment system starts operation. The WHO (2006) guidelines also suggest that each country should establish national criteria and procedures that suit its epidemiological, social, and economic needs.

The United States Environmental Protection Agency (USEPA) (2012) guideline explicitly notes that setting a tolerable virus concentration in reclaimed wastewater (‘virus limit’) is not recommended for the following reasons: 1) viruses are well reduced by appropriate wastewater treatments, 2) identification and enumeration of viruses is time- and labor-consuming, 3) detection of infectious viruses in water is further labor- and time-consuming, 4) molecular-based virus detection does not always indicate the presence of infectious viruses, and 5) waterborne viral infections due to reclaimed water have not been documented. Instead of setting a virus limit, the control of viral infectious diseases caused by contaminated reclaimed wastewater is to be achieved by the multiple-barrier system, which coincides with the WHO guideline. Based on the USEPA guidelines for water reuse, several U.S. state governments have set performance targets for virus removal in wastewater reclamation. In the Groundwater Replenishment Reuse Project (GRRP) of the state of California, where the most stringent and intimate regulations related to recycled water have been established, a 12 LR of viruses along with a 10 LR of both Giardia cysts and Cryptosporidium oocysts in the wastewater is required when treated wastewater is used for groundwater recharge intended for indirect potable reuse (California State Water Resources Control Board, 2015). The reductions should be accomplished by three or more successive treatment process units, each of which can reduce 1–6 log_{10} of viruses. In the state of Texas, where direct potable reuse (DPR) of reclaimed water is being implemented, 12, 10, and 10 LRs of viruses, Cryptosporidium, and Giardia, respectively, are recommended as a baseline for DPR when untreated wastewater is used as a source (Texas Water Development Board, 2015). These required LR values were developed based on the general approach presented in “Potable Reuse: State of the Science Report and Equivalency Criteria for Treatment Trains”, which was prepared as part of the Water Reuse Research Foundation Project 11-02 (WRRF Project 11-02) (Texas Water Development Board, 2015). The general approach for determining the required pathogen LR used available data obtained from literatures and calculated drinking water concentrations needed to meet a health goal in the USEPA Surface Water Treatment Rule (SWTR), which is a risk of 1 infection per 10,000 exposed individuals per year (Texas Water Development Board, 2015). On another front, Texas Commission on Environmental Quality (TCEQ) has established minimum (or baseline) LR and/or inactivation targets for the DPR, which are 8, 5.5, and 6 LRs of viruses, Cryptosporidium, and Giardia, respectively, when wastewater treatment plant effluent is used as a source, although these baseline LR targets are considered a starting point for the TCEQ approval process and may be revised based on data collected from the wastewater effluent in question (Texas Water Development Board, 2015).
In Australia, viruses are considered to be the most important pathogens in water and water treatment systems are designed considering virus removal on a preferential basis. The Australian guideline is based on a study of quantitative microbial risk assessment (QMRA) conducted to determine the required virus reduction level for reclaimed water treatment (NRMMC, 2006). In this assessment, rotavirus was selected as a representative virus because of its prevalence, high infectivity, and the availability of a reliable dose-response model. In this analysis, the required reduction level varied from 2.1 to 6.5 \( \log_{10} \) depending on the degree of human contact. Again, the multiple-barrier system is adopted by the Australian national guidelines (NRMMC, 2006), as with WHO (2006) and USEPA (2012). The advantage of the multiple-barrier system is that the simultaneous failure of multiple process units is not probable and even if one process unit becomes dysfunctional, the remaining barriers should be capable of providing sufficient protection. Based on the Australian national guidelines (NRMMC, 2006), several Australian states have set criteria for virus control in wastewater reclamation. For example, the state of Victoria has set a criterion that the performance of each process unit is determined by evaluating its virus reductions (The state of Victoria, 2008). A variety of enteric viruses that can cause waterborne infection, e.g., enterovirus, adenovirus, rotavirus, norovirus and other caliciviruses, hepatitis A virus, astrovirus, and coronavirus, are listed as examples of viral contaminants in the guidelines of Queensland, Australia (The state of Queensland, 2008). In the state of Queensland, a 6.5 \( \log_{10} \) of viruses in treated wastewater is necessary to be classified as the highest quality reclaimed water (class A+), which is more stringent than the required reductions for either bacteria (5 \( \log_{10} \)) or protozoa (5 \( \log_{10} \)). If the reclaimed water is used for augmentation of drinking water supplies, an 8 \( \log_{10} \) of viruses along with an 8 \( \log_{10} \) of bacteria and protozoa is required according to QMRA studies.

Some countries in Europe, including Cyprus, France, Greece, Hungary, Italy, Portugal, and Spain, have guidelines or regulations for wastewater reuse (Raso, 2013; Sanz and Gawlik, 2014). Among them, only France has set a regulation regarding viral contamination, in which a 2 to 4 \( \log_{10} \) of F-specific bacteriophage by reclamation processes is required. In the Netherlands, a unique QMRA-based viral regulation in drinking water is implemented, but viruses in reclaimed water are not regulated (Smeets et al., 2009) because fresh water resources are rich and the use of reclaimed water is mostly limited to industrial purposes (Rietveld et al., 2011; Paranychianakisa et al., 2015). In Singapore, approximately 30% of the water supply is provided by a reclaimed water program called NEWater. Secondary treated wastewater is sequentially treated by microfiltration, reverse osmosis (RO), and UV for producing NEWater (PUB, 2010). No specific regulation for reclaimed water quality is present in Singapore, but NEWater has been shown to meet WHO and USEPA standards for drinking water and to be negative for enteroviruses (PUB, 2010). NEWater is not supplied directly as drinking water but is thought to be safe for direct potable use.

These examples indicate that the multiple-barrier system serves as a global basic concept for the regulation of pathogenic viruses in wastewater reclamation and reuse, in which these viruses are not being regulated by virus concentration limits in the treated wastewater, but rather are managed by the sequential use of technological units for wastewater treatment and disinfection where the virus removal efficiency of each treatment unit has previously been determined. The required virus removal efficiency (performance target) is determined by QMRA, and subsequently it is required to combine sequential wastewater treatment and disinfection practices in such a way that the sum of pre-determined virus removal efficiencies exceeds the performance target. Under this concept of the multiple-barrier system, two important issues must be addressed: how to determine the virus removal efficiency of treatment steps prior to operation and how to ensure that the pre-determined performance targets are met or exceeded in daily operation. These two issues are discussed in the following sections.

### 3. Virus removal efficiency of wastewater treatment technologies

Solid removal techniques such as filtration, flocculation, coagulation, and sedimentation, and disinfection techniques such as UV irradiation and ozonation are regarded as effective for reducing viruses in wastewater and producing virologically safe reclaimed water; however, the values of virus removal efficiency for these processes are heavily dependent on operation conditions, such that efficiencies can vary greatly even within the same reactor for a given treatment (Teunis et al., 2009). It is necessary for water engineers to know the “average” virus removal efficiency of each wastewater treatment unit as a virus LR credit in order to design efficient wastewater reclamation systems under the concept of multiple-barrier system (Fig. 1).

In this section, we employed a meta-analysis approach to obtain the representative values of virus removal efficiency for wastewater.
treatment process units. We selected norovirus (genogroup I and II), rotavirus, and enterovirus as enteric viruses of interest and papers investigating the removal efficiency of these viruses in wastewater treatment processes were collected using a systematic review protocol. Google Scholar, PubMed, and Web of Knowledge were used as search engines in this study. Relevant articles published between 2000 and 2015 were found in all three databases using the search terms “removal efficiency” and “wastewater treatment” combined with each viral genus name of Norovirus, Rotavirus and Enterovirus. All articles obtained from PubMed and Web of Knowledge coincided with those from Google Scholar, therefore all the article numbers indicated below are the results obtained from Google Scholar. Norovirus, Rotavirus and Enterovirus combined with the above-mentioned terms gave 261, 397, and 281 retrievals, respectively (Table S1).

A meta-analysis methodology was employed to estimate how effectively viruses are reduced by two widely-employed wastewater treatment technologies: membrane bioreactors (MBRs) and activated sludge (AS). Data obtained from Google Scholar was processed according to the PRISMA flow diagram used in clinical medicine with some modifications (Moher et al., 2009). The PRISMA flow diagram explains the process of selection of relevant articles among the records obtained via web search (Figure S1). After identification of records, they were screened to eliminate non-English records, articles without virus data or quantitative virus data, doctoral dissertations, book chapters, review papers, posters, conference proceedings, and other technical reports. The number of articles selected by the initial screening were 29, 23, and 16 for norovirus, rotavirus, and enterovirus, respectively (Table S1).

After the screening, information required for the calculation of averaged virus LR values was extracted from the full text articles, including quantitative data of virus reductions (average and standard deviation) and the size of samples used in the calculation of virus reductions. Table S1 summarizes the articles selected for the meta-analysis stage. Additionally the data in one article was directly obtained from one of the authors (Katayama et al., 2008). Arithmetic mean and standard deviation values of LR were calculated from the available data when these values were not specified explicitly in the articles. The ImageJ program developed by the National Institutes of Health was used for data extraction from figures if necessary. Extracted data of LR were separated into that of MBR and AS processes. Meta-analysis calculation and Forest plot construction were performed using a Microsoft Excel software package originally developed by Neyeloff et al. (2012), with some modifications. The step-by-step calculation procedure is described in the Supplementary materials. Sample numbers considered for calculation, data extraction, and calculation methods are summarized in Table S2.

The forest plot of LR values of norovirus GII by MBR with 95% confidence interval (CI) values is shown in Fig. 2. Four articles were selected based on the availability of quantified LR data (Sima et al., 2011; Simmons et al., 2011; Chaudhry et al., 2015; Miura et al., 2015) (Table S2). These studies investigated virus concentrations in the influent and effluent of an MBR process by quantitative reverse-transcription PCR (RT-qPCR) and LR values, each calculated as a ratio of virus concentration in influent to that in effluent at each sampling event, were presented. LR values were extracted from these studies only when viruses were detected in both influent and effluent samples. We excluded paired data of virus concentrations in influent and effluent from the calculation when either the influent or effluent sample was negative (non-detect) for virus quantification. Since viruses are not detected from an effluent sample when highly efficient virus removal is achieved, this practice may lead to the exclusion of superior LR values (higher virus removal efficiencies) from the calculation. The reasonable treatment of datasets including non-detects is discussed in the following section. The mean values of norovirus GII LR varied between 0.96 (Miura et al., 2015) and 4.16 (Chaudhry et al., 2015), which may reflect differences between these MBR configurations and their operational conditions (Table S2). Since the PCR for norovirus does not discriminate genotypes within a genogroup, the fluctuation of virus removal efficiency between studies may also reflect that different genotypes or strains of norovirus GII were removed dissimilarly, although genotype- or strain-dependent removal properties have not been thoroughly investigated (Miura et al., 2015). Chaudhry et al. (2015) reported the gradual increase of LR when the time after membrane wash was increased from 0 to 24 h, which implies that the level of membrane fouling is one of the determinant factors of virus LR. The hydraulic retention time (HRT) is another influential factor for virus reduction in MBR (Campos et al., 2013), but not all of the selected publications specified the details of operational conditions including HRT. Among the retrieved papers, Sima et al. (2011) and Chaudhry et al. (2015) did not specify HRT values of the reactors, because these studies did not focus on the relationship between HRT and virus removal efficiency. Since these determinant factors of virus removal efficiency in wastewater treatment units may be beneficial information for wastewater engineers, it is recommended that future publications with regards to virus LR include detailed operational conditions (HRT, time after membrane wash, etc.) of the wastewater treatment units.

Fig. 2. Removal efficiency of norovirus genogroup II by membrane bioreactors. Black square is the mean value of virus log reduction (LR) obtained in each study, and a bar indicates 95% confidence interval (CI). The sample number in each study is indicated in a parenthesis next to 95% CI values. The total LR value is obtained by the calculation procedure indicated in Supplementary materials.
Since heterogeneity was detected via Q-statistics (Eq. (S4)) between studies of norovirus GII reduction in MBR, a random effect model utilized by Neyeloff et al. (2012) was applied (see Eqs. (S5) to (S7)) in order to calculate a representative value of 3.35 LR (95% CI between 2.39 and 4.30, Fig. 2). Some articles were excluded from this representative value calculation; for example, Zhou et al. (2015) investigated the concentrations of multiple virus types including norovirus GII before and after an MBR process by RT-qPCR, but no information about sample size in the calculation of average LR was described, thereby making it impossible to include the presented LR data in the meta-analysis. da Silva et al. (2007) also analyzed the removal efficiency of norovirus GII in MBR, but all effluent samples were norovirus GII-negative and could not be included in the meta-analysis. All manuscripts excluded in this meta-analysis because of the lack of required information are likely trustworthy and useful as case studies for virus removal in wastewater, but without key experimental information it is not possible to utilize such data for calculating the average value of virus LRs by meta-analysis in the present study. It is strongly recommended that arithmetic mean and standard deviation values of LR as well as sample size, which are necessary for meta-analysis calculations, be clearly described in the publication, otherwise LR datasets cannot be included in the calculation of representative values of virus removal efficiency.

We retrieved three articles related to the reduction of enteroviruses in MBR processes (Ottoson et al., 2006; Simmons et al., 2011; Miura et al., 2015) (Table S2). Q-statistics detected heterogeneity between these studies as with norovirus GII; therefore, the random effect model was again employed to calculate a representative virus removal value of 2.71 LR with a 95% CI between 1.52 and 3.89 (Fig. 3). The broad CI may be attributable to different operational conditions (Table S2), and/or different removal efficiencies among viral species within the genus Enterovirus. The RT-qPCR assay used for enterovirus in these studies targets the 5′ non-coding region, which is highly conservative among enteroviruses and includes more than 100 serotypes. This means that the virus removal efficiency obtained in each study is the representative removal efficiency of multiple enterovirus types in each MBR process. Zhou et al. (2015) was again not involved in the meta-analysis, because of the lack of sample size information. Francy et al. (2012) reported the removal efficiency of enterovirus in MBR, but there was only one enterovirus-positive sample in the MBR effluent, therefore this study was not included in the meta-analysis.

With the exception of norovirus GII and enterovirus, we could not retrieve a sufficient number of publications regarding the removal of human enteric viruses (rotavirus, adenovirus, aichivirus, astrovirus, hepatitis A virus and sapovirus) in MBR that met the described systematic review criteria. It may be possible to supplement this lack of datasets for human enteric viruses by providing additional phage removal information; however, we found that the majority of publications regarding phage removal in wastewater treatment units were based on the infectious phage concentration in wastewater samples measured by cell culture methods (e.g., Purnell et al., 2015). It is not common to directly compare the LR values calculated based on genome copy to those calculated from infectious units, because the reduction of infectious units can be caused by both the physical removal and disinfection of viruses. For this reason, we chose not to include phage reduction datasets in this study.

Four articles were selected for rotavirus reduction in AS processes (Li et al., 2011; Prado et al., 2011; El-Senousy et al., 2015; Qiu et al., 2015) (Table S3). The mean values obtained in each study ranged between 1.31 (Prado et al., 2011) and 2.15 (El-Senousy et al., 2015) (Fig. 4). Q-statistics detected heterogeneity between these studies as well, and a representative removal efficiency of 0.87 LR with 95% CI between 0.20 and 1.53 was calculated. Kitajima et al. (2014) reported the removal efficiency of rotavirus in two wastewater treatment plants employing AS processes, but the removal efficiency was obtained for the combined process of AS and chlorine treatment. Since this study aims to calculate the virus LR in each treatment unit, we did not use the datasets in Kitajima et al. (2014) in the meta-analysis.

Four articles were also obtained pertaining to norovirus GI and GII removal in AS processes (da Silva et al., 2007; Katayama et al., 2008; Nordgren et al., 2009; Flannery et al., 2012) (Table S3). The representative removal efficiency of norovirus GI in AS processes was 1.48 with 95% CI between 0.96 and 2.00 (Fig. 5), while that of norovirus GII was 1.35 with 95% CI between 0.52 and 2.18 (Fig. 6). These results indicate that the removal efficiencies of gastroenteritis viruses (rotavirus, norovirus GI and GII) by AS are very comparative, and about 1 to 1.5 LRs are expected on average. The systematic review process eliminated some articles about norovirus reduction in AS processes. For example, Campos et al. (2013) reported the norovirus concentration in influent and effluent samples of AS processes, but did not provide the sample number and SD values of the concentration, which are indispensable for the meta-analysis. La Rosa et al. (2010) also reported the norovirus concentration in influent and effluent samples of AS processes, but did not provide SD values.

With the exception of rotavirus and noroviruses, we could not retrieve a sufficient number of publications regarding the reduction of human enteric viruses (adenovirus, astrovirus, aichivirus, hepatitis A virus, and sapovirus) in MBR, but all effluent samples were norovirus GII-negative and could not be included in the meta-analysis. All manuscripts excluded in this meta-analysis because of the lack of required information are likely trustworthy and useful as case studies for virus removal in wastewater, but without key experimental information it is not possible to utilize such data for calculating the average value of virus LRs by meta-analysis in the present study. It is strongly recommended that arithmetic mean and standard deviation values of LR as well as sample size, which are necessary for meta-analysis calculations, be clearly described in the publication, otherwise LR datasets cannot be included in the calculation of representative values of virus removal efficiency.
virus, and sapovirus) in AS processes that met the described systematic review criteria. Although we did not include information regarding the removal efficiencies of bacteriophages from wastewater in the present study due to the reason described above for MBR, the meta-analysis of phage LR values in wastewater treatment processes may be beneficial in the discussion of the human health impact from wastewater reuse and should be addressed in future publications.

The examples of meta-analysis in this section help to define the shape of the dataset that needs to be taken to facilitate calculating virus removal efficiency of a wastewater treatment unit. One of the essential qualifications is to explicitly describe the treatment of non-detects. Since any pathogenic viruses can be removed by wastewater treatment to some extent, the quantification datasets of viruses in treated wastewater commonly include a significant number of non-detects. Even for untreated wastewater samples, pathogenic viruses are frequently not detected because of diel and annual variability. For example, noroviruses are frequently detected in untreated wastewater during the epidemic season, but not during other times of year (Pérez-Sautu et al., 2012). These non-detects do not allow us to calculate the virus removal efficiency and, therefore, the meta-analysis examples above employed only datasets where viruses in untreated and treated wastewater samples were both quantified and could be used to calculate the log-ratio of virus concentrations before and after a given treatment. This approach, in general, may underestimate the true virus removal efficiency value because viruses are not detected from an effluent sample when highly efficient virus removal is achieved as described above. Substitution of the non-detect data with specific values such as the limit of quantification, the half value of quantification limit, or zero has been used as a classical approach for dealing with non-detects, but the substitution gives inaccurate estimation of distribution parameters when the distribution of concentration is predicted (Helsel, 2006).

It has been proposed that datasets including non-detects or so called "left-censored" datasets can be analyzed by Bayesian estimation of the posterior predictive distribution of pollutant concentrations in environmental samples (Paulo et al., 2005). We have extended the Bayesian model to estimate the posterior predictive distribution of virus-

![Fig. 4.](image)

**Fig. 4.** Removal efficiency of rotaviruses by activated sludge processes. Black square is the mean value of virus log reduction (LR) obtained in each study, and a bar indicates 95% confidence interval (CI). The sample number in each study is indicated in a parenthesis next to 95% CI values. The total LR value is obtained by the calculation procedure indicated in Supplementary materials.

![Fig. 5.](image)

**Fig. 5.** Removal efficiency of norovirus genogroup I by activated sludge processes. Black square is the mean value of virus log reduction (LR) obtained in each study, and a bar indicates 95% confidence interval (CI). The sample number in each study is indicated in a parenthesis next to 95% CI values. The total LR value is obtained by the calculation procedure indicated in Supplementary materials.
removal efficiency (Kato et al., 2013; Ito et al., 2015). These models employ a parametric probabilistic distribution such as a log-normal distribution for expressing pollutant concentrations, which may give inaccurate estimations when the observed data is skewed (Shoari et al., 2015). It is necessary to test the robustness of these statistical models and their applicability to environmental survey data.

While datasets were collected for human enteric virus removal in MBR and AS in this study, under the systematic review criteria employed in this study we found that there were a limited number of publications, if any, about other wastewater treatment processes that fulfilled our prerequisites for meta-analysis. In the multiple-barrier system, wastewater engineers have to connect wastewater treatment units to establish a wastewater reclamation system that can achieve the target LR values, which means that the accumulation of virus LR information in other potential wastewater treatment units (e.g. reverse osmosis (RO), disinfection practices, etc.) that include all of the required information for meta-analysis is very critical and mandatory in the future study.

4. Regulations for validating virus removal efficiency in wastewater reclamation processes

Wastewater engineers can design a system for wastewater reclamation that can achieve the required levels of virus removal by combining virus LR credit of each wastewater treatment unit under the concept of multiple-barrier system. In this section we discuss the issue of how to validate that the employed wastewater treatment processes are working correctly and achieving the pre-evaluated performance targets for the safe management of wastewater reuse.

Although USEPA (2012) does not require the monitoring of viruses in wastewater effluent, some states in the U.S. classify reclaimed water into several classes based on their degree of treatment and quality of the final product; virus monitoring is included in these regulations. The state of California has established sophisticated regulations related to recycled water, and stipulated how to validate each of the treatment processes used to meet the requirements. According to the California Code of Regulations (Title 22), a GRRP sponsor must prepare an Operation Optimization Plan that identifies and describes the approaches for operations, maintenance, analytical methods, and monitoring necessary for the GRRP to meet the requirements using pathogens of concern or microbial surrogate parameters that verify the performance of each treatment process to achieve its credited LR, as well as report monitoring results to the regulatory agency (State Water Resource Control Board, 2015). To meet these regulatory requirements, the first option for a GRRP sponsor may be to monitor microbial indicators or surrogate parameters in effluent. There is a long history of usage of conventional indicator bacteria, including total coliforms and Escherichia coli, for such monitoring; however, the absence of indicator bacteria in treated wastewater does not necessarily indicate the absence of viral pathogens because viruses are typically more resistant to wastewater treatments (Kitajima et al., 2014). The selection of appropriate indicators or surrogates is thus important to ascertain the reliability of the treatment. WHO (2006) and ATSE (2013) suggested selecting bacteriophages, especially somatic coliphages and F-specific bacteriophages, as viral process efficiency indicators. Recent studies reported that the frequent and abundant appearance of pepper mild mottle virus in wastewater (Rosario et al., 2009), and used this virus as an enteric virus surrogate for evaluating the virus LR (Symonds et al., 2014). Other viruses, such as adenoviruses and Aichi virus have been also proposed as surrogates for wastewater treatment removal of enteric viruses (Hata et al., 2013; Kitajima et al., 2014). The issue of proper use of indicators or surrogates for monitoring viral LR values in wastewater treatment processes is still in open discussion, and further investigation is needed to select the most appropriate and affordable indicators or surrogates for validating virus removal efficiency in wastewater reclamation systems. Since multiple viruses should be compared to test their appropriateness as viral LR monitoring indicators, the micro- and nano-fluidic qPCR system for virus quantification may be a powerful tool because of its cost-effective and time-saving features (Ishii et al., 2014; Coudray-Meunier et al., 2016).

The state of Arizona requires virus monitoring for class A+ reclaimed water, which can be used for any purpose excluding 1) direct potable reuse, 2) when the water is not treated by designated techniques (filtration, denitrification, and disinfection after secondary treatment), or 3) when the class A+ reclaimed water is used after mixing with lower classes of water. Arizona’s regulations stipulate that virus concentrations should be less than the detection limit value for 4 sampling events during a 7-month monitoring period (Arizona Department of Environmental Quality, 2008). In the state of Florida, the acceptable virus concentration in reclaimed water varies between 0.04 and 14 per 100 liters depending on virus type, with rotavirus corresponding to the lowest concentration (USEPA, 2012). To meet these regulatory requirements, the monitoring of viruses in reclaimed wastewater has to be performed constantly in the daily operation of wastewater reclamation and reuse systems, and the first choice mean in the monitoring may be the molecular biology techniques including PCR. Although time- and cost-saving PCR systems have been recently proposed (Ishii et al., 2014; Coudray-Meunier et al., 2016), a highly frequent monitoring of viruses in reclaimed wastewater, such as on a daily basis, is not affordable and realistic. Further technical innovation in the virus
and UV. Fluorescent dyes such as rhodamine WT are potentially useful. Particulate matter can shield viruses from disinfectants such as chlorine and viral reductions by 5 log10 at a full-scale wastewater treatment plant (Adelman et al., 2011). This means that the infectivity reduction of human norovirus by any disinfection practices cannot be evaluated at the moment. Recently, Jones et al. (2014) reported that co-cultivation with an enteric bacterium strain enhanced the norovirus infection of B cells, and the applicability of this methodology to the other norovirus strains is currently being tested. Culture-independent approaches for evaluating the inactivation of non-cultivable viruses have been proposed (Hamza et al., 2011), and some of them may be available in some specific settings (Sano et al., 2015); however, there is no consensus as to which methodology for culture-independent testing is the most appropriate for the evaluation of infectivity reduction of non-cultivable viruses.

One of the possible solutions for the issues caused by the insufficient technological innovation in the monitoring of virus presence and infectivity can be seen in the regulation of the state of California, where novel and unique regulations have been established also in various applications of reclaimed wastewater other than GRRP. The California Code of Regulations (Title 22) stipulate three levels of recycled water quality, which are 1) disinfected secondary-2 recycled water, 2) disinfected secondary-3 recycled water, and 3) disinfected tertiary recycled water (Table S4). The disinfected tertiary recycled water has the highest hygiene quality, and can be used for food crops, parks and playgrounds, school yards, residential landscaping, unrestricted golf courses, and so forth. The disinfected tertiary recycled water that has not received conventional treatment can be used also for non-restricted recreational impoundments. As indicated in Table S4, the disinfected tertiary treated water is a filtered and subsequently disinfected wastewater with either chlorine at a CT (the product of total chlorine residual and modal contact time measured at the same point) of not less than 450 mg-minutes per liter at all times with a modal contact time of at least 90 min, based on peak dry weather design flow, or a disinfection process that has been demonstrated to reduce the infectious titer of the F-specific bacteriophage MS2 or poliovirus by 5 log10 in the combination with the filtration process. The use of other viral indicators that show comparable resistance against chlorination to poliovirus is also possible. Recent publication reported that free chlorine treatment at a CT value of 22 mg-minutes per liter was enough to inactivate bacteriophage MS2 by 5 log10 at a full-scale wastewater treatment plant (Adelman et al., 2016). The enacted CT value in the Title 22 (450 mg-minutes per liter with a minimum contact time of 90 min), which is more than ten-times larger than that are sufficient for 5 log10 reduction of infectivity of bacteriophage MS2, gives a sufficient oxidative stress to destroy virus particles and ensures the safety of disinfected water in terms of virus infection risk, and may exempt the obligation of virus monitoring in reclaimed wastewater that is obliged in several state regulations.

Non-microbe parameters can also be potential indicators for virus reduction. For example, turbidity can be an indicator for protozoan and viral reductions by filtration processes (USEPA, 2012). USEPA also suggests that the removal of suspended matter is related to virus removal because many viruses are particulate-associated, and that such particulate matter can shield viruses from disinfectants such as chlorine and UV. Fluorescent dyes such as rhodamine WT are potentially useful to assess the efficiency of viral removal by RO membranes; however, the assay sensitivity of rhodamine WT analysis should be improved for this application. Some of these non-microbe indicators can be monitored using real-time sensors, thus very compatible with water management systems based on Hazard Analysis and Critical Control Point (HACCP), in which multiple parameters are determined as the important monitoring points for ensuring the water safety. The WHO Sanitation Safety Plan (SSP) employs the concept of HACCP, and is one of the strong options for governments that do not have any wastewater reuse guidelines in operation. Another HACCP-based WHO plan, the Water Safety Plan (WSP), can also be applicable when the reclaimed wastewater is used as a drinking water source either directly or indirectly. The Water, Sanitation & Hygiene (WaSH) Safety Plans proposed by the United Nations Children’s Fund (UNICEF) are a congregative approach for public health, which also employs a HACCP-like system (Sanderson and McKenzie, 2011). The HACCP concept was developed in the food industry, and control parameters for pathogen inactivation such as D-values (decimal reduction time, which is the time required to reduce 1 LR of the heat-exposed microorganisms at a given temperature) and Z-values (temperature required for achieving 1 LR more in D-value) are investigated in the study field of predictive microbiology, one of the disciplines within food microbiology (McMeekin et al., 2013). Because of the increased complexity of wastewater matrices compared to food, the approaches of predictive microbiology have not been fully applied to water virology. For the full introduction of SSP, WSP or WaSH Safety Plans for the safe usage of reclaimed wastewater, predictive microbiology-like approaches need to be introduced for identifying appropriate indicators of virus LR in the HACCP-based systems for wastewater reclamation.

5. Conclusions

Current guidelines for wastewater reclamation operations have proposed that virus infection risks for any type of reclaimed wastewater usage are to be regulated by the multiple-barrier system, in which sequential wastewater treatment processes are designed in a way that the sum of virus removal efficiencies for each process exceeds a predetermined performance target, which is a total virus removal efficiency calculated by virological risk assessment. A meta-analysis approach is useful for obtaining the averaged value of virus LR in each wastewater treatment unit; however, there are several academic issues that should be further addressed including 1) how to treat left-censored datasets in the calculation of virus removal efficiency, and 2) what indicators and methodologies are appropriate for validating virus removal and disinfection performance in the daily operation of wastewater reclamation systems. The frameworks of SSP, WSP and WaSH Safety Plan, employing HACCP along with the multiple-barrier system, must enhance the efficacy of the risk management of virus infection through reclaimed wastewater.

A competing financial interests declaration

The authors declare no competing financial interests.

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