



## Review

# Bacteriophage removal efficiency as a validation and operational monitoring tool for virus reduction in wastewater reclamation: Review



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## ABSTRACT

The multiple-barrier concept is widely employed in international and domestic guidelines for wastewater reclamation and reuse for microbiological risk management, in which a wastewater reclamation system is designed to achieve guideline values of the performance target of microbe reduction. Enteric viruses are one of the pathogens for which the target reduction values are stipulated in guidelines, but frequent monitoring to validate human virus removal efficacy is challenging in a daily operation due to the cumbersome procedures for virus quantification in wastewater. Bacteriophages have been the first choice surrogate for this task, because of the well-characterized nature of strains and the presence of established protocols for quantification. Here, we performed a meta-analysis to calculate the average log<sub>10</sub> reduction values (LRVs) of somatic coliphages, F-specific phages, MS2 coliphage and T4 phage by membrane bioreactor, activated sludge, constructed wetlands, pond systems, microfiltration and ultrafiltration. The calculated LRVs of bacteriophages were then compared with reported human enteric virus LRVs. MS2 coliphage LRVs in MBR processes were shown to be lower than those of norovirus GII and enterovirus, suggesting it as a possible validation and operational monitoring tool. The other bacteriophages provided higher LRVs compared to human viruses. The data sets on LRVs of human viruses and bacteriophages are scarce except for MBR and conventional activated sludge processes, which highlights the necessity of investigating LRVs of human viruses and bacteriophages in multiple treatment unit processes.

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## Abbreviations

ATSE	Australian Academy of Technological Sciences and Engineering
CAS	conventional activated sludge process
CI	confidence interval
df	degree of freedom
DO	dissolved oxygen
<i>E. coli</i>	<i>Escherichia coli</i>
GRRP	Groundwater Replenishment Reuse Project
HRT	hydraulic retention time
LRV	log <sub>10</sub> reduction value

MBR	membrane bioreactor
MF	microfiltration
MLSS	mixed-liquor suspended solids
NTU	nephelometric turbidity unit
PFU	plaque forming unit
SRT	solids retention time
SD	standard deviation
TMP	transmembrane pressure
UF	ultrafiltration
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WWTP	Wastewater treatment plant

## 1. Introduction

Reported outbreaks of viral infectious diseases caused by insufficiently treated wastewater (Bernard et al., 2014; Okoh et al., 2010; Sinclair et al., 2009) emphasizes the importance of wastewater treatment as a barrier for the virus transmission, especially for reclamation and reuse (Adefsoye et al., 2016; Zhang et al., 2016). To ensure the microbiologically safe usage of reclaimed wastewater, multiple-barrier concept has been employed in international and domestic guidelines for wastewater reclamation (ATSE, 2013; US Environmental Protection Agency, 2012; WHO, 2006). In the multiple-barrier concept, each unit process of wastewater treatment is assigned a credit value of pathogen reduction efficiency, and the total pathogen reduction efficiency of a treatment process chain is calculated as the sum of log<sub>10</sub> reduction values (LRVs) of each process (Sano et al., 2016). For example, in the Groundwater Replenishment Reuse Project (GRRP) of the state of California, USA, performance target LRVs of 12 for viruses and 10 for both *Giardia* cysts and *Cryptosporidium* oocysts, are required when treated wastewater is used for groundwater recharge intended for indirect potable reuse (California State Water Resources Control Board, 2015). The Texas Water Development Board has proposed that water reclamation plants achieve LRV of 12 for viruses for direct potable reuse and has assigned 6 log<sub>10</sub> upper end reduction of viruses by UV disinfection combined with advanced oxidation process (Texas Water Development Board, 2015). Wastewater engineers are required to combine a number of unit processes to exceed these performance target values when designing wastewater reclamation systems under the multiple-barrier concept.

During the operation of wastewater reclamation systems, there is a necessity to monitor the plant performance to make sure the particular plant provides recycled water of expected quality as stipulated by guidelines (US Environmental Protection Agency, 2012; Victoria Department of Health, 2013). Four stages of a wastewater reclamation monitoring system are: 1) baseline monitoring to assess risks and impacts of the recycled water on the environment; 2) performance monitoring to validate performance

required by the water quality management plan; 3) operational monitoring to ensure the system is operating within the design specification; and 4) verification monitoring at the end to confirm that the quality of recycled water complies with the water quality management plan and whether any modifications are necessary (Australian Environment Protection and Heritage Council, 2006). Bacteriophages have been the most widely used microbial parameter for the second (validation) and third (operational) monitoring stages with respect to virus reduction efficiency in wastewater treatment processes. The USEPA guideline suggests that MS2 coliphage be used for on-site validation (US Environmental Protection Agency, 2012). The Australian guidelines recommend that indigenous *Escherichia coli* be monitored mandatorily and coliphages or other pathogens such as adenovirus or enterovirus be also monitored as representatives of viral contaminants (Australian Environment Protection and Heritage Council, 2006). The state of Victoria, Australia provides guidelines on the usage of indigenous somatic coliphages or F-specific RNA (FRNA) bacteriophages, or seeded MS2 coliphage as suitable surrogates for enteroviruses when indigenous or seeded enteroviruses are not used in the validation process (Victoria Department of Health, 2013). MBR validation protocol published by WaterVal™ program suggests that both somatic coliphages and FRNA bacteriophages must be used to validate MBR systems (WaterSecure, 2017).

There are compelling reasons for the selection of bacteriophages for the monitoring over the usage of the human enteric viruses. Some investigations reported that bacteriophages were present in similar or higher numbers in water environments when enteric viruses were present (Hartard et al., 2015; Lucena et al., 2004). In a previous meta-analysis study, Pouillot et al. (2015) reported a strong positive correlation ( $R^2 = 0.8$ ) between LRVs of male-specific coliphages and human norovirus GII (Pouillot et al., 2015). Bacteriophage enumeration results can be obtained within 12 h with simpler techniques as opposed to enteric virus enumeration techniques that requires more time, cost and labor (Lucena et al., 2006; Zhang and Farahbakhsh, 2007). Recent research efforts aimed at reducing the process time required for bacteriophage enumeration

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