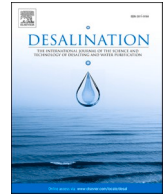




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## Sources, fates and treatment strategies of typical viruses in urban sewage collection/treatment systems: A review

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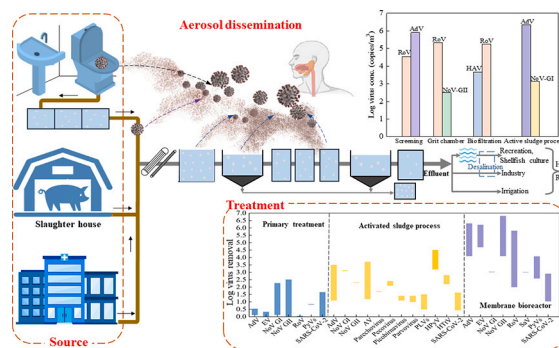
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### HIGHLIGHTS

- Characteristic of sewer systems was the key factor affecting virus concentration.
- HAdV and NoV were most frequently detected viruses in the effluent of WWTPs.
- Aerosolization in sewer systems and at WWTPs might be a main reason for spreading.
- “Primary treatment+MBR+chlorine” was recommend for a better removal of SARS-CoV-2.

### GRAPHICAL ABSTRACT



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### ABSTRACT

The ongoing coronavirus pandemic (COVID-19) throughout the world has severely threatened the global economy and public health. Due to receiving severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a wide variety of sources (e.g., households, hospitals, slaughterhouses), urban sewage treatment systems are regarded as an important path for the transmission of waterborne viruses. This review presents a quantitative profile of the concentration distribution of typical viruses within wastewater collection systems and evaluates the influence of different characteristics of sewer systems on virus species and concentration. Then, the efficiencies and mechanisms of virus removal in the units of wastewater treatment plants (WWTPs) are summarized and compared, among which the inactivation efficiencies of typical viruses by typical disinfection approaches under varied operational conditions are elucidated. Subsequently, the occurrence and removal of viruses in treated effluent reuse and desalination, as well as that in sewage sludge treatment, are discussed. Potential dissemination of viruses is emphasized by occurrence via aerosolization from toilets, the collection system and WWTP aeration, which might have a vital role in the transmission and spread of viruses. Finally, the frequency and concentration of viruses in reclaimed water, the probability of infection are also reviewed for discussing the potential health risks.

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## 1. Introduction

The eruption of atypical pneumonia [coronavirus disease 2019 (COVID-19)] in December 2019 led to the global infection of over 429 million individuals and more than 5.9 million deaths as of February 2022. In addition to addressing the enormous economic damage incurred, a particular emphasis needs to be placed on environmental concerns and public health relevant to the fate of viruses. Thus, researches on the transmission of typical viruses in sewage collection, treatment, and desalination systems have attracted growing attention throughout the world.

The majority of viruses exhibit several unique characteristics, such as small size, resistance to disinfection, low infectious dose, refractoriness to antibiotics, and proclivity for adaptive mutation [1]. To date, more than 700 types of waterborne viruses have been detected in natural water and wastewater samples, and their long-range transmission from environmental media to hosts is the main cause of the outbreak of terrible waterborne diseases such as gastroenteritis, cardiac abnormalities, conjunctivitis, meningitis, respiratory diseases and hepatitis [2,3]. It is estimated that annually 1.4–1.9 million deaths are closely related to the prevailing waterborne diarrheal diseases associated with viruses already identified in natural or engineered water and wastewater systems [4,5].

The main sources of viruses released into wastewater may include hospitals, slaughterhouses, residential areas and so forth. In particular, the principal sources of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in wastewater systems are feces/urine of infected individuals and hospital sewage [6–8]. Once the viruses enter the sewer system, a subsequent decline in viral load is detected as a consequence of the dilution effect of other wastewater, reaction with the chemicals (e.g., disinfectants and detergents) and virus inactivation under water physicochemistry variation (e.g., pH, temperature, and solids content) [9–11]. Hence, septic and collection systems are extremely important with respect to the collection, storage, treatment, neutralization and stabilization of virus-containing wastewater [12]. Once viruses enter the wastewater treatment plants (WWTPs), conventional primary and secondary treatment may no longer be capable of achieving an absolute removal of viruses because viral genomes and extracellular proteins are unlikely to be denatured easily [13]. Thus, many advanced treatment units have been explored for the further treatment and elimination of these viruses within wastewater. For instance, the moving-bed biofilm reactor and sequencing batch reactor have been proven to be ideal secondary treatments for SARS-CoV-2 RNA removal from wastewater [14]. Chemical disinfection is a powerful way to inactivate viruses in tertiary treatment [15], where chlorination and UV irradiation are expected to provide high accessibility for SARS-CoV-2 inactivation based on experience with other classes of SARS viruses [16,17]. However, mechanistic insights into the removal trends of typical viruses during the operation of the available treatment units of WWTPs, as well as the dose-effect response of specific viruses to disinfectants and their inactivation kinetics of coronaviruses, still need further exploration. In addition, numerous studies have proven that typical enteric viruses and SARS-CoV-2 remain in WWTP effluent and present a high health risk during reuse and desalination [17]. Thus, obtaining further understanding of the effects of different treatment methods on virus removal in wastewater treatment, WWTP effluent reuse and desalination, and sewage sludge treatment is vital for controlling the spread of the virus and reducing the risk of wastewater-to-human transmission.

The main objectives of this study were to: (1) review the occurrence and concentration distributions of viruses in wastewater; (2) compare the efficiency and mechanisms of virus removal during the primary, secondary and tertiary treatments of WWTPs; (3) summarize the occurrence and removal of viruses in treated effluent reuse and desalination as well as in the different sewage sludge treatments; and (4) discuss the dissemination of viruses throughout the entire sewage collection, transmission and treatment process and assess the public

health risks associated with reuse of treated wastewater. This work can aid us in risk assessment, virus inactivation and management during current and future viral outbreaks.

## 2. Methodology

The review was prepared based on a literature search for documents published until 14 February 2021 in the following databases: PubMed, Scopus, ScienceDirect, and Web Science Core Collection. The keywords employed for the search are presented in Table S1. In this first stage of the research, the title of each article was evaluated, and information about the quantification of typical viruses in wastewater, sewage, feces, urine samples and sewer system was selected, as well as information related to the concentration and removal of typical viruses found in sewage/sludge treatment units. In total, 618 articles were selected, and their abstracts were screened to verify whether they truly contained the information described above. Then, 412 articles were excluded because they did not contain the required information, and finally 246 eligible articles were screened and used as references for this review. In addition, another 25 articles were added due to a screening of the selected articles and the reviewers' suggestions.

## 3. Occurrence of viruses in wastewater

Viruses, generally with an average size ranging from 18 nm to 1500 nm, are microscopic pathogenic agents [18]. The reports of the International Committee for the Taxonomy of Viruses in 2016 revealed that 7 orders, 104 families, 505 genera, and 3286 species of viruses have been detected, including enveloped viruses, (such as severe acute respiratory syndrome coronavirus (SARS-CoV)) and other nonenveloped enteric viruses (including norovirus (NoV), rotavirus (RoV), hepatitis A virus (HAV), hepatitis E virus (HEV), adenovirus (AdV), polyomavirus (PyVs), astrovirus (AV), coxsackievirus (CV), poliovirus (PV), sapovirus (SaVs) and others) [19]. These viruses can be transmitted by contaminated water (SARS-CoV-2 can be transferred via both the waterborne and nonwaterborne pathways) [13], which lead to the spread of various human diseases, such as gastroenteritis, conjunctivitis, fever, meningitis, heart anomalies, paralysis, and hepatitis (Table 1).

Viruses cannot replicate in aquatic environments, thus the excretion of viruses via the feces and urine of humans/animals is their first entry point into the environmental cycle. Specifically, the nonenveloped particles excreted through the feces of infected humans or animals (slaughterhouses) have been regarded as one of the main pathways by which virus particles are released into the environment. Quasi-enveloped particles originating from blood might be another important pathway of virus excretion into aquatic environments, especially via slaughterhouse wastewater emissions. For instance, viral RNA from SARS-CoV-2 [42] has been recently detected in the feces of infected humans even 26 days after their recovery [31]. It is recognized that a person with an enteric viral infection may excrete as many as  $10^{14}$  viral particles per day, and this may exceed  $10^{15}$  for some infection cases [43,44].

Overall, eight viral families (*Astroviridae*, *Coronaviridae*, *Parvoviridae*, *Circoviridae*, *Herpesviridae*, *Anelloviridae*, *Caliciviridae*, and *Picobirnaviridae*) and six previously known viruses (feline CoV type 1, felid herpes 1, feline calicivirus, feline NoV, feline panleukopenia virus and picobirnavirus) detected in the feces of animals and are infectious to human beings [45]. For instance, the high concentrations of chicken/turkey parvoviruses detected in chicken stools ( $1.97 \times 10^2$ – $1.07 \times 10^{10}$  genome copies·g<sup>-1</sup>) and slaughterhouses ( $1.90 \times 10^5$ – $8.14 \times 10^5$  genome copies·mL<sup>-1</sup>) highlight the importance of controlling virus emissions that occur via animal fecal contamination [46]. Furthermore, hospital wastewater has been described as another important source of disease-causing agents, particularly RoV, which is associated with diarrhea mortality among children younger than 5 years (128,515 deaths annually) and among all ages (228,047 deaths annually) [47]. The survey by

Kargar et al. revealed that RoV has frequently been detected in hospital sewage samples (40%), which is much higher than the level found in urban sewage samples (33.33%) [48]. Moreover, many hospitals do not properly treat their wastewater, especially those in developing countries, before it is discharged into their collection systems, seriously threatening the safety of aquatic environments [49].

According to the work of Korajkic et al. and Muirhead et al., the Zika virus exhibited a much higher decay rate under a temperature of >20 °C than under a temperature of <15 °C [50,51]. Prolonged viral persistence was observed at lower temperature for nonenveloped viruses [52]; in contrast, enveloped viruses are more susceptible to temperature as they contain a lipid bilayer membrane (envelope) surrounding the protein capsid [53,54]. Undoubtedly, viruses that enter the collection system via complex underground pipes partially maintain their infectivity before reaching WWTPs [55,56], despite the operational temperatures, pH and wastewater characteristics/composition that would influence their survival [57]. The survival times of typical viruses in sewage at different temperatures are shown in Fig. 1. The representative enveloped (SARS-CoV-2) and unenveloped (MNV) viruses were selected to linear fitting with temperatures (Fig. S1), and the R<sup>2</sup> were 0.98 and 0.82, respectively; and the obtained absolute values of slope were 0.613 and 0.055, respectively, which verified that enveloped viruses were more sensitive than nonenveloped viruses.

#### 4. Species and concentrations of viruses in collection systems

Viruses have been widely detected in raw domestic wastewater, regardless of whether the wastewater is from urban or rural areas. For instance, some households in developed countries utilize drinking water from private wells and rely on onsite systems for wastewater treatment

[62,63]. A significant correlation has been reported between wastewater-borne illnesses and the presence of private wells and/or onsite wastewater systems [64]. On the other hand, the release of raw sewage, due to the overflow of combined sewers, also contributes to the emission of viruses into surface waters [65]. Information on the specifics and distributions of typical viruses in wastewater in sewer systems, especially those found in raw domestic wastewater, septic tanks, combined sewer systems and the influent of WWTPs, is collected and summarized in Table 2.

The concentration of the viruses within the wastewater of the different collection units varied widely. For instance, concentration of enterovirus (EV), RoV and NoV in raw domestic wastewater were 10<sup>4</sup>–10<sup>5</sup>·L<sup>-1</sup>, 200–1000·L<sup>-1</sup> and 2 × 10<sup>5</sup>·L<sup>-1</sup>, respectively [66,72]; in regard to septic tanks, the concentrations of the viruses in the influent were found to be 10–10<sup>2</sup> virus·L<sup>-1</sup> [67], 10–10<sup>2</sup> virus·L<sup>-1</sup> [70] and 10<sup>5</sup> virus·L<sup>-1</sup>, respectively [67]. Although the viruses would be partially diluted in the septic tanks, it is estimated that the concentration of viruses in the septic tanks would reach 10<sup>7</sup>–10<sup>10</sup>·L<sup>-1</sup> even with only one infected individual in a household [75,76]. Furthermore, those viruses would be transferred into WWTPs. For example, the concentrations (in genome copies·L<sup>-1</sup>) of EV, AdV, RoV, NoV and AV detected in the influent of WWTPs were 2.2 × 10<sup>3</sup>–7.9 × 10<sup>3</sup>, 10<sup>6</sup>–10<sup>7</sup>, 10<sup>6</sup>–8.9 × 10<sup>6</sup>, 5.6 × 10<sup>2</sup>–8.3 × 10<sup>3</sup> and 10<sup>6</sup>–10<sup>8</sup>, respectively [71,73,77,78].

Overall, the concentration distribution of viruses within the different collection units was seriously affected by pipeline quality, the areal percentage distribution of the combined collection system, extreme rainfall conditions, and the living standards of the households [68,79–81]. A publication revealed that rainfall in Chicago, which carries viruses in amounts comparable to those of sewers, led to a significant virus concentration increase in the influent of WWTPs as well as

**Table 1**  
Virus concentrations in sewage from different sources.

Virus	Characteristics [20]	Symptoms and diseases [21,22]	Viral load at patients feces	Virus concentration in slaughterhouses	Virus concentration in hospital wastewater
Nov	27–35 nm; non-enveloped, spherical, non-segmented, positive-sense, ssRNA virus	Diarrhea, vomiting, nausea, dehydration, fever, abdominal pain, gastroenteritis	2.5 × 10 <sup>6</sup> –3.9 × 10 <sup>9</sup> genome copies·g <sup>-1</sup> [23]	NoV were not detected [24], while NoV GII were detected 14.2% of the pigs feces [25].	3.6 × 10 <sup>2</sup> , 3.1 × 10 <sup>2</sup> genome copies·mL <sup>-1</sup> [7].
RoV	70 nm; non-enveloped, icosahedral, segmented, positive-sense dsRNA virus	Diarrhea, gastroenteritis	10 <sup>10</sup> genome copies·g <sup>-1</sup> [26]	GARV: 0.8% [25].	1.7 × 10 <sup>3</sup> , 4.1 × 10 <sup>6</sup> genome copies·mL <sup>-1</sup> [7].
HAV	27–34 nm; non-enveloped, icosahedral, non-segmented, positive-sense ssRNA virus	Inflammation of the liver which causes severe pain, fever, vomiting, incapacitation of the patient	/	/	2.1 × 10 <sup>4</sup> genome copies·mL <sup>-1</sup> [7].
HEV	27–34 nm; non-enveloped, spherical, non-segmented, positive-sense, ssRNA	Non-bacterial gastroenteritis in humans	2.1 × 10 <sup>4</sup> –7.7 × 10 <sup>7</sup> genome copies·g <sup>-1</sup> [27]	1.3 × 10 <sup>-1</sup> –6.4 × 10 <sup>3</sup> genome copies·mL <sup>-1</sup> [27]	55.6% [28].
AdV	60–90 nm; non-enveloped, icosahedral, non-segmented, dsDNA virus	Gastroenteritis, conjunctivitis, respiratory disease	/	PAdV: 1.56 × 10 <sup>3</sup> genome copies·mL <sup>-1</sup> [29]	5.0 × 10 <sup>3</sup> , 1.0 × 10 <sup>3</sup> genome copies·mL <sup>-1</sup> [7].
SARS-CoV	60–200 nm; enveloped, spherical, non-segmented, positive-sense, ssRNA virus	Respiratory and enteric symptoms	10 <sup>5.6</sup> –10 <sup>8.1</sup> genome copies·g <sup>-1</sup> [30,31]	/	0.633 genome copies·mL <sup>-1</sup> in wastewater of the adjusting tank [32].
PyVs	40–50 nm; non-enveloped, icosahedral, non-segmented dsDNA virus	Sarcoma, cancer	/	OPyV: 9.81 × 10 <sup>-1</sup> genome copies·mL <sup>-1</sup> [32]	45.0% [33].
AV	27–32 nm; non-enveloped, spherical, non-segmented, positive-sense, ssRNA virus	Gastroenteritis	2.8 × 10 <sup>5</sup> –1.6 × 10 <sup>11</sup> genome copies·mL <sup>-1</sup> [34]	20.8% [25].	11.7% of the children stool samples with a diagnosis of gastroenteritis [35].
CV	20–30 nm; non-enveloped, icosahedral, non-segmented, positive-sense, ssRNA	Meningitis, respiratory disease	Ct value: 21.9–38.4 [36]	/	25 × 10 <sup>5</sup> genome copies·mL <sup>-1</sup> in CA6-infected children stool [37].
PV	20–30 nm; non-enveloped, icosahedral, non-segmented, positive-sense, ssRNA virus	Fever, paralysis, meningitis, poliomyelitis	4.7 × 10 <sup>2</sup> –2.1 × 10 <sup>5</sup> genome copies·g <sup>-1</sup> [38]	/	0.61% of pediatric patients stool [39].
SaVs	41–46 nm; non-enveloped, icosahedral, non-segmented, positive-sense, ssRNA virus	Dehydration, vomiting, abdominal pain	3.46 × 10 <sup>5</sup> –2.09 × 10 <sup>10</sup> genome copies·g <sup>-1</sup> [23]	GIII SaVs:78% [40].	29.4% [41].

Notes: NoV, norovirus; RoV, rotavirus; HAV, hepatitis A virus; HEV, hepatitis E virus; AdV, adenovirus; SARS-CoV, severe acute respiratory syndrome coronavirus; PyVs, polyomavirus; AV, astrovirus; CV, coxsackievirus; PV, poliovirus; SaVs, sapovirus; dsRNA, double-stranded RNA; ssRNA, single-stranded RNA; dsDNA, double-stranded DNA; NoV GII, norovirus GII; GARV, group A rotavirus; PAdV, Porcine adenovirus; OPyV, Ovine polyomavirus; Ct, Cycle threshold.

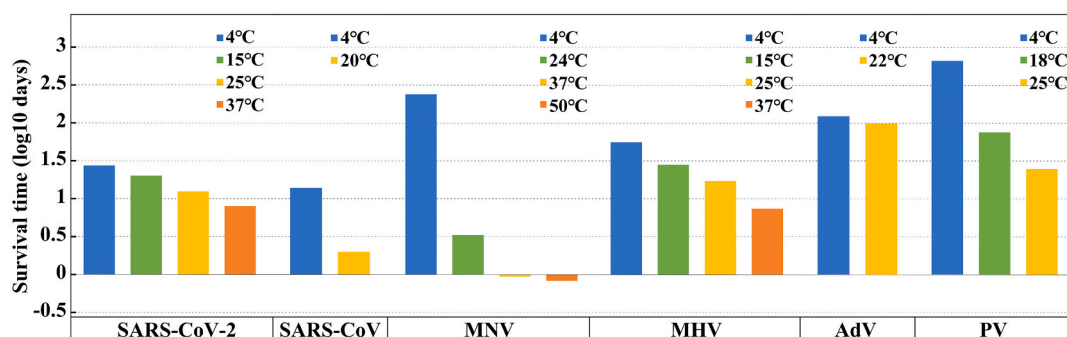


Fig. 1. Persistence of typical virus in wastewater at varying temperature [57–61].

(SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SARS-CoV, severe acute respiratory syndrome coronavirus; MNV, Murine Norovirus; MHV, Mouse hepatitis virus; AdV, adenovirus; PV, poliovirus)

Table 2  
Concentration of the typical viruses in the collection system (per liter).

Virus	Raw domestic wastewater	Influent of septic tank	Combined sewer system	WWTPs influent
EV	10 <sup>4</sup> –10 <sup>5</sup> [66]	10–10 <sup>2</sup> [67]	6 × 10 <sup>3</sup> –1.74 × 10 <sup>5</sup> [68]	3 × 10 <sup>2</sup> –1.1 × 10 <sup>6</sup> [69]
RoV	2 × 10 <sup>2</sup> –10 <sup>3</sup> [66]	10–10 <sup>2</sup> [70]	/	10 <sup>6</sup> –8.9 × 10 <sup>6</sup> [71]
AdV	/	/	5.3 × 10 <sup>3</sup> [68]	2 × 10 <sup>4</sup> –6.7 × 10 <sup>8</sup> [69]
NoV	2 × 10 <sup>5</sup> [72]	10 <sup>5</sup> [67]	2.3 × 10 <sup>3</sup> [68] (NoV GI) 6.31 × 10 <sup>5</sup> –1.61 × 10 <sup>6</sup> [68] (NoV GII)	5.6 × 10 <sup>2</sup> –8.3 × 10 <sup>3</sup> [73] 5.5 × 10 <sup>5</sup> –6 × 10 <sup>6</sup> [74]

Notes: EV, enterovirus; RoV, rotavirus; AdV, adenovirus; NoV, norovirus.

in natural water bodies via overflows of the combined sewer [65]. In contrast, no obvious virus emission during rainfall was observed in Tokyo Bay due to the extensive construction of confluence systems [82,83]. In addition, Katayama et al. reported that significant seasonal concentration variation of EV was detected in wastewater [84]. For instance, NoV GI and NoV GII were found to be more abundant in wastewater during the winter (from Nov. to Mar.) than in the summer (190–200·mL<sup>-1</sup> vs. 4.9–9.1·mL<sup>-1</sup>).

## 5. Removal trends of viruses in different WWTP unit processes

### 5.1. Primary treatment process

The primary treatment units of WWTPs mainly include bar screens and grit chambers, which involve a settling process under a very short hydraulic retention time (HRT) for the purpose of removing colloidal and other solid organic materials. Those viruses, exhibiting colloidal structures and a negative charge under neutral pH conditions [85] and thus can be easily removed via electrostatic adsorption [86] (Fig. 2), and the agglomerated particles always have a larger diameter and higher density, which benefit the sedimentation and subsequent virus removal. It was reported that as many as 75% of viruses, such as PV (serotypes 1, 2, and 3), CVB (serotypes 1, 2, 3, 4, and 5), echovirus (serotypes 3, 14, and 22), and reovirus, could be removed during the primary treatment of WWTPs [18,87], with a decrease in EVs, RoVs, and NVs of 0.2–0.4 log occurring through a fine screen [71]. In contrast, other publications showed that the primary treatments of WWTPs were less effective at virus removal, with an average removal rate of less than 10%, due to their short HRT [88,89]. The very recent work of Balboa et al. stated that the concentration of SARS-CoV-2 RNA of the primary settler effluent exhibited a lower value (< detection limit–4.2 genome copies·mL<sup>-1</sup>) than that of the WWTP influent (2.15–9.8 genome copies·mL<sup>-1</sup>), as well as the frequent detection of SARS-CoV-2 RNA in the sludge of the primary

setter (up to 24 genome copies·mL<sup>-1</sup>), clearly revealing the adsorption of the positive charged amino groups of the virus onto the negative charged carboxyl groups of the sludge samples [90,91].

To further enhance the removal rate of typical viruses in primary treatment units of WWTPs, nanoparticles with different characteristics have been explored for the purpose of the in situ removal/inactivation of viruses [92]. The recent work of Mazurkow and Yuzbasi et al. found that the application of copper (copper oxide) nanoparticles as the active phase and plate-shaped porous alumina as the carrier material achieved 99.9% removal of bacteriophage MS2 [93]. Moreover, the formation of cation bridges between the metal Ca<sup>2+</sup>/Mg<sup>2+</sup> (Ca<sup>2+</sup> exhibited a stronger tendency than Mg<sup>2+</sup>) and carboxylate groups of both the natural organic matter and the MS2 capsids played a key role in MS2 reduction [94]. Overall, the two sorption mechanisms, hydrophobic interactions and electrostatic interactions, played major roles in the attachment of viruses to biosolids [95].

### 5.2. Secondary treatment processes

#### 5.2.1. Performance of conventional biological treatment

The predominant mechanisms for virus removal in the secondary (biological) treatment units of WWTPs mainly include reversible binding/adsorption onto particles or flocs (such as suspended bacteria and algae, etc.) [96], filtration or internalization by larger organisms (e.g., nanoflagellates), and inactivation. Besides the characteristics of viruses [97], the binding, internalization and inactivation removal of viruses are closely related to the operational parameters and surrounding environmental conditions, such as pH, temperature, ionic strength and wastewater hardness [98–101].

For example, according to one of the earliest works by Clarke et al., approximately 90% of PV type 1 virus and 98% of CV A9 virus could be efficiently removed in an activated sludge system under a 6–7 h HRT [102]. In addition, the removal efficiency of NoV GI, under a high-purity oxygen-modified activated sludge system, was found to be lower than that of NoV GII, EV serotypes and male-specific coliphages (0.95 log vs. 1.48 log) [103]. On the other hand, the optimized Ludzack-Ettinger activated sludge system exhibited a much higher reduction of viruses (3.1 log or 99.92% for GI and 2.3 log or 99.5% for GII) than those of trickling filters, biological aerated filters and humus tanks [104]. Moreover, a case study conducted in Tunisia revealed that the upgrading of the traditional activated sludge process, via extended aeration in the oxidation channel, enhanced the bulk removal of HAV viruses from 32.43% to 91.47% [105].

In addition, long HRT WWTPs would benefit the overall removal of the viruses in wastewater. For instance, an as high as 1 log reduction of viruses in wastewater was achieved for a pond system under every 14.5–20.9 days of retention [106]. Theoretically, the initial rapid decline of virus concentration resulted from both adsorption and biodegradation by biomass, while the following gradual decline was due

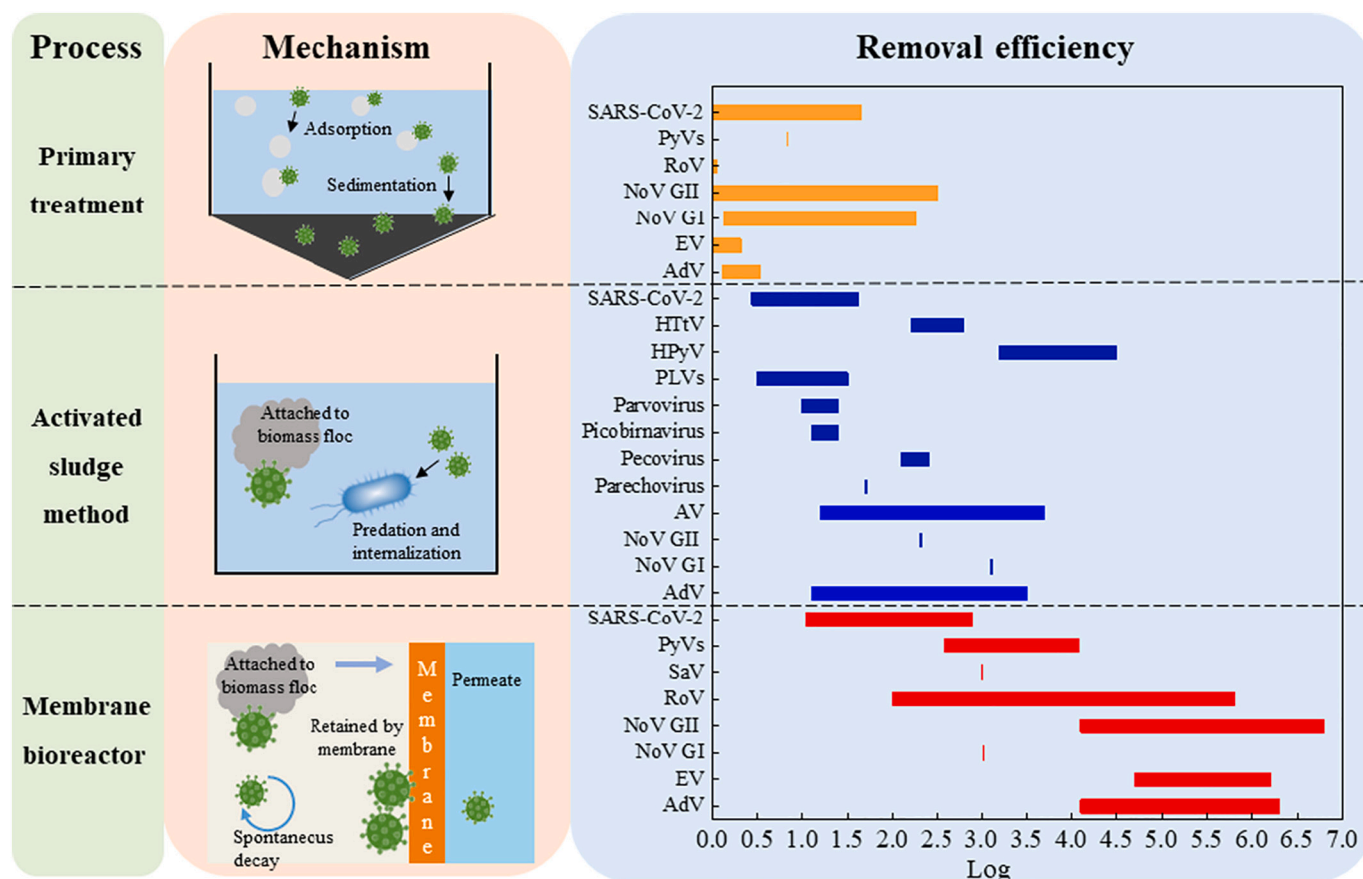


Fig. 2. Mechanism and log removal efficiency of different viruses during different WWTP units operation [69,73,104,108,121–129]. (SARS-CoV-2, severe acute respiratory syndrome coronavirus2; PyVs, polyomavirus; RoV, rotavirus; NoV GII, norovirus GII; NoV GI, norovirus GI; EV, enterovirus; AdV, adenovirus; HTtV, human torque teno virus; HPyV, Human polyomavirus; PLVs, primate lentivirus; AV, astrovirus; SaV, sapovirus).

to only degradation. Specifically, the biodegradation process could be well described by the Eckenfelder model (first order reaction), which might be the main reason for the excellent performance of the virus reduction under a longer HRT condition [107]. Furthermore, the performance of WWTP operation was deeply affected by virus characteristics, and a removal trend of human polyomavirus (HPyV) (3.65 log) > *Microviridae* (2.81 log)  $\approx$  human adenovirus (HAdV) (2.79 log) was observed via a comparison of the operation of three WWTPs that employed Bardenpho processes [108]. It should be noted that complicating factors contribute to the wide disparity in virus removal, as evidenced by the relevant data on reduction (0.3–4.5 log) measured in the secondary treatment of WWTPs (Fig. 2).

### 5.2.2. Performance of membrane bioreactors

The majority of membrane bioreactors (MBRs) exhibit an excellent performance in virus removal [109], and the main mechanisms can be summarized as: (1) size exclusion, electrostatic repulsion, and sorption onto the membrane; (2) pore blocking and adsorption onto the biomass layer; (3) adsorption and predation by the suspended biomass; and (4) spontaneous decay and inactivation [110]. For example, an average removal rate of 4.1–6.3 log for AdV and 3.5–4.8 log for EV could be achieved in the operation of a full-scale aerobic MBR using a 0.04  $\mu\text{m}$  membrane, whereas 3.67 and 3.40 log, respectively, were observed for a 0.4  $\mu\text{m}$  membrane [110]. Moreover, the removal efficiency of viruses should greatly improve once the biofilm attaches to the membrane surface, as evidenced by the average 2.1 log virus removal rate of the 21-day continuously operated membrane, nevertheless, whereas it was only 0.3 log during the initial operational stage (9 h of continuous operation) [111]. Although membrane fouling contributes to virus rejection, it is

detrimental for WWTP operation because of the negative effect of the permeate flux [112,113]. Thus, backwashing is necessary for the operation of the MBR system; here, and it should be pointed out that backwashing partially deteriorated the overall performance of the MBR in virus removal (declined of 0.5 log) [114]. Hence, seeking a balance between virus particle rejection and membrane permeability will be an arduous task for the practical operation of WWTPs [115]. On the other hand, microorganism predation and enzymatic breakdown also contribute to virus removal [116], as evidenced by the noteworthy correlation between the bulk removal of SaVs and the mixed liquor suspended solids concentration in a pilot-scale MBR operation [117]. In summary, the abovementioned fluid flux, transmembrane pressure, chemical characteristics of biomass and the reduction of membrane pore size can significantly affect virus removal in the MBR system.

Overall, the removal efficiency of WWTP treatments is closely related to the characteristics of the viruses. Specifically, primary treatment was efficient for NoV GI (up to 2.26 log) and NoV GII (up to 2.5 log) removal and activated sludge treatment was efficient for HPyV (up to 4.5 log), AV (up to 3.7 log), NoV GI (up to 3.12 log) and AdV (up to 3.5 log) removal. It should be noted that primary treatment was more effective for the removal of SARS-CoV-2 than activated sludge treatment (1.65 log vs 1.62 log), emphasizing the importance of primary treatment in WWTPs especially during the COVID-19 pandemic. MBR systems exhibited better virus removal (2–6.8 log) than the traditional biological wastewater treatment systems (Fig. 2). Moreover, enveloped viruses are more likely to be removed in MBR system relative to nonenveloped viruses [118,119]. Thus, MBR has been recommended as one of the most effective approaches for virus removal during WWTP operation [120].

### 5.3. Tertiary treatment processes

#### 5.3.1. Ultrafiltration

Generally, as high as a >4 log reduction of viruses could be efficiently achieved in ultrafiltration (UF) membrane filtration via size exclusion [130], especially for SARS-CoV-2 virus [131]. The adsorption of viruses to solids, a formation of a cake layer on the membrane surface, and subsequent trapping of viruses played a key role in achieving a high removal efficiency of viruses in the UF system [132,133]. Specifically, UF membranes with a slightly negative charge benefit virus elimination more than those with a neutral charge [134]. It should be noted that the nominal pore size of UF membranes is not small enough to remove NoV [135], AdV [135], RoV [136], and bacteriophages [137,138]. Hence, the reduction of viruses can be improved via the use of coagulation as a pretreatment for UF systems. For example, Lee et al. [139] reported that the mean removal rate of MS2 during coagulation-UF operation in wastewater was 2.1 log higher than that with UF alone, and this was closely related to the efficient adsorption of MS2 onto aluminum floc particles and the subsequent trapping by UF.

#### 5.3.2. Sand filtration

Viruses could be efficiently removed by slow sand filtration (SSF) via combined biological and physical treatment mechanisms [140–142], as evidenced by relatively high removal rates of 99.0% for MS-2 (28 nm) and 99.9% for PRD-1 (65 nm) [143]. The main mechanisms involved in the thin biologically active Schmutzdecke (filter cake formed on filter surface) are closely related to the combination processes of straining (related to filter bed resistance), attachment (association with sticking efficiency) and predation (related to biologic activity) [144]. The performance of SSF in virus removal also depended on the chemical characteristics of the water sources, operational temperature, the surface ripening of the Schmutzdecke and hydraulic parameters (e.g., filtering material, filter depth, hydraulic load (HL), HRT and feeding schedules) [145]. For instance, Anderson et al. [146] found virus removal to increase with a continuous increase in filter depth, water temperature, and operational HRT and with a decline in the HLs by evaluating the removal rate of sixteen MS2 during biologically mature pilot-scale slow sand filter operation. Since the majority of virus removal occurred within the Schmutzdecke, maintain a high operational efficiency of Schmutzdecke via the operational parameter optimization played a key role in enhancing virus removal [147].

#### 5.3.3. Disinfection

The disinfection of viruses by damaging genome- and protein-mediated functions to inhibit their infectivity [148] has been proven to be the most efficient and convenient approach to virus elimination before WWTP effluent is discharged into aquatic environments. Powerful disinfection approaches, including ultraviolet (UV), ozonation, chlorination and chlorine dioxide oxidation, have been widely applied and proven to be efficient technologies for SARS-CoV deactivation [149].

##### i. UV disinfection

UV radiation inactivated the viruses mainly via the following two pathways: (1) damage of nucleic acids (DNA/RNA) through the formation of pyrimidine dimers, and (2) inhibition of virus replication and transcription through the reaction of photoproducts [150]. Overall, UV radiation exhibited excellent inactivation performance for RoV, HAV, calicivirus, PV and CVB5 [151], whereas it was less efficient for HAdV type 2 (one of the most UV light-resistant enteric viruses). Recent work shows that SARS-CoV-2 is highly susceptible to UV irradiation [152], and SARS-CoV-2 could be effectively inactivated by UVC within 9 min of operation even under a  $5 \times 10^6$  TCID<sub>50</sub>-mL<sup>-1</sup> viral load. The inactivation of viruses during UV reaction is not only related to virus characteristics but also closely related to water turbidity [153]. Therefore, the

elimination or reduction of the particles in the wastewater can significantly improve UV disinfection efficiency.

##### ii. Ozonation

Ozonation inactivated viruses by directly attacking the DNA or RNA [154] or via direct oxidation by the formed free radicals [155]. Generally, ozonation requires less contact time to achieve the same inactivation efficacy of viruses as traditional chlorination (1/10 times that of chlorine) [156]. Specifically, the susceptibility of the selected five enteric viruses [coxsackievirus B5 (CVF), CVEnv1, and CVEnv2], HAdV, and echovirus 11] toward ozone decreased in the following order: CVEnv2 > echovirus 11 > HAdV > CVF  $\approx$  CVEnv1 [157]. The disinfection performance of ozonation is seriously affected by operating parameters, such as pH, alkalinity and organic content (as free radical triggers, promoters and scavengers).

##### iii. Chlorine-based disinfectants

Chlorine and chlorine dioxide have been widely applied for virus inactivation, especially for f2 bacteriophages, PV, and HAV. Specifically, chlorine exhibited excellent performance in destroying the genome and proteins of viruses [148], whereas the destruction of the genome has been regarded as the primary role involved in chlorine dioxide inactivation (especially for poliovirus, EV71 and hepatitis A virus) [158–160]. Meanwhile, the denaturation of virus proteins, such as human rotavirus (HRoV), also plays a key role in chlorine dioxide disinfection [161]. Cromeans et al. systematically compared the performance of chlorine on the inactivation of EV2, EV40, EV41, CVB3, CVB5, ekovirus 1, ekovirus 11 and murine NoV, and found CVB5 to show the strongest resistance to chlorine, whereas murine NoV exhibited the least resistance [162]. Similarly, the resistance of typical viruses to chlorine dioxide declined as follows: HRoV > coxsackie virus > echovirus > PV > f2 phages > monkey RoV [15,163–165]. Compared to chlorine dioxide, chlorine exhibited a much higher inactivation efficiency for SARS-CoV and SARS-CoV-2, which could be proven by the observation of complete inactivation of SARS-CoV by 20 mg·L<sup>-1</sup> chlorine after a 1 min reaction [58,166]. Inactivation by chlorine dioxide for virus removal is temperature- and pH-dependent, and the virucidal efficiency of EV 71 was higher at pH 8.2 than at pH 5.6 and pH 7.2. Similarly, better inactivation was also observed at 36 °C than at 4 and 20 °C [159].

It should also be pointed out that the disinfection methods mentioned above still need further improvement during their practical utilization. For example, the decline in the light intensity of UV radiation after long-term operation [167] and the formation of reaction intermediates and stable disinfection byproducts (DBPs) (haloacetic acid, trihalomethanes, chloramines, chlorate, chlorite and other toxic byproducts) during the use of chlorine-based disinfectants [168] have been widely reported. To compare the sensitivity of typical viruses to disinfectants, the performance of typical disinfections under different operational parameters is summarized in Tables 3 and 4. The UV doses commonly applied for water and wastewater treatment are between 30 and 40 mJ/cm<sup>2</sup>, and these doses were effective for many viruses except for AdV due to that double-stranded DNA viruses are likely the most resistant viruses to UV light disinfection [169]. In addition, chlorine dioxide was not as effective as expected in the inactivation of SARS-CoV at the disinfection dose applied for WWTPs, and ozone is a highly effective disinfectant for virus control (Table 4).

As noted above, traditional WWTPs are not specifically designed for the purpose of entirely removing viruses; thus, the viruses remaining in WWTP effluents negatively affect their recreational, irrigation, and potable- and nonpotable water reuse [170]. This has become the main challenge to most developing countries because they cannot be easily equipped with advanced wastewater treatment technologies [171]. Typical virus concentrations and positive detection rates in the effluent from WWTPs of different continents are shown in Fig. 3. According to

**Table 3**

Relative sensitivity of enteric viruses to chlorine, chlorine dioxide, and ozonation disinfections [15].

	Chlorine	Chlorine dioxide	Ozonation
HRoV	L	L	L
f <sub>2</sub>	L	M	H
CV	M	L	H
Echo	M	L	H
PV	H	M	L
SA11	H	H	L

Note: HRoV, Human rotavirus; CV, coxsackievirus; Echo, echovirus; PV, poliovirus; SA11, simian rotavirus; H: high, M: middle, L: light.

the data of positive samples (%), viruses with a positive detection rate (%) more than 50 were AiVs (54), EVs (92), JCPyV (85.4), SaVs (88) for America Continent and were EVs (58.3), HAstV (83), HAdV (75), NoV GI (61.6), NoV GII (92.85), RoV (66.7), SARs-CoV-2 (75) for Europe and were HAdV (89), AiV (91.6), HAstV (66.7), NoV GII (58) for Asia and were HAdV (78), HBoV (57.5), HPyV (82.4), NoV (83.3) for Africa, respectively, in which HAdV and NoV were frequently detected in Europe, Asia and Africa; although they were occasionally detected in America Continent, the log concentrations (genome copies·L<sup>-1</sup>) were as high as 6.45 and 6.1, respectively, highlighting their risks in WWTP effluent. In addition, it is worth noting that the frequency and concentration of SARS-CoV-2 were relatively high in Europe (with positive detection rates of 75%) and Aisa (with a concentration of 2 × 10<sup>3</sup> genome copies·L<sup>-1</sup>).

**Table 4**

Inactivation efficiency, operational parameters of the typical disinfectants for different viruses inactivation.

Viruses	UV			Ozonation					Chlorine dioxide			
	Dosage (mJ·cm <sup>-1</sup> )	Removal	Ref.	Parameters	Ozone	Contact time	Removal	Ref.	Concentration ppm	Treatment time	Removal	Ref.
PV-1	30	3.0 log	[196]	5 °C pH 7	0.37 mg·L <sup>-1</sup>	5 min	4.5 log	[197]	85 µM	1 min	4.0 log	[198]
	9	2.0 log	[199]									
	18	3.43 log										
	24	4.75 log										
	35	5.7 log										
Ad 40	9	0.2 log	[199]	5 °C pH 7	0.60 mg·L <sup>-1</sup> ·min	/	4.0 log	[165]	15 °C, pH 6 0.92 mg·L <sup>-1</sup> ·min		4.0 log	[200]
	18	0.68 log										
	24	0.87 log										
	35	1.21 log										
	90	3.0 log										
Ad 2	120	4.0 log		5.1 × 10 <sup>-6</sup> -4.1 × 10 <sup>-3</sup> mg·min·L <sup>-1</sup>			2.0 log	[157]	1.0 ppm	15 s	1.5 log	[203]
	78	2.0 log	[169]									
	119	3.0 log										
	160	4.0 log										
	30 (medium-pressure)	2.19 log	[201]									
90 (medium-pressure)	5.36 log		0.77-1.10 mg·min·L <sup>-1</sup>	4.0 log								
Simian Rotavirus (SA11)	42	4.0 log	[204]	4 °C pH 6	0.17 mg·L <sup>-1</sup>	8 s	4.0 log	[205]	0.5 mg·L <sup>-1</sup> ; pH 10	15 s	>4.0 log	[206]
				4 °C pH 7	0.1 mg·L <sup>-1</sup>	32 s	4.0log					
				4 °C pH 8	0.1 mg·L <sup>-1</sup>	6 s	4.0 log					
FCV	36	4.0 log	[207]	5 °C pH 7	0.01-0.03 mg·L <sup>-1</sup> min		4.0 log	[165]	0.8 mg·L <sup>-1</sup>	2.1 min	4.0 log	[208]
HAV	16	4.0 log	[204]	pH 7.0,	1.0 mg·L <sup>-1</sup>	24 s	4 log	[209]	0.2 mg·L <sup>-1</sup>	19.5 min	4.0 log	[208]
									0.8 mg·L <sup>-1</sup>	0.43 min	4.0 log	
									0.4 mg·L <sup>-1</sup>	19.58 min	4.0 log	
CVB5	27	3.0 log	[201]	1.0 × 10 <sup>-5</sup> -8.0 × 10 <sup>-3</sup> mg·min·L <sup>-1</sup>			2.0 log	[202]	0.6 mg·L <sup>-1</sup>	1.00 min	4.0 log	[208]
									0.4 mg·L <sup>-1</sup>	2.41 min	4.0 log	
									0.2 mg·L <sup>-1</sup>	3.73 min	4.0 log	
SARS-CoV	3.75	0.9 log	[210]	/	/	/	/	/	10 mg·L <sup>-1</sup>	1 min	0.25 log	[58]
	37.5	3.1 log								5 min	0.5 log	
	75	>3.3 log								10 min	>6.0 log	
	112.5	>3.3 log								20 min	>6.0 log	
	225	>3.3 log							40 mg·L <sup>-1</sup>	1 min	1.25log	
	292	1.0 log	[211]							5 min	>6.0 log	
	1048	>4.0 log								10 min	>6.0 log	

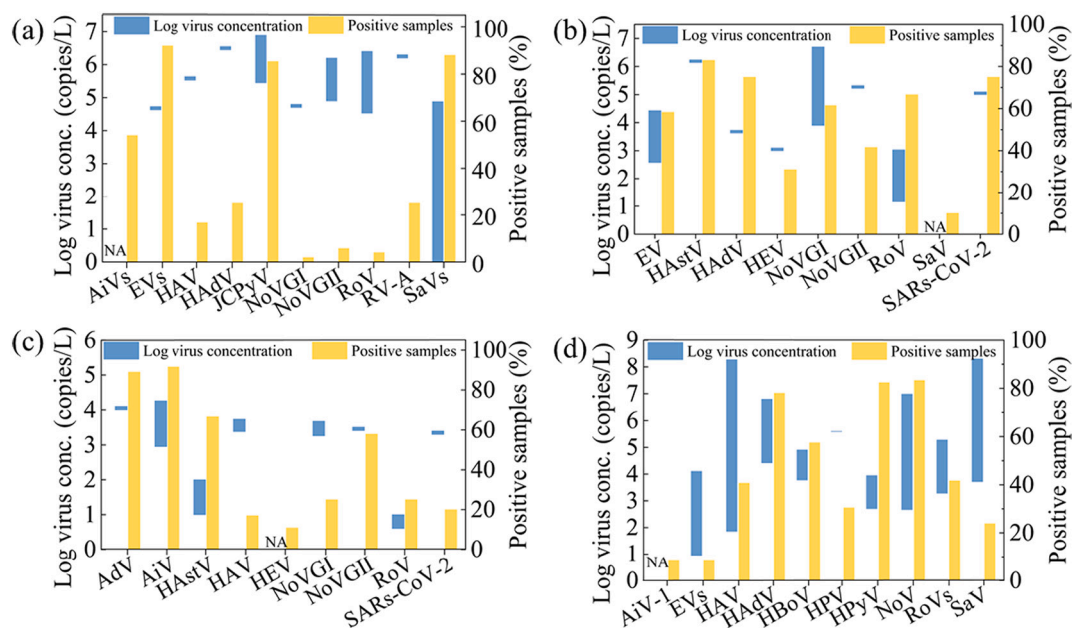
Note: PV-1, poliovirus 1; Ad 40, adenovirus type 40; Ad 2, adenovirus type 2; FCV, feline calicivirus; HAV, hepatitis A virus; CVB5, coxsackievirus B5; SARS-CoV, severe acute respiratory syndrome coronavirus.

5.4. Reuse and desalination

As mentioned above, typical enteric viruses have been frequently detected in treated WWTP effluent, which is of great concern in low-income and arid/semiarid countries, where have to reuse the effluents of WWTPs for agriculture and industry to alleviate serious water scarcity conditions [212,213]. In addition, the WWTP effluents are typically discharged into receiving water (sea, lake, river, etc.). Thus, we need to understand the occurrence and removal performance of viruses in sewage reuse and desalination. Briefly, the traditional treatment technologies of reclaimed water for irrigation mainly included coagulation, sedimentation, filtration and disinfection [214]. In contrast, membrane-based desalination technologies, especially reverse osmosis (RO) and nanofiltration (NF), have also been widely applied in agricultural applications throughout the world in recent years to meet salt content demands of agricultural irrigation water. Research has shown that NF membrane desalination technology can remove 95% of viruses [215]. Reclaimed water for industry used for cooling, district heating, etc. requires desalination according to the standard of water conductivity, and that for the steel industry is less than 400 µS·cm<sup>-1</sup> [216]; and the technical route of “UF + RO” has been regarded as one of the best technologies for desalination [217], where as high as a 2.3-2.9 log reduction rate of viruses could be achieved [129].

In coastal areas, NoV GI, NoV GII, SaV, EV, RoV, HAdV, HAV, and HPyV can be detected in seawater due to the treated sewage discharge, with the concentration of 2 × 10<sup>2</sup>-1 × 10<sup>6</sup> genome copies·L<sup>-1</sup> [218,219]. The recent work of Dias et al. identified viruses in all 48 water samples obtained from four beaches, among which 43% water





**Fig. 3.** Concentration and positive detection rates of typical viruses in WWTP effluent of (a) American Continent [7,129,172–176], (b) Europe [177–181], (c) Asia [182–190], (d) Africa [191–195]. (EVs, enterovirus; HAV, hepatitis A virus; HAAdV, human adenovirus; JCPyV, JC Polyomavirus; NoV GII, norovirus GII; NoV GI, norovirus GI; RoV, rotavirus; RV-A, rotavirus A; SaVs, sapovirus; HAstV, Human astrovirus; HEV, hepatitis E virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; AdV, adenovirus; AiVs, Avian influenza virus; HBoV, Human bocavirus; HPV, Human papillomavirus; HPyV, human polyomavirus; NoV, norovirus).

samples were positive for HAAdV and 23% were positive for JC Polyomavirus (JCPyV) [220]. Similarly, Moresco et al. analyzed 132 samples of coastal water, and found that 55% were positive for HAAdV, 51.5% for HAV, 7.5% for HuNoV GI, 4.5% for HuNoV GII, and 3% for JCPyV [218]. To obtain reclaimed water from seawater, RO, membrane distillation, forward osmosis (FO), and electrodialysis (ED) are sustainable and cost-effective desalination technologies. Specifically, RO was capable of achieving the highest log removal of viruses (3.52 to 4.40 log) among the different approaches (1.93 to 3.05 log virus removal for NF) due to its small “pore sizes” and significant size exclusion; it is interesting to note that operational pressure has a relatively weak effect on the log removal of viruses [221]. Practically, the adoption of hybrid system in seawater desalination, such as the FO-ED hybrid system, exhibits excellent performance and consumes very little energy compared to other hybrid systems [215]. Unfortunately, no data have been reported for hybrid systems in virus removal.

Recent work on the reliability of solar stills for wastewater treatment found that 90% of the virus can be removed by heating the WWTP effluent to 70 °C and denaturing proteins at high temperatures [17], and the pilot-scale solar could remove 4.5 log (99.997%) of HAAdV-5 in the distillation process [222]. Furthermore, the emergence of nanotechnology improved the distillation mechanism greatly, and might help alleviate water issues by solving the technical challenges that removing viruses [223], creating potential avenues for addressing the virus concerns via the combination traditional desalination and emerging materials.

### 5.5. Sludge treatment and disposal

The majority of the viruses (including SARS-CoV-2) in the wastewater were eventually be transferred into the sewage sludge due to their excellent ability to bind to solids and colloids [224], as evidenced by the high and steady concentration of viruses observed in primary and thickened sludge (Table 5). The removal characteristics of the viruses in the sludge samples during subsequent treatment and disposal highly depended on the treatment techniques (composting, aerobic digestion, anaerobic digestion and air-drying) used.

The average survival time of viruses within a compost pile ranged from 34 to 44.5 h [225], and a long composting HRT (10–15 days) would lead to a 3–4 log reduction of viruses under an operational temperature of 55–70 °C [130]. Aerobic conditions, accompanied by the heat generated within composting systems, might be the main reason for virus inactivation [226]. Although the utilization of anaerobic digestion alone was ineffective in the elimination of EV (1 log reduction) and SARS-CoV-2, the combination of mesophilic anaerobic digestion and thermal hydrolysis has been regarded as one of the most effective approaches to virus inactivation [226]. Similarly, the recent work of Serra-Compte et al. found the log removal of viruses during traditional digestion to be negative (mean values of  $-0.17 \pm 0.89$ ), while it was  $1.69 \pm 0.27$  for thermal hydrolysis+anaerobic digestion, which is closely related to the inactivation of the viruses by saturated steam at 150–160 °C [227]. For sludge air-drying, the infectivity of viruses declined with the continuous increase in solid content [228]; moreover, such inactivation was irreversible because of the disintegration of viral particles and release of RNA genomes. It should be pointed out that the proteases produced by indigenous microbes in sludge have a potentially significant role in the decay of enteric viruses in sewage sludge [229].

## 6. Dissemination of viruses during the entire wastewater collection, transmission and treatment process

The dissemination of viruses during the entire sewage wastewater collection, transmission and treatment process mainly includes fecal-oral transmission, aerosol transmission and direct contact [238–240]. The fecal-oral contamination route of viruses spreading via the improper treatment of wastewater plays a predominant role in the spread of viruses in low-income regions [241]. Due to a lack of wastewater sanitation and sewer systems in low-income regions, billions of people have limited or no toilet facilities and suffer from unsafe sanitation [242], and the consequential public contact with infected waste or wastewater facilitates the transmission of viral diseases via the incidental fecal-oral route. Importantly, an increasing number of researchers have proven that the fecal transmission is the main transmission route of SARS-CoV-2 [240]. Since high volumes of bioaerosols can be generated through the

**Table 5**  
Concentration of typical viruses in sewage sludge samples.

Virus	Raw sludge (genome copies·kg <sup>-1</sup> )	Anaerobically digested sludge (genome copies·kg <sup>-1</sup> )	References
EV	10 <sup>5</sup> –10 <sup>7</sup> (Primary sludge) 3 × 10 <sup>5</sup> (Secondary sludge)	2 × 10 <sup>2</sup> –2.1 × 10 <sup>5</sup>	[230]
NoV GI	/	5.0 × 10 <sup>7</sup>	[231]
Nov GII	1.6 × 10 <sup>4</sup> –4.90 × 10 <sup>5a</sup> (Primary sludge)	1.50 × 10 <sup>8</sup>	[231,232]
RoV	8.00 × 10 <sup>3</sup> –8.00 × 10 <sup>5a</sup> (Primary sludge)	1.4 × 10 <sup>4</sup> –4.85 × 10 <sup>5</sup>	[230,232]
HAdV	1.80 × 10 <sup>5</sup> –1.20 × 10 <sup>10a</sup> (Primary sludge) 9.40 × 10 <sup>9</sup> –3.90 × 10 <sup>11</sup> (Thickened sludge)	9.10 × 10 <sup>7</sup> –6.90 × 10 <sup>9</sup>	[231–233]
HAV	Activated sludge: 3.10 × 10 <sup>5</sup> –5.20 × 10 <sup>5a</sup>	/	[232,233]
AdV	Activated sludge: 5 × 10 <sup>4</sup> –1.3 × 10 <sup>7a</sup>	/	[233]
EV	5.47 × 10 <sup>7</sup> –1.15 × 10 <sup>8</sup> (Primary sludge) 5.66 × 10 <sup>6</sup> –1.44 × 10 <sup>7</sup> (Activated Sludge) 1.44 × 10 <sup>7</sup> –2.40 × 10 <sup>8</sup> (Thickened Sludge)	2.60 × 10 <sup>7</sup> –7.60 × 10 <sup>7</sup>	[231,234]
HPyV	/	7.40 × 10 <sup>7</sup> –2.5 × 10 <sup>8</sup>	[231]
Parechovirus	/	2.51 × 10 <sup>7</sup> –2.51 × 10 <sup>8</sup>	[235]
SARS-CoV-2	1.17 × 10 <sup>4</sup> –4.02 × 10 <sup>4a</sup> (Primary and activated sludge)	8.13 × 10 <sup>1</sup> –8.13 × 10 <sup>4</sup>	[236,237]

Note: EV, enterovirus; NoV GI, norovirus GI; NoV GII, norovirus GII; RoV, rotavirus; HAdV, human adenovirus; HAV, hepatitis A virus; AdV, adenovirus; HPyV, human polyomavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

<sup>a</sup> Expressed in genome copies·L<sup>-1</sup>.

high pressure and turbulence of toilet flushing, aerosol inhalation also plays a key role in sewage-associated virus transmission in communities because inhalation exposure of bioaerosols is approximately 10<sup>5</sup> times higher than dermal exposure [243]. In addition, the improperly sealed (dried out) floor drains lead to a vertical transmission of virus-laden aerosols throughout the building collection system, facilitating the spread of viruses [244] (Fig. 4a). Additionally, the leakage of wastewater from septic tanks and collection systems allows the direct discharge of viruses into receiving water bodies (such as streams, rivers, ponds, estuaries, lakes and groundwater) [245], which increases the incidence of direct human contact with virus-loaded sewage.

The mechanical rotation and aeration of wastewater during the primary and secondary treatment creates bubbles with diameters of approximately 60–600 μm that travel as much as ~8 cm above the wastewater surface of the aeration tanks [246,247]. Thus, aerosol inhalation or direct contact with infectious viral particles leads to a higher exposure risk of viruses for WWTP workers (Fig. 4b). Approximately 94% of viruses in wastewater have been detected in a liquid phase rather than on biosolid surfaces [248]; thus, aerosolization might be the main pathway of virus spread in WWTPs [249]. In addition, the microorganisms in sludge easily escape into the air and accumulate in the dewatering room during the sludge dewatering process, which also threatens the safety of workers in WWTPs [250]. Although accurate qualitative and quantitative studies of microbial concentrations in

WWTPs are currently lacking, it is clear that the concentration of microbial aerosols is significantly affected by temperature, wind speed and other factors [187]. The recent work of Gholipour et al. revealed that the annual infection risk ranged from 1.1 × 10<sup>-2</sup> to 2.3 × 10<sup>-2</sup> per person per year (PPPY) among wastewater workers and was higher than the WHO recommended level (10<sup>-3</sup> PPPY) [251]. From the above, we can conclude that the aerosols generated during wastewater treatment contributed to the transmission of viral infections; thus, it is urgently necessary to further control the aerosol inhalation of viruses in WWTPs, especially during the ongoing global COVID-19 pandemic.

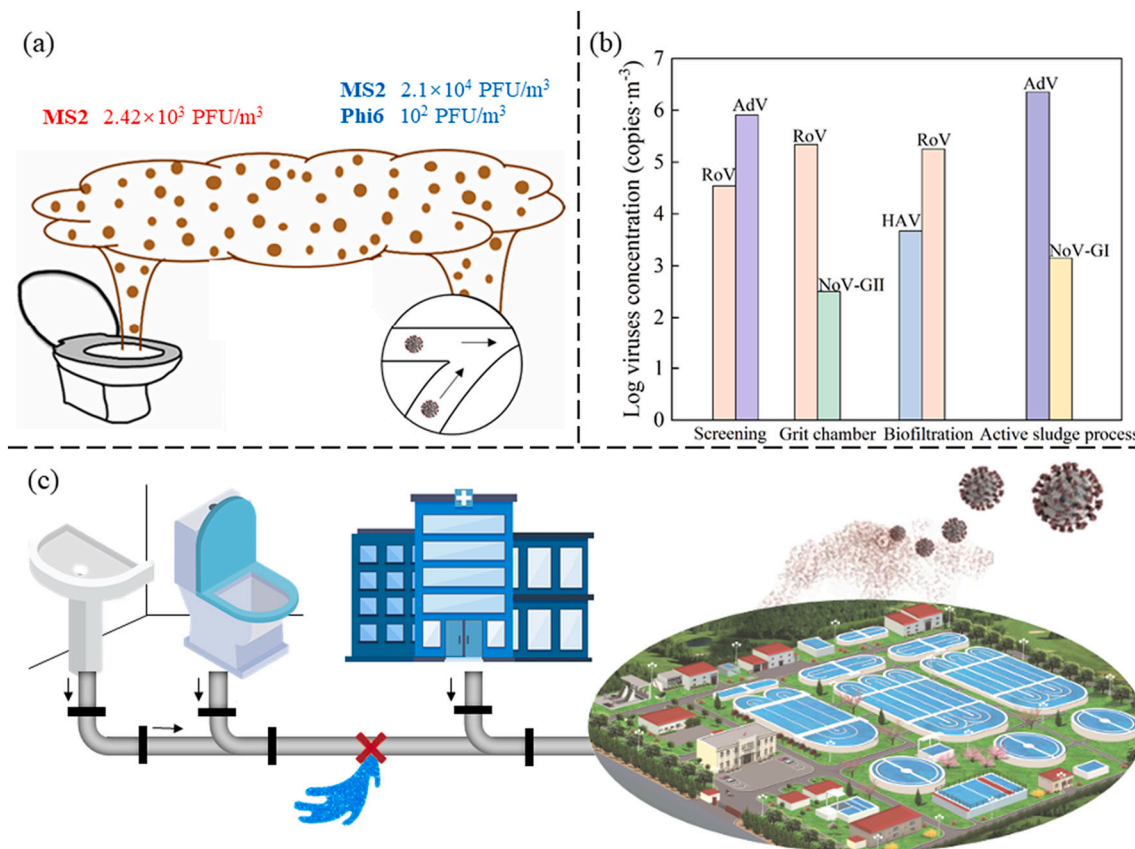
## 7. Public health risks associated with the reuse of (inadequately) treated wastewater

Discharge of treated or inadequately treated WWTP effluent into surface and natural water bodies poses a public health risk of viral infection, especially during the utilization of sewage-contaminated surface water for irrigation, recreation, shellfish culture, etc. due to the low infectious doses of viruses and their ability to persist for long periods of time outside their hosts [256,257]. Ingestion through food contamination and inhalation through the respiratory system were the means through which the pathogens viruses can enter the human body [258], evidenced by the investigation in the United States between 2009 and 2013 reporting that viruses caused 68% of foodborne outbreaks [259].

For agricultural irrigation, the probability of illness (DALY pppy) estimated of the concentrations of both HAdV and NoV GII in both tertiary effluents were 1.44 × 10<sup>-3</sup> and 1.94 × 10<sup>-3</sup> for WWTP1, respectively, whereas 2.09 × 10<sup>-4</sup> and 2.99 × 10<sup>-4</sup> were observed for WWTP2, which haven't met the threshold of ≤10<sup>-6</sup> DALY pppy for an acceptable level of risk for irrigation [180]. To achieve an annual health-based target of the tolerable annual disease burden values, the concentration of AdV in irrigation water needs to ≤6.9 × 10<sup>-5</sup> genome copies·mL<sup>-1</sup> and < 1.5 × 10<sup>-2</sup> genome copies·mL<sup>-1</sup> for EVs [260]; and the concentration of NoV GII in irrigation water needs to ≤1.8 log genome copies·mL<sup>-1</sup> [261]. Furthermore, in most developing regions, there is a concern that waterborne infection risk may increase from the reuse of untreated or partially treated sewage. The average DALY was 7.15 × 10<sup>-4</sup> for maize, 7.09 × 10<sup>-4</sup> for barley and 7.17 × 10<sup>-4</sup> for paddy rice cultivation at the concentration of ADV, EV, NoV GI, NoV GII, NoV GIV, and RV in irrigation water that were almost the same virus concentrations in raw sewage [262]. Additionally, it is estimated that at least one type of virus has been detected (with the highest concentration of 7.8 × 10<sup>5</sup> GC·m<sup>-3</sup>·h<sup>-1</sup> found for HEV) in the air above irrigated plots, [263], which emphasized the risk of inhalation of pathogen bioaerosols to public health. These reports showed that polluted irrigation water is infectious to not only farmers but also crop consumers.

In the recreational surface waters, the daily risk of viral infection at either of the investigated beaches ranged from 0.2 to 2.4/1000 swimmers; moreover, as high as 0.9%–1.5% infection risk for child swimmers taking three swims per day over a season was obtained using AdV as the risk quantitative microbial assessment model [264]. According to a case study conducted in Singapore, the mean probability of illness caused by NoV was measured as 0.61% for adults and 0.89% for children during primary contact recreation, and as 0.28% and 0.48%, respectively, for HAdV; in contrast, the mean probability of illness were 0.16% for NoV and 0.068% for HAdV during secondary contact recreation [265]. In most developing countries, such as Bangladesh, wastewater enters directly into the rivers, which increase the people's vulnerability to waterborne diseases. The risks of illness were ranged from 7 to 10% for NoV and 12 to 17% for RoV from a single exposure of bathers. The overall risk of illness at the rivers was slightly higher in children (9–19%) compared to adults (7–16%), that was higher than that in developed countries/regions [266].

The improper intake of raw or lightly cooked bivalve shellfish (oysters, clams, and mussels) can lead to illness spreading if these



**Fig. 4.** Dissemination of virus via aerosol route in the whole process of sewage collection, transmission and treatment [252–255].

(a) Concentrations of MS2 and Phi6 in aerosols generated in lab-scale models of toilet and converging sewer pipes. (b) Concentrations of typical viruses in aerosols generated from different units of WWTP. (c) Overview of potential virus dissemination via aerosol route during wastewater collection, transmission and treatment. (Different font colors in (a) and column colors in (b) indicate that the data come from different articles) (RoV, rotavirus; AdV, adenovirus; NoV GII, norovirus GII; HAV, hepatitis A virus; NoV GI, norovirus GI.)

shellfish are harvested from coastal areas impacted by sewage discharges. For example, as high as a 10% detection frequency of EV genomes was observed in the wild shellfish in Morocco growing near the outlet of a sewage drainage system [267]. According to Campos et al. the total concentration of NoV in shellfish, predicted by the model at 300:1, 1000:1 and 5000:1 ratios of estuarine water to sewage effluent, was 1200, 600, and 200 genome copies·g<sup>-1</sup>, respectively [268]. Epidemiological evidence indicates that most cases of shellfish-related illness are gastroenteritis caused by viruses, especially for NoV GI and NoV GII [269].

To guarantee the safe implementation of the WWTP effluent reuse, countries and organizations have implemented different regulations and guidelines on pathogens spread control [270], where the fecal coliforms and *Escherichia coli*, rather than virus, were the main pathogens indicators for the reuse of treated wastewater; thus, the inclusion of the virus dissemination index in the discharging guidelines and regulation of WWTPs should be seriously considered.

## 8. Challenges and future perspectives

The ongoing COVID-19 pandemic has clearly revealed the challenges and knowledge gaps in the viability and the reduction/inactivation mechanisms of viruses during wastewater collection, transmission, treatment and reuse/desalination processes. Future works in this regard could focus on the following:

- Public health departments should promote wastewater surveillance programs for monitoring the typical virus concentrations over their

entire journey from source (especially for hospital wastewater) through collection (especially for septic tanks and combined sewer systems) and treatment, especially for SARS-CoV-2 and its variants, to avoid virus dissemination.

- The outbreak of viral diseases is mostly clustered; thus, these residential areas should have an effective septic tank system, properly sealed floor drains and bathroom vent pipes, and operable drain traps in flush toilets to reduce the atomization of wastewater infected with viruses and prevent secondary dissemination.
- Since enveloped viruses (e.g., SARS-CoV-2) are more susceptible to temperature, the removal of enveloped viruses at low water temperatures is a challenge for WWTPs. In addition, studies on the persistence of enveloped viruses in wastewater, removal efficiency and inactivation mechanisms during wastewater treatment, especially in disinfection (UV, chlorine, chlorine dioxide, ozone, etc.), are still scarce and require additional exploration.
- Construction and operation of primary treatment units of WWTPs are extremely essential for the bulk removal of viruses, especially for SARS-CoV-2. Meanwhile, MBR is more effective in viruses reduction than conventional secondary treatment. However, UV is not as effective as expected in the inactivation of AdV, and the same is true of chlorine dioxide for SARS-CoV-2. Thus, “primary treatment+MBR+chlorine” was recommend for a better removal of viruses in practical operation of WWTPs.
- Diffused aeration is more reliable than surface aeration due to lower gaseous emissions of viral RNAs [271]; thus, we strongly recommend that diffused aeration be extensively used in the secondary treatment of WWTPs. In addition, the impact of SARS-CoV-2 spreading by

airborne bioaerosols is ambiguous; thus, studies of fecal bioaerosols as a possible SARS-CoV-2 transmission route should be conducted to aid the prevention and spread of current and future pandemics.

## 9. Conclusion

This review highlighted the occurrence, removal and dissemination of typical viruses in wastewater during their collection, transmission, and treatment in WWTPs. The occurrence of typical viruses (including SARS-CoV-2) among the different sources (patient feces, slaughterhouse wastewater, and hospital wastewater) underlines a significant role of wastewater in the transmission of viruses. The wide range of concentration distributions of viruses in collection systems and the effects of combined sewer systems provide critical information for quantitative risk assessment and the upgrading of sewers. By comparing the efficiency, mechanisms and influencing factors of typical virus removal in WWTP units, we provided useful data for the parameter optimization of virus inactivation. The membrane-based desalination technologies used prior to effluent reuse are highly efficient for virus removal. However, wastewater is discharged into receiving waters without being inadequately treated, and the reuse of WWTP effluent still pose a risk of dissemination of virus-related diseases to humans. The majority of viruses in wastewater are eventually transferred into sewage sludge; the combination of thermal hydrolysis and anaerobic digestion was the most effective method for the reduction of viruses. Fecal-oral aerosol transmission and direct contact are key mechanisms of the fecal droplet respiration transmission of viruses, and aerosolization in sewer systems and WWTPs may be a concern during the ongoing global COVID-19 pandemic.

## CRedit authorship contribution statement

Jian-ju Li: Conceptualization, Writing-Original Draft, Writing-review & editing. Jing Liu: Investigation, Visualization, Writing-original draft. Hang Yu: Investigation, Writing-original draft, Writing-review & editing. Wei-xin Zhao: Visualization, Writing-review & editing. Xin-hui Xia: Investigation, Writing-review & editing. Shi-jie You: Conceptualization, Project administration. Jun Zhang: Conceptualization, Supervision. Hai-long Tong: Conceptualization, Writing-Original Draft, Writing-review & editing. Liang-liang Wei: Conceptualization, Writing-Original Draft, Writing-review & editing, Project administration, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.desal.2022.115798>.

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