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Review Article

Occurrence, fate and removal of SARS-CoV-2 in wastewater: Current knowledge and future perspectives

Bhavini Saawarn, Subrata Hait *

Department of Civil and Environmental Engineering, Indian Institute of Technology Patna, Bihar 801 106, India

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ABSTRACT

The coronavirus disease 2019 (COVID-19), a pandemic of global concern, is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Recently, many studies have documented the detection of SARS-CoV-2 in human excreta and wastewater. The presence of SARS-CoV-2 in human excreta and wastewater poses serious implications for wastewater treatment. Thus, this review aims to understand the fate of SARS-CoV-2 in the urban water cycle and its inactivation in different stages of treatment in wastewater treatment plants (WWTPs) for effective control to prevent any recurrence of the outbreak. The viral load of SARS-CoV-2 in feces of individuals tested positive has been reported to be in the range of 10^4–10^8 copies/L depending on the infection stages. In the wastewater, dilution of feces results in the decrease of the viral load in the range of 10^2–$10^{6.5}$ copies/L. Monitoring of SARS-CoV-2 in WWTP samples following the wastewater-based epidemiology (WBE) can complement real epidemiological data from clinical testing to help to monitor disease outbreaks in a community. Though promising, high uncertainty involved with the WBE technique warrants further research for reliable and quantitative information. Inactivation of SARS-CoV-2 in WWTPs depends on the operational parameters and is generally enhanced by the tertiary treatment and disinfection techniques with a higher dosage. However, the risk of SARS-CoV-2 dissemination by the treated effluent intended to be disposed of or reused in the urban water cycle needs to be assessed with respect to the extent of viral infectivity.

1. Introduction

In December 2019, a bunch of cases of pneumonia of unidentified etiology was reported to the World Health Organization (WHO) from Wuhan city, Hubei Province of China [1]. The unidentified causative agent was then isolated from the throat swab sample of a patient and referred to as a novel coronavirus by the Chinese Centre for Disease Control and Prevention (CDC) and was subsequently named 2019-nCoV [1]. Later on, the characterization of the virus by whole-genome sequencing of RNA extracted from Bronchoalveolar-lavage fluid also showed the association of the outbreak with the virus, named 2019-nCoV [2]. The International Committee on Taxonomy of Viruses (ICTV) later officially designated the virus as SARS-CoV-2 due to its genetic resemblance with severe acute respiratory syndrome coronavirus (SARS-CoV) [3]. Phylogenetic analysis of SARS-CoV-2 showed it to have ˃85% genomic similarity with two bat derived SARS-like CoV (bat-SL-CoVZC45 and bat-SL-CoVZXC21). It is also showed that SARS-CoV-2 is distantly linked to SARS-CoV and the Middle-East respiratory syndrome coronavirus (MERS-CoV) with a genetic similarity of79% and 50%, respectively [2,4–6]. On 11th February, 2020, this coronavirus-associated acute respiratory disease pandemic was named as the coronavirus disease 2019 by the WHO and abbreviated as COVID-19 [7]. The clinical symptoms of the disease were identified as fever, cough, dyspnea, myalgia, headache, sore throat, diarrhea, and rhinorrhea, which, in severe cases, may lead to pneumonia, acute respiratory distress syndrome (ARDS), and multi-organ dysfunction [5,8]. On 11th March, 2020, COVID-19 was declared a global pandemic by the WHO [9]. The developmental stage of COVID-19 is illustrated in Fig. 1. The SARS-CoV-2 infection has escalated to almost all the countries across the globe, reporting 52,588,524 confirmed cases with a death toll of 1,292,078 at the time of writing this article [14].

SARS-CoV-2, the seventh coronavirus known to cause human disease, is a single-stranded, positive-sense RNA virus with helical symmetry from the *Coronaviridae* family, *Betacoronavirus* genus, and *Sarbecovirus* subgenus [4,6]. It has spike (S) glycoproteins on its surface, which uses angiotensin-converting enzyme 2 (ACE2) receptors as its host receptor [15–18]. The SARS-CoV-2 S protein recognizes human ACE2 (hACE2) with similar binding affinity as SARS-CoV S protein and

* Corresponding author.
E-mail address: shait@iitp.ac.in (S. Hait).

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enters the cells through endocytosis. This suggests an efficient human to human transmission [6,16,17,19,20]. ACE2 receptors are highly expressed in the lungs. Therefore, SARS-CoV-2 infects the alveolar epithelial cells and affects the lower airways [21]. The estimated basic reproductive number (R_0) of COVID-19 (the average number of secondary cases caused by the primary case in susceptible population) measures the transmissibility of the virus to be between 1.5 and 6.68 (1.4–2.5 as estimated by WHO) [22,23]. This exceeds the reproductive number estimated for SARS-CoV (R_0: 2–5) [23,24].

The spread of COVID-19 is primarily believed to be through respiratory droplets in the form of aerosols and contact transmission. However, recent evidences show the shedding of the viral RNA of SARS-CoV-2 through bodily excreta like feces, urine, which are subsequently discharged into the wastewater. Further, the presence of viable SARS-CoV-2 in the excreta raises concern of the possible spread of the disease through different environmental compartments. Pertaining to the detection of SARS-CoV-2 in human discharge, recent studies have also shown fecal-oral transmission as a possible alternative route [25–28]. Currently, studies have indicated the presence of SARS-CoV-2 in wastewater in multiple geographical regions. The reports of the detection of viral RNA in wastewater highlight the requirement of an effective environmental surveillance. Wastewater-based epidemiology (WBE) as a public health surveillance tool involves the screening of wastewater at the community-level and serves as an early warning system. A cost-effective way to assess the spread of infection by monitoring the viral load in the wastewater, it contains feces excreted from both symptomatic and asymptomatic individuals, and, therefore, overcomes the limitations of clinical surveillance [25,29]. Further, it is imperative to understand the fate of SARS-CoV-2 in the urban water cycle and its inactivation in different stages of treatment in the wastewater treatment plants (WWTPs) for effective control to prevent any recurrence of the outbreak. In these contexts, this review comprehensively reports and summarizes the occurrence of SARS-CoV-2 in human excreta and wastewater, along with the environmental factors affecting the virus survivability. Currently used methods for the enumeration of SARS-CoV-2 in wastewater are outlined and compared. In addition to introducing the WBE as an approach for early indication of the infection, the fate of SARS-CoV-2 in the urban water cycle is comprehensively explored. Moreover, the efficacy of various treatment techniques at different stages of WWTPs in removing SARS-CoV-2 is critically reviewed. Future research perspectives on the comprehensive performance assessment of wastewater treatment processes are presented to alleviate the risks of any future outbreak.

2. SARS-CoV-2 in wastewater

2.1. Gastrointestinal (GI) symptoms of COVID-19

Human coronaviruses, as has been identified, cause various gastrointestinal (GI) symptoms in addition to the respiratory tract disease. Previous researches have reported the existence of SARS-CoV RNA in the excreta i.e., feces and urine samples of infected individuals, with diarrhea being the most common GI symptom [30–33]. The studies conducted for SARS-CoV-2 also showed a similar result [28,34–36]. Other symptoms include nausea, vomiting, anorexia, abdominal pain, and gastrointestinal bleeding [28,37–40]. A recent study conducted on 138 COVID-19 patients reported that 10% (14 patients) experienced diarrhea and nausea prior to the onset of fever and dyspnea [41]. Another study conducted on confirmed COVID-19 cases demonstrated that out of 1141 patients, 16% showed GI symptoms with a loss of appetite being the most common symptom followed by nausea and vomiting [42]. Further, the presence of digestive symptoms in the absence of any respiratory tract diseases too has been reported [43]. Many reasons that may seem to cause the digestive symptoms are identified. However, the exact mechanism of the symptoms pertaining to gastro-intestine, which occurs due to SARS-CoV-2 still remains unclear. Various clinical investigations of COVID-19 suggest that the enteric symptoms of the virus may be attributed to the invaded ACE2 expressing enterocytes, which, in turn, can lead to malabsorption, unbalanced intestinal secretion, and activated enteric nervous system, thus resulting in diarrhea [42,44,45]. SARS-CoV-2 invade the human body by binding to the ACE2 receptor, which is expressed not only in alveolar type 2 cell of the lung, but also in the upper esophagus, stratified epithelial cells, and absorptive enterocytes of the ileum and colon [35,42,43]. Apart from this, other reasons that may give rise to digestive symptoms are: firstly, SARS-CoV-2 directly or indirectly injures the digestive system by the chain reaction of inflammatory responses [43]. Secondly, the virus changes the composition and function of the intestinal flora, which affects the respiratory tract [43]. Correspondingly, the respiratory tract flora affects the digestive tract. Both these processes take place through immune regulation, known as the gut-lung axis [43]. The assertions of these factors are indicative of the presence of GI symptoms in COVID-19 patients. Thus, along with the respiratory tract, the possibility of a potential route of the infection through the digestive system is also suggested.

2.2. Shedding of SARS-CoV-2 in human excreta

The intestinal ailments like diarrhea are indicative of the release of SARS-CoV-2 through human feces. Recently, many studies of COVID-19 cases confirmed the occurrence of SARS-CoV-2 RNA in human excreta (feces, anal/rectal swab, and urine of infected individuals as summarized in Tables 1–3, respectively). Wang et al. [46] investigated the bio-distribution of SARS-CoV-2 in the different tissues of the patients and showed the presence of the viral RNA in 29% of the total collected samples of feces. The study also reported $< 2.6 \times 10^7$ copies/L viral loads for stool samples. Further, Ling et al. [27] examined the stool specimens of 66 patients and detected the presence of viral nucleotide in 82% of the patients. The study, moreover, showed that despite the throat swabs having been tested negative, the feces or urine specimens might

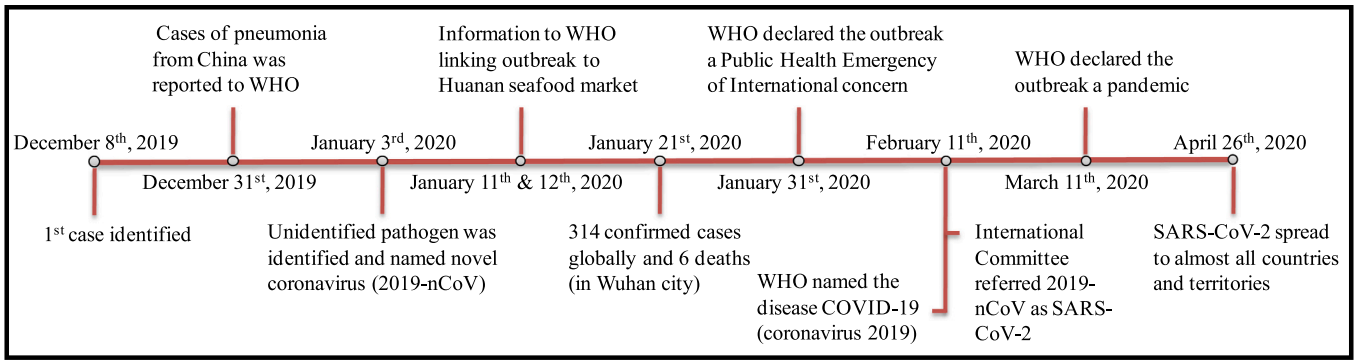

Fig. 1. Developmental stage of COVID-19 [1,7,10–13].

Table 1
Occurrence of SARS-CoV-2 in human feces.

Number of patients	Detection method	Positive rate[a]	Viral load (copies/L)	Other observations	References
153	rRT-PCR[b]	44/153 (29%)	$< 2.6 \times 10^7$	Live SARS-CoV-2 detected in the feces	[46]
4	Cell-culture	2/4 (50%)	–		
65	rRT-PCR	31/65 (48%)	–	GI symptoms- 58/95 (61%) tested positive 65 patients tested for SARS-CoV in feces, 42 were with GI symptoms, and 23 without GI symptoms	[28]
42	rRT-PCR	28/42 (67%)	–	64% (18/28) patients positive even after pharyngeal swab tested negative Duration of viral shedding from feces after negative throat swabs- 7 days	[47]
59	–	9/59 (15%)	1.26×10^8 (with diarrhea) 7.9×10^6 (without diarrhea)	Viral RNA detected in 39% with diarrhea and 9% without diarrhea Meta-analysis of 60 studies (4243 patients) detected 48.1% patients in feces and 17.6% with GI symptoms	[37]
73	rRT-PCR	39/73 (53%)	–	Duration time of positive stool test- 1–12 days 23% patients tested positive in stool after negative respiratory samples	[48]
10	RT-qPCR[c]	10/10 (100%)	–	Diarrhea in 80% of patients	[49]
14	rRT-PCR	5/14 (36%)	–	–	[50]
13	rRT-PCR	5/13 (38%)	–	Discharged COVID-19 patients Feces of 2 tested positive up to 15 days	[51]
74	rRT-PCR	41/74 (55%)	–	Positive for an average of 27.9 days	[52]
17	RT-PCR[d]	9/17 (53%)	5.5×10^4–1.21×10^5	–	[53]
6	rRT-PCR	5/6 (83%)	–	Prolonged virus RNA shedding from 2 to > 30 days	[54]
1	RT-PCR	1/1 (100%)	–	Asymptomatic child	[55]
66	–	54/66 (82%)	–	–	[27]
9	RT-PCR	8/9 (89%)	Up to 10^8 copies/g-feces	No live virus isolated	[56]
5	RT-PCR	2/5 (40%)	6.3×10^5–1.3×10^8 copies/g-feces	–	[57]
1	Cell-culture	1/1 (100%)	–	Live virus isolated in the feces sample	[58]
1	rRT-PCR	1/1 (100%)	–	Detected positive on day 7th	[26]
3	rRT-PCR	3/3 (100%)	–	Pediatric patients Longer duration of virus in feces (>28 days) than respiratory tract (14 days)	[59]
96	RT-qPCR	55/96 (57%)	–	Median duration of virus in feces (22 days) longer than respiratory samples (18 days)	[60]
3	rRT-PCR	3/3 (100%)	–	Pediatric patients Positive stool sample after 10 days of recovery	[61]
1	rRT-PCR	1/1 (100%)	1.7×10^9–4.1×10^{10}	Patient was 27-day old neonate	[62]
35	rRT-PCR	32/35 (91%)	–	Pediatric patients Viral shedding over 70 days in some children	[63]
15	qRT-PCR	11/15 (73%)	–	Positive stool sample for longer duration than respiratory tract sample	[64]
8	RT-PCR	4/8 (50%)	–	–	[65]
15	rRT-PCR	11/15 (73%)	3.86×10^6 copies/L (median)	–	[66]

[a] Positive rate of viral RNA in the samples;
[b] rRT-PCR: Real time reverse transcriptase polymerase chain reaction;
[c] RT-qPCR: Quantitative reverse transcriptase polymerase chain reaction;
[d] RT-PCR: Reverse transcriptase polymerase chain reaction.

Table 2
Occurrence of SARS-CoV-2 in rectal/anal samples.

Number of patients	Detection method	Positive rate	Viral load (copies/L)	Other observations	References
10	rRT–PCR	8/10 (80%)	5.27×10^7–5.27×10^{10}	Pediatric patients	[67]
31	RT-qPCR	14/31 (45%)	–	Shift from oral positive during early detection to anal swab positive during late infection	[68]
1	RT-qPCR	1/1 (100%)	–	Asymptomatic case	[69]
9	RT-qPCR	2/9 (22%)	4.47×10^5–5.42×10^7	–	[70]
1	rRT–PCR	1/1 (100%)	–	Asymptomatic pediatric case	[71]
1	rRT–PCR	1/1 (100%)	–	Samples remained positive from onset to 28 days	[72]

test positive as was the scenario in the case of urine samples of 3 patients. Cheung et al. [37] also reported the presence of the viral RNA in stool samples after respiratory samples identified negative. The median fecal viral load of 1.26×10^8 copies/L was recognized in patients with diarrhea. In the study, the viral genome was found in 39% of patients with diarrhea having 1.26×10^8 copies/L viral load and in 9% of patients without diarrhea with the virus concentration of 7.9×10^6 copies/L. Additionally, Zhang et al. [68] reported an increased number of viral RNA in stool and rectal swabs than in the oral swabs collected in the later phase of the disease. It, however, has also been reported that the number of cases with positive urine samples has been far less in comparison to the feces samples as shown in Table 3. This observation has been put forth by Lo et al. [49] as well indicating that though the positive viral genome is not present in the urine samples, it exists in the

Table 3
Occurrence of SARS-CoV-2 in human urine.

Number of patients	Detection method	Positive rate	Viral load (copies/L)	Other observations	References
72	rRT-PCR	0/72	–	–	[46]
10	rRT-PCR	0/10	–	–	[47]
9	RT-qPCR	1/9 (11%)	3.22×10^5	–	[70]
10	RT-qPCR	0/10	–	–	[49]
13	rRT-PCR	0/13	–	–	[51]
58	RT-PCR	4/58 (7%)	–	Urine samples of 3 patients tested positive despite negative throat swabs	[27]
9	RT-PCR	0/9	–	–	[56]
5	rRT-PCR	0/5	–	–	[57]
1	RT-PCR	1/1 (100%)	–	–	[73]
96	RT-qPCR	1/96 (1%)	–	–	[60]
1	rRT-PCR	1/1 (100%)	–	Viral RNA excreted for > 10 days	[62]
10	RT-PCR	0/10	–	–	[65]

feces.

The viral shedding from the digestive system has been reported to continue for a longer period than from the respiratory tract [74]. Some studies have indicated prolonged fecal shedding of viral RNA from 1 week to up to 5 weeks even after the respiratory swabs identified negative [47,52,59,61,75]. Zhang et al. [76] reported that the average duration of viral shedding in feces was up to 22 days, while the duration was 10 days for nasal-throat mixed swabs. Additionally, viral RNA was reported to be present in the fecal discharge of an asymptomatic child 17 days after the exposure [55]. In the course of another study, the presence of the viral genome was ascertained in the stools of an asymptomatic patient for 42 days in spite of the throat samples being tested negative [69]. The clinical diagnosis conducted on 10 pediatric patients for SARS-CoV-2 RNA in rectal swabs tested positive in 80% of children even after the negative nasopharyngeal test [67]. Therefore, shedding of the viral genome in human excreta of symptomatic as well as asymptomatic patients for a prolonged period highlights the probability of fecal-oral transmission and shows the need for an assessment of both fecal and throat samples to enhance the diagnostic sensitivity.

2.3. Methods of SARS-CoV-2 RNA detection in wastewater

Presently, real-time reverse-transcriptase polymerase chain reaction (rRT-PCR), quantitative RT-PCR (RT-qPCR), and nested RT-PCR are the techniques that are employed for the detection of SARS-CoV-2 RNA [77–80]. RT-PCR mainly targets different genomic regions of SARS-CoV-2, including the open reading frame (ORF) 1a and ORF1b regions, and the nucleocapsid (N), spike (S) protein, RNA-dependent RNA polymerase (RdRP), or envelope (E) genes [79,81]. Medema et al. [82] demonstrated the use of RT-PCR against a single fragment of E gene and three fragments of the nucleocapsid protein gene (N1-3) to detect SARS-CoV-2 in the wastewater of seven sites. The results showed the detection of N1 primer set in the samples of six sites, N3 at five sites, and E primer set at four sites, thus, proving the presence of the virus in the wastewater. While N3 and E were reported to be the two sensitive primer sets for the detection of SARS-CoV-2, N1 was identified to be the most sensitive set.

La Rosa et al. [83] brought into use two different nested RT-PCR assays targeting ORF1a, and one targeting the S gene. One real-time RT-qPCR assay, targeting the RdRp to identify the existence of SARS-CoV-2 was also brought into use. The result showed that 50% of the sampled wastewater was determined to be positive for the viral RNA. On the other hand, through RT-qPCR no positive results were obtained. Thus, no quantitative data was provided. However, the order of positive samples was further confirmed by directly sequencing qPCR. The study conducted by Ahmed et al. [25] enumerated viral RNA copies using RT-qPCR and showed 22% positive results in the samples collected from WWTP. Through the study, a median range of 171–1090 infected persons in the catchment was estimated using the Monte Carlo simulation. It also suggested the development of an effective methodology and molecular assay for the enveloped virus to improve the sensitivity and accurateness of wastewater surveillance.

As RT-PCR essentially requires thermal cycler, an alternative isothermal amplification technique that eliminates the need for a thermal cycler, such as reverse transcription loop-mediated isothermal amplification (RT-LAMP), is developed. This method allows the viral RNA detection to be easier, rapid, and cost-effective [24,81,84]. Moreover, a study has been conducted to estimate the viral infectivity using an engineered cell line in wastewater having a high vulnerability to SARS-CoV-2 [24]. This assessment is also being done employing RT-qPCR following the treatment by ethidium monoazide bromide (EMA) or propidium monoazide (PMA) to detect viruses [24]. Though the nucleic acid-based PCR test methods have high specificity and sensitivity, they are resource and time-intensive considering the requirement of sending samples to the laboratory, costly instrumentation facility, demand of skilled manpower and a long turnaround time for analysis, which are not ideal for onsite, real-time monitoring is intended. To overcome these impediments, new simple and cheaper tests like the clustered regularly interspaced short palindromic repeats (CRISPR) based assay, enzyme-linked immunosorbent assay (ELISA), paper-based indicator method are being developed for the rapid detection of SARS-CoV-2 in wastewater. The CRISPR-based assay like CRISPR-Cas12-based lateral flow assay has been developed as a more rapid substitute to real-time RT-PCR assay for COVID-19 infection [85]. Further, the ELISA method based on the concept of antigen binding to its specific antibody bringing out a change in color or fluorescence as a result of enzymatic activity has also been developed for the detection of SARS-CoV-2 [86,87]. On a similar line, paper-based analytical devices relying on a color change based on the correlation between the virus concentration and the number of aggregated particles formed by antibody-antigen binding upon the addition of antibody-conjugated fluorescent submicron particles have also turned up to be cost-effective tools for rapid detection of viruses and other pathogens [87–89]. These paper-based devices involve simple folding of the paper-based devices in multiple ways without a pump or power supply [88,89]. However, the concern remains about the sensitivity of such on-site, rapid testing methods without a robust filtration step to remove biological materials including bacteria from the wastewater samples before analysis. As the rapid tests do not amplify genetic material rather antibodies bind to a particular target, the introduction of an amplification step in such tests perceivably could help address the problem.

2.4. Occurrence of SARS-CoV-2 in wastewater

Amidst the COVID-19 pandemic, many cases have demonstrated the shedding of SARS-CoV-2 RNA in human excreta, thereby proving that wastewater might also contain the viral RNA. Table 4 summarizes the occurrence of SARS-CoV-2 in wastewater along with the methods of concentration and detection. As evident from Table 4, most of the recent studies indicated the detection of the viral nucleotide in wastewater in the countries with high as well as low prevalence of COVID-19 cases. For instance, in Australia, where comparatively lesser number of cases was reported, studies showed the presence of SARS-CoV-2 in 22% of the wastewater samples with the viral concentration being as low as

Table 4
Detection and occurrence of SARS-CoV-2 in wastewater.

Country	Type of water sample	Quantity of sample (mL)	Methods		Target gene	Positive rate	Maximum concentration (copies/L)	References
			Concentration method	Viral detection method				
USA	Untreated Wastewater	150	Corning Spin X-ultrafiltration	RT-qPCR	N	7/7 (100%)	$> 3 \times 10^4$	[90]
	Untreated Wastewater	1,000	Ultrafiltration and adsorption - elution using electronegative membranes	RT-qPCR	N	2/7 (29%)	1.5×10^3	[91]
	Treated Wastewater Secondary					0/4		
	Tertiary					0/4		
	Untreated Wastewater	–	PEG[a] precipitation	RT-qPCR	S, N	10/14 (71%)	$> 2 \times 10^5$	[92]
India	Untreated Wastewater	50	PEG[a] precipitation	RT-qPCR	ORF1ab, N, S	2/2 (100%)	0.78×10^2, 8.05×10^2	[29]
	Untreated wastewater	–	Adsorption	rRT-PCR	RdRp, ORF1ab, E, S, N	6/17 (35%)	–	[93]
France	Untreated Wastewater	–	Ultracentrifugation	RT-qPCR	E	23/23 (100%)	$> 10^{6.5}$	[94]
	Treated wastewater					6/8 (75%)	$\sim 10^5$	
Spain	Untreated Wastewater	200	Aluminum hydroxide adsorption-precipitation	RT-qPCR	N	35/42 (83%)	3.4×10^4	[95]
	Treated Wastewater Secondary					2/18 (11%)		
	Tertiary					0/12		
Italy	Untreated Wastewater	250	PEG/dextran	Nested RT-PCR[b], and RT-qPCR	ORF1ab, S, RdRP	6/12 (50%)	–	[83]
Netherlands	Untreated Wastewater	250	Ultrafiltration of centrifuged supernatant	RT-PCR	N, E	14/24 (58%)	–	[82]
Turkey	Untreated wastewater	250	Ultracentrifugation, PEG[a] 8000 adsorption, electronegative membrane, and ultrafiltration	RT-qPCR	RdRp	7/9 (78%)	9.33×10^4	[96]
Israel	Untreated wastewater	200–400	Primary: PEG[a] or Alum precipitation; Secondary: Amicon ultrafiltration	RT-qPCR	E	10/26 (38%)	–	[97]
UAE	Untreated Wastewater-WWTP	50	Ultrafiltration columns, and PEG[a]	RT-qPCR	–	28/36 (78%)	7.5×10^2–3.4×10^4	[98]
	Untreated Wastewater- 38 other locations					(85%)	2.86×10^2–2.90×10^4	
Japan	Untreated wastewater	200	EMV[c], membrane-adsorption-direct RNA extraction	Nested RT-PCR, and RT-qPCR	ORF1ab, N, S	0/5	–	[99]
	Secondary treated Wastewater	5,000				1/5 (20%)	2.4×10^3	
Australia	Untreated Wastewater	100, 200	Electronegative membranes for RNA extraction; Ultrafiltration	RT-qPCR	N	2/9 (22%)	1.2×10^2	[25]

[a] Polyethylene glycol;
[b] Nested RT-PCR: Nested reverse transcriptase polymerase chain reaction;
[c] Electronegative-vortex.

1.2×10^2 copies/L [25]. A similar study conducted in UAE reported the viral load in influents of 11 WWTPs and 38 other locations across the country [98]. Results showed that the viral load in wastewater from the WWTPs ranged between 7.5×10^2 and 3.4×10^4 copies/L. The virus was also detected in 85% of untreated wastewater samples collected from other locations with viral loads in positive samples ranging from 2.86×10^2 to 2.90×10^4 copies/L. On the other hand, in India, where the number of cases was quite high, studies detected the presence of viral RNA in all the influent samples with the maximum concentration of the virus as 8.05×10^2 copies/L [29]. Similarly, in France, Wurtzer et al. [94] conducted a study on untreated as well as treated wastewater and reported 100% positive rate with the viral concentration $> 3.2 \times 10^6$ copies/L in the former and 75% with concentration $\sim 10^5$ copies/L in the latter. Furthermore, in Japan, a study conducted by Haramoto et al. [99] showed 20% of the secondary-treated effluent samples having a viral load of 2.4×10^3 copies/L while no influent samples tested positive. As suggested by the study, the discrepancies could be due to the difference in sample volumes as the volume of influent and treated wastewater was 20 mL and 5,000 mL, respectively. These studies confirmed the viral RNA to be present in wastewater. However, assessment of the impacts of different parameters like temperature, pH, retention time, etc. on SARS-CoV-2 must also be estimated.

In the previous outbreak of the coronavirus, studies were conducted to analyze the perseverance of the virus in the changing environment. In their study, Wang et al. [100] showed that SARS-CoV maintained the vitality for 14 days at the temperature of 4 °C but was inactivated after 2 days at the temperature of 20 °C. Gundy et al. [101] too demonstrated the faster inactivation of 99.9% human coronavirus (HCoV-229E) at 23 °C (within 10 days) as compared to that occurring at 4 °C (> 100 days). The study also indicated the factors influencing the survival of coronaviruses in wastewater treatment plants to be temperature (temperature-sensitive), organic matter and suspended solids (adsorbed to the particles), and aerobic microorganisms (increase the inactivation rate). Apart from this, the presence of oxidants (strong oxidants like chlorine cause inactivation) and exposure to light (solar and UV inactivation) also affect the persistence of the virus [83,101–103].

2.5. Effect of varying environmental conditions on the persistence of SARS-CoV-2 in wastewater

In line with the assessment of the effect of the aforesaid fluctuating environmental conditions for SARS-CoV, attempts have been made to explore the survivability of SARS-CoV-2 in wastewater under varying seasonal temperatures [93,104]. Hart and Halden [104] discussed the temperature sensitivity of SARS-CoV-2 and reported that in winter, the viral genome persisted for a longer duration of time as compared to that in summer. The study also mentioned that virus detection was influenced by in-sewer travel time and suggested the half-life of the virus to be in between 4.8 and 7.2 h at 20 °C. La Rosa et al. [83] reviewed the effect of temperature on CoV and suggested a rapid decline in the viral load at a higher temperature (23–25 °C) than at a lower temperature (4 °C). Chin et al. [105] also demonstrated the temperature sensitivity of SARS-CoV-2. A decline in the stability of the virus from 14 days at 4 °C to 5 min at 70 °C was hereby seen in different clinical samples. However, Arora et al. [93] detected the viral genome in the wastewater samples at the ambient temperature of 45 °C. This corroborates well with the findings of Goswami et al. [106] elucidating an increasing linear trend between the COVID-19 cases on the one hand and the average temperature and average relative humidity in many Indian states on the other.

Further, the effect of relative humidity on the survivability of SARS-CoV-2 has been emphasized while studying the inactivation of viruses from *Coronaviridae* family [107]. Moreover, SARS-CoV has been reported to retain its viability at a temperature range of 22–25 °C and relative humidity of 40–50% for 5 days [108]. This viability is extirpated at a higher temperature (38 °C) and higher relative humidity (> 95%). Additionally, the study conducted by Chin et al. [105] reported the stability of the virus at a pH range of 3–10, which is an important criterion in wastewater. The studies, thus, brought to attention the influence of different environmental conditions on the survivability of SARS-CoV-2 in the wastewater. Nonetheless, further study is warranted to determine the stability and distribution of the virus in various water matrices.

3. Fate of SARS-CoV-2 in the urban water cycle

Many recent researches have established the presence of SARS-CoV-2 in wastewater. Originating from both symptomatic and asymptomatic patients' excreta, SARS-CoV-2 is transported to the WWTP. The wastewater released from quarantine centers, hospitals, or households with COVID-19 patients is seen to play a potential role in the spread of infection. Recently, many attempts have been made to detect SARS-CoV-2 RNA in municipal wastewater samples from the countries like Australia, France, India, Israel, Italy, Japan, the Netherlands, Spain, Turkey, the USA, UAE, and many other countries around the globe [25,29,82,83,90–99]. The viral load of SARS-CoV-2 in feces of patients tested positive has been reported to be in the range of 10^4–10^8 copies/L. In the wastewater, dilution of feces results in the decrease of the viral concentration in the range of 10^2–$10^{6.5}$ copies/L. Although the survivability of the virus in the wastewater is not explored much, few studies have isolated culturable virus from the feces of infected patients [46,58]. In the developing countries like India and other underdeveloped countries which have low sewage treatment coverage, contaminated wastewater is discharged into the receiving water bodies. This water, if consumed, can infect healthy people. The wastewater treatment systems, which have surface water and groundwater as their sources, have chances of getting viral contaminated water supply. This can be caused due to combined sewer overflows, discharge of partially treated wastewater or because of the fecal shedding of viral genome from infected patients in close proximity to the water source. A study conducted in Italy to detect the SARS-CoV-2 RNA in wastewater and rivers showed the presence of viral nucleotide in the influent but not in the effluent from the WWTP [109]. However, the viral RNA was detected in the receiving rivers, thus, showing the limited efficacy of the sewerage system. Furthermore, the detection of the viral RNA in blood, sputum, saliva and the stool samples, can also contaminate water [21,53,67,68]. The infection, nonetheless, can be curbed in the contaminated wastewater, if treated properly in WWTPs. This can be inferred from the studies which have shown negative results in the effluent samples after secondary or tertiary treatment [91,95]. However, the leakage from sewers, septic tanks, and cesspits can act as the source of viral contamination to the environment. For instance, the contaminated water from a leaking sewage pipe was transformed into aerosols and caused infections during the SARS outbreak in Hong Kong in 2003 [110]. Further, the risk of infection of SARS-CoV-2 via the direct or indirect contact with treated wastewater effluent reused for irrigation and recreational activities in the urban water cycle cannot be ruled out without proper research as the extent of viral infectivity in treated effluent is not conclusive yet. The potential route of SARS-CoV-2 in the urban water cycle has been depicted in Fig. 2.

4. Wastewater-based epidemiology (WBE)

The wastewater-based epidemiology (WBE) is a significant epidemiological tool used to detect the movement of viruses in a community and to understand the disease outbreak status by monitoring the viral load in a given catchment area [24,111]. It offers a reliable and practical approach to assess the virus prevalence and helps in minimizing the global domino effects (such as accessible health care, economic and financial stabilities, food security, etc.) of a pandemic like COVID-19 [112]. According to a study, such a surveillance strategy is useful for the prior detection of the outbreak. Constant regulation of the timely change in concentration and diversity of virus in wastewater coupled with monitoring metabolites and biomarkers for population adjustments, help in the development of early warning system [113]. It further allows for detecting the areas of onset of the outbreak, the subsequent spread, and can also be used to identify the hotspots of the disease [113,114]. The capability of wastewater surveillance to trace the virus in mild, subclinical or asymptomatic cases which otherwise remain undetectable by clinical monitoring, provide an unbiased evaluating method in determining the viral circulation in a community [24,25,29,83]. This approach was first developed in 2001 to track the consumption of illicit drugs. However, now it covers a broad spectrum of particles, including viruses in the wastewater [111,115–118]. During the past viral outbreak, WBE has proven to be a valuable tool for finding the enteric viruses like poliovirus, hepatitis A virus, and norovirus [89].

SARS-CoV-2 shows asymptomatic and oligo-symptomatic effects, which are not likely to be determined in clinical testing. With limited diagnostic capacity, the estimated extent of the infection remains largely uncertain [119]. The scrutiny of wastewater can provide for an unprejudiced method of assessing the spread of contamination even in ill-resourced regions where low prevalence may lead to underreporting [120]. Therefore, WBE may prove critical in developing countries with limited medical assistance and poor health infrastructure. Fig. 3 shows the detection of SARS-CoV-2 in the wastewater from a community using the environmental surveillance approach. WBE is an integrated technique comprising the extraction, analysis, data processing, and interpretation of biological marker shedded from human excreta in wastewater for acquiring extensive health information at the community level. Untreated wastewater is generally collected from wastewater treatment plants (WWTPs) serving the communities in a well-defined geographical sewerage catchment. Urban sewerage system provides more indicative samples of the community as the whole served population contributes to wastewater collected by any WWTP. Human viruses are inherently served as biological markers owing to the presence of their DNA or RNA in wastewater. It is postulated that the detection and quantification of SARS-CoV-2 as the biomarker in community wastewater indicate the presence and scale of infection in near real-time. Thus, the prevalence of SARS-CoV-2 infection within the sewerage catchment can be estimated using a mass balance based on the

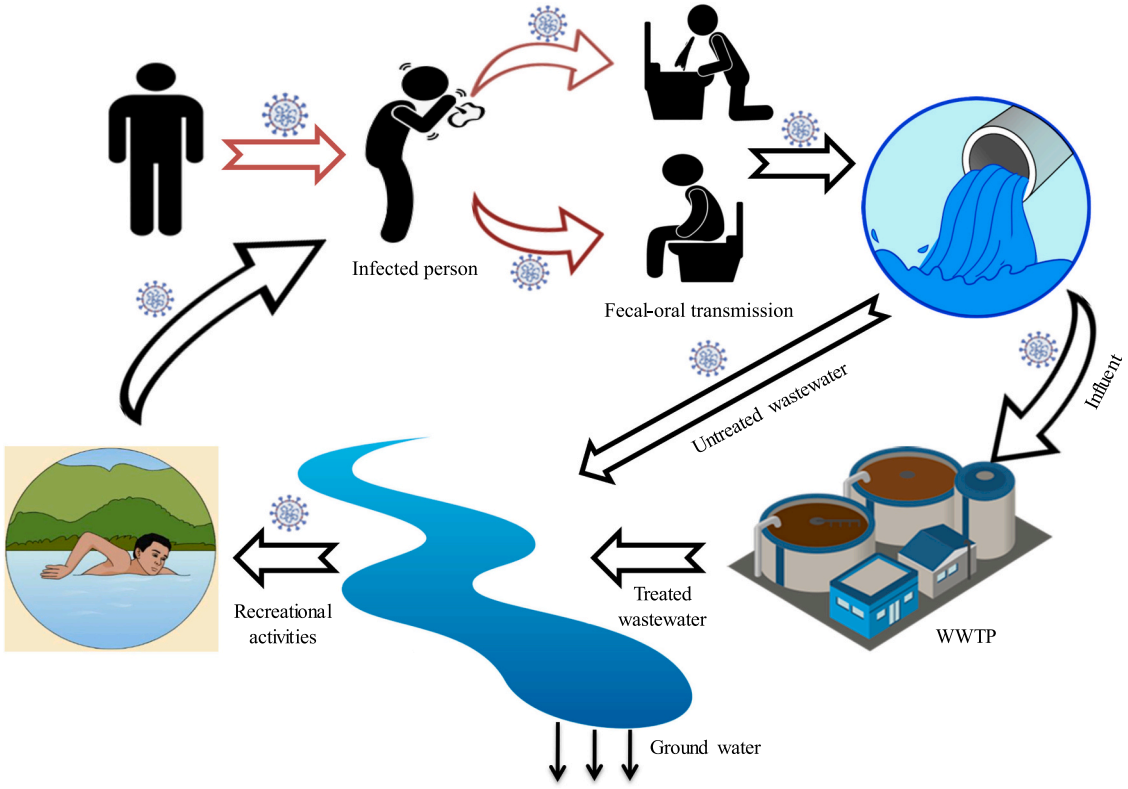

Fig. 2. Fate of SARS-CoV-2 in the urban water cycle.

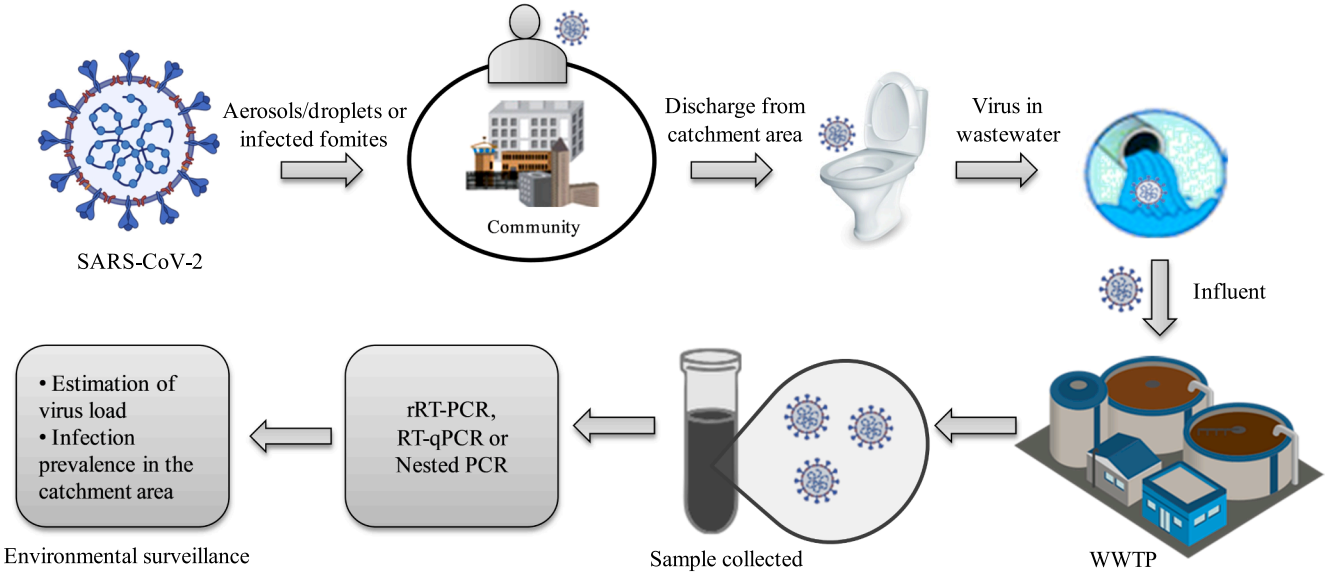

Fig. 3. Surveillance of SARS-CoV-2 in wastewater.

total viral RNA shedding in wastewater each day and the number of SARS-CoV-2 RNA copies in human excreta by an infected individual each day following the Eq. (1) [25]:

$$Persons\ \text{infected} = \frac{\left(\frac{RNA\ \ copies}{Liter\ \ of\ \ wastewater}\right) \times \left(\frac{Liter\ \ of\ \ wastewater}{day}\right)}{\left(\frac{g\ \ of\ \ feces}{person-day}\right) \times \left(\frac{RNA\ \ copies}{g\ \ of\ \ feces}\right)} \quad (1)$$

SARS-CoV-2 RNA was detected in the wastewater even before the first cases were reported by the local authorities [95]. WBE data can, thus, efficiently be employed in predicting community transmission based on the presence of viral RNA in wastewater even at low levels as well. A USA-based study adopted the environmental surveillance approach for phylogenic analysis to infer viral ancestry and monitor viral prevalence in the community [90]. Another study carried out in France presented a positive relation between the viral genome units in wastewater with the positive cases of infection. It also reported that viral genomes could be detected prior to the onset of the exponential growth of the epidemic [94]. Currently, many ongoing studies show WBE as the primary warning system for alleviating the spread of contamination and

for implementing more focused and balanced social and health measures [89,113,120]. However, there are many uncertainties involved with the WBE technique that affect the wastewater surveillance data to quantitatively resemble the confirmed cases of COVID-19 at any geographical location. Uncertainties involved with the WBE technique include the estimation of population, different excretion or shedding rate by infected individuals, decay of viral biomarker during conveyance in the sewer system, influent wastewater flow variations among others [121]. The estimation of the contributing population to wastewater samples is a crucial step for the applicability of WBE. Population normalization is, thus, important to confirm that a considerable increase in viral load in wastewater does not correspond to population increase in the sewerage catchment. It has been reported that the population dynamics may induce significantly higher uncertainties in smaller populations than the large populations [122,123]. The viral concentration and the duration of shedding vary within the individuals and across time. Also, not all infected individuals shed viral genome in excreta [95]. Further, the shedding rate of viruses in the excreta is influenced by several factors like the duration, the stage of infection, age [124]. Moreover, the catchment size and associated diurnal variation in influent wastewater flow along with the decay of viral biomarker during conveyance in the sewer system must be suitably factored in the WBE [125,126]. Dilution due to precipitation and infiltration of surface water and ground water into the sewers leads to a decrease in viral load in wastewater. WBE data is susceptible to over- or under-estimation if the temperature-induced decay of viral biomarker in wastewater is not considered during conveyance through the sewerage system. Additionally, the lack of optimized and standardized protocol for virus detection, lack of laboratory coverage and sensitive detection assay, low virus concentration method efficiency, inconsideration of temperature effects on virus detectability, etc. are some of the limitations of the wastewater surveillance approach [24,120]. Thus, the WBE can be used as a complementary monitoring technique to be integrated with the real epidemiological data from clinical testing to acquire reliable information about the prevalence of infection in the community and help to monitor disease outbreaks.

5. Removal of viruses including SARS-CoV-2 in wastewater treatment plants (WWTPs)

The SARS-CoV-2 has been reported to be extensively present in the excreta of infected patients as presented in Tables 1–3. Thus, the influent wastewater reaching the centralized WWTPs via the sewerage system is likely to contain SARS-CoV-2 RNA with considerable viral load as reported in Table 4. The treated effluents from WWTPs are typically either discharged in receiving water bodies or reused for purposes like irrigation and recreation. Prior to using reclaimed water, avoiding the dissemination of the virus in the environmental media is of utmost importance. Ahmed et al. [127] reported that the virus reduction of 6–7 $\log_{10}$ would be required through treatment plants for recycled water to be reused for irrigation. The study also mentioned that for indirect potable reuse, a 12 $\log_{10}$ reduction in virus concentration would be needed, whereas an additional removal of 2–3 $\log_{10}$ would be required for portable and non-portable use of recycled water. In this context, this section particularly deals with the assessment of the efficacy of various physical, chemical, and biological techniques and unit operations commonly employed at the different stages of the treatment viz. primary, secondary, and tertiary stages in the WWTPs for the removal of SARS-CoV-2 to produce safe water for reuse and recycle. The primary treatment stage consists of physical unit operations like screening, grit chamber, and primary sedimentation for the removal of essentially the suspended solids present in water. The secondary treatment stage comprises of biological treatment processes for the removal of biodegradable organic matter and suspended solids. The tertiary treatment stage in WWTPs involves physico-chemical treatment processes to further reduce residual organics, turbidity, nutrients, and pathogens. However, the research efforts to assess the removal of SARS-CoV-2 in various stages of conventional WWTPs are limited. The efficacy of the removal of SARS-CoV-2 in various treatment processes at different treatment stages of WWTPs is summarized in Table 5. As the viruses present in wastewater are extremely diverse with a variety of genome types, structures, replication cycles, and pathogenicity, the efficacy of removal of SARS-CoV-2 during wastewater treatment is inferred based on the mechanism of removal of other viruses, especially the human enteric viruses considering limited research being conducted.

5.1. Primary treatment

Primary treatment in a WWTP comprising of physical processes involves the removal of fixed and volatile suspended solids from wastewater through physical barriers [130]. Gravitational sedimentation process governed by the settling velocity is employed in this stage to accomplish the removal of suspended solids. The adsorption of viral particles onto the coarse suspended solids accompanied by gravitational settling is regarded as the major mechanism for virus removal in the primary treatment stage [131]. Thus, virus removal is accomplished with the increased settling velocity of suspended particles with a resultant larger diameter owing to the adsorption of virus particles. Previous studies have shown an effective removal of enteric viruses by sedimentation process [131–134]. Gerba [132] reported that up to 50% of the enteric viruses with 1.8–2.7 h of settling time could be removed through physical treatment. Similar results have also been reported by Aulicino et al. [135]. Recently, the study conducted by Balboa et al. [128] showed the occurrence of the SARS-CoV-2 RNA in 25% of the effluent samples using a primary settler. However, scarce research has been conducted to highlight the efficacy of primary treatment involving physical processes in WWTPs for the removal of SARS-CoV-2. It can be inferred based on the available scientific literature that the gravitational settling appears to be insufficient for the complete removal of viruses from the wastewater in the primary treatment stage of a WWTP.

5.2. Secondary treatment

Secondary treatment methods involve biological processes through which biodegradable organic matter and suspended solids are removed from the wastewater. Biological techniques like activated sludge process (ASP), membrane bioreactor (MBR), moving bed biofilm reactor (MBBR), sequencing batch reactor (SBR), treatment ponds, etc. are generally employed as a part of the secondary treatment stage in the WWTPs [130]. Studies conducted in the past have shown a higher level of removal of the enteric viruses through secondary treatment methods than the primary treatment methods. Further, it has been reported that coronavirus survival was slightly higher in primary treated effluent than that in secondary treated effluent owing to the protection provided to viruses by the presence of higher organics in primary treated effluent [100,136]. Aulicino et al. [135] reported around 91% removal of enteroviruses using the ASP technique. Further, a reduction in the viral load to a level of $< 2.5 \times 10^4$ copies/L was attained after the secondary treatment of wastewater employing the ASP technique for SARS-CoV-2 [95]. Adsorption of viruses onto the organic biomass and removal by settling in the secondary clarifier has been ascribed as major mechanism for the removal of viruses in the ASP [137]. Further, enveloped viruses are more likely to be attached with the organic biomass and removed than the non-enveloped viruses though no conclusive data is yet available for SARS-CoV-2 being an enveloped virus [101,138]. Moreover, extracellular enzymatic activity by hydrolases and proteases in the biological treatment processes like ASP is likely to inactivate SARS-CoV-2 as in case of other viruses [138–140]. Membrane bioreactor (MBR), comprising of a suspended-growth bioreactor and membrane filtration, has gained attention for secondary treatment of wastewater to achieve high quality effluent with considerable removal of viruses [141]. Francy et al. [142] analyzed the effectiveness of MBR technique

Table 5
Efficacy of treatment processes in different stages of wastewater treatment plants (WWTPs) for the removal of SARS-CoV-2 RNA.

Country	Number of WWTPs	Sampling (grab or composite)	Treatment Stage		Processes used	Samples collected	Results	References
Spain	6	Grab	Secondary treatment		Activated sludge	18	Positive samples: 2/18 Viral load: $< 2.5 \times 10^4$ copies/L	[95]
			Tertiary treatment		Coagulation, Flocculation, Sand filtration, Disinfection (UV, NaClO)	13	Positive samples: 0/13	
France	3	–	–		–	8	Positive samples: 6/8 Viral load: $\sim 10^5$	[94]
USA	2	Grab and composite	Secondary treatment Tertiary treatment		Activated sludge Chlorine disinfection	4	Positive samples: 0/4	[91]
Spain	1	Grab	Water line	Primary treatment	Primary settler	4	Positive samples: 1/4	[128]
				Secondary treatment	SBR	5	Positive samples: 0/5	
				Tertiary treatment	Chemical removal, microfiltration			
			Sludge line	Sludge treatment	Gravity thickening and centrifuge, thermal hydrolysis, anaerobic digestion	35	Positive samples: 14/35	
Chile	2	Composite	–		–	8	Positive samples: 3/8	[129]
Japan	1	Grab	Secondary treatment		Activated sludge	1	Positive samples: 1/5 Viral load: 2.4×10^3 copies/L	[99]
India	6	–	–		MBBR (site 5) SBR (site 6)	–	All negative	[93]

along with ASP for the removal of enteric viruses from wastewater. The study showed that the median log removal in the case of MBR was 3.02 to greater than 6.73 for pathogens including enteric viruses. Additionally, the log removal of pathogens in the range of 1.53–4.19 by the ASP was also reported by the same study. In another study conducted by Simmons et al. [143], the MBR technique as a secondary treatment option was able to achieve the log removal values (LRV) of 4.8, 6.3, and 6.8 for noroviruses, adenoviruses, and enteroviruses, respectively. In similar lines, Arora et al. [93] highlighted the MBBR and SBR as effective secondary treatment options for the removal of SARS-CoV-2 RNA from wastewater. Role of biomass with attachment to biological solids, enzymatic action, microbial predation, size retention and exclusion by membrane and cake layer formation, and membrane backwashing have been attributed as the major mechanisms of virus removal and inactivation in the MBR [139,144,145].

Analogous to other viruses, the removal of SARS-CoV-2 during the biological treatment of wastewater in the secondary stage of the WWTPs is likely to be governed by various operating parameters like hydraulic retention time (HRT), biological solids retention time (BSRT), and environmental parameters like temperature, pH [101,146,147]. Attempts were made in the past to evaluate the effect of HRT on the efficiency of virus removal. A study conducted by Feachem et al. [148] concluded that wastewater treatment ponds were capable of reducing enteric virus concentrations by 1–2-$\log_{10}$ units for every five days of retention at temperature exceeding 25 °C, suggesting that 30-day pond system would achieve at least 6-$\log_{10}$ virus reduction. Another similar study conducted by Shuval et al. [149] reported virus removal to be as high as 2–4-$\log_{10}$ units in a 20-day pond system with temperatures above 20 °C. Furthermore, a comprehensive analysis done by Verbyla and Mihelcic [131] revealed weak to moderate correlation of virus removal with the HRT in the wastewater treatment pond systems. The study reported that an average of 1-$\log_{10}$ reduction of viruses was achieved for every 14.5–20.9 days of retention, even though the 95th percentile value of the data showed 54 days of retention for each $\log_{10}$ reduction of viruses. However, a recent study demonstrated about 90% removal of SARS-CoV-2 in 36 h in wastewater [150]. This result suggests that retention time longer than the typical HRT of biological wastewater treatment processes favors the removal of SARS-CoV-2 in the WWTPs. Thus, it can be inferred based on the present state-of-the-art that the longer retention time in the WWTPs could be effective for the inactivation of SARS-CoV-2 in wastewater and this effect is expected to be more pronounced at higher temperatures.

5.3. Tertiary treatment

The tertiary treatment stage in WWTPs includes physico-chemical treatment methods to further reduce organics, turbidity, nitrogen, phosphorus, metals, and pathogens. This stage involves processes like coagulation, flocculation, filtration, nanomaterial-based treatment, chlorination, ultraviolet (UV) disinfection, ozonation, etc. [151]. The study conducted by Shirasaki et al. [152] evaluated the removal of human enteric viruses (adenovirus type 40, coxsackievirus B5, and hepatitis A virus) by coagulation-sand filtration showing a reduction of 1–3 $\log_{10}$. Apart from removal of pollutants and emerging contaminants like ciprofloxacin, oxytetracycline, sodium dodecyl sulfate [153–156], nanomaterials like carbon nanotubes (CNTs), titanium dioxide (TiO2) and zero-valent iron (ZVI) have also been employed for the removal and inactivation of viruses in wastewater [157–159]. As compared to only 2-$\log_{10}$ removal of SARS-CoV-2 in treated effluent in a study [94], a complete virus removal in secondary treated effluent treatment has been reported in another study [95]. These contrasting results suggest that effective deactivation of SARS-CoV-2 is not achieved and thereby indicate the requirement of subsequent disinfection of secondary treated effluent before disposal or reuse to reduce viral contamination. In terms of disinfection techniques, chlorination and UV irradiation have been used to effectively remove the viruses from wastewater. Collivignarelli et al. [160] reviewed the researches on the inactivation of SARS-CoV and HCoV-229E in wastewater employing disinfection techniques to understand the response of SARS-CoV-2 to the treatment. The study inferred that the inactivation of SARS-CoV was achieved in 30 min at the residual chlorine concentration of greater than 0.5 mg/L or ClO_2 concentration of 2.19 mg/L. This inference corroborates with the study conducted by Chin et al. [105] showing complete inactivation of SARS-CoV-2 using 1:99 diluted household bleach after 5 min of contact

time. Simhon et al. [161] studied the use of chlorine and UV irradiation at 5 different WWTPs reporting 0.3–1.3 LRV for enteroviruses and noroviruses. The LRVs achieved for coliphage in the study were in the range of 2.5–3. A similar case was reported by Tree et al. [162] elucidating the polioviruses reduction by 1 log after 30 min with the chlorine dose of 8 mg/L and within 5 min at the dose of 16 mg/L. On the other hand, 1.76 LRV was reached at the chlorine dose of 16 mg/L after 30 min. Further, enteroviruses were reduced by the LRVs of 0.35 and 1.2 at the chlorine dose of 8 and 16 mg/L, respectively, within the contact time of 30 min. Further, Arora et al. [93] also explored the potential of sanitization using disinfectants like NaOCl and other chlorine agents to inactivate or attenuate viruses in hospital wastewater and reported the effectiveness of disinfecting agents for virus removal. It has been reported that chlorine-based disinfection techniques and UV irradiation are likely to more effective on the enveloped viruses like SARS-CoVs owing to their lipoproteinaceous bilayer envelopment as compared to non-enveloped viruses [100,101,163]. However, the presence of organics in secondary treated effluent is expected to provide hindrance against disinfection of SARS-CoV-2 by acting as a physical barrier [136, 164]. Further, the presence of organics and nitrogenous compounds in secondary effluent will lead to consumption of chlorine-based disinfectants and thereby reduction in total residual chlorine for viral disinfection. Ozonation is another method that has efficiently been used for the inactivation of viruses. Due to high demand and unwanted byproducts, ozone has been used as a disinfectant in the place of chlorine in the effluents used for irrigation and for surface discharge [165]. The study conducted by Wang et al. [166] highlighted the efficacy of ozonation in the removal of human pathogenic viruses. The research showed a reduction of around 55–91% adenovirus, 85–100% norovirus GII, and 99–100% astrovirus 4 and parvovirus using ozonation. The formation of free radicals and subsequent oxidation of DNA or RNA lead to inactivation of viruses [167,168]. As in case of chlorination, a significant reduction of oxidation capacity for virus inactivation occurs because of the presence of organics in secondary effluent. The presence of residual enteric viruses in disinfected secondary effluent has been reported with a cause of concern for reuse [169–171]. Therefore, the inadequate virus removal and inactivation during the pandemic with greater viral load might lead to viral transmission through reuse unless suitably disinfected with higher dosage.

Recent studies have reported the presence of SARS-CoV-2 RNA in the effluent from centralized WWTPs and the surface water bodies receiving the treated effluent and thereby indicating a public health risk [29,94, 172]. The risk of infection of SARS-CoV-2 via the direct contact or aerosolization of the treated effluent upon disposal or reuse cannot be neglected without the assessment of the extent of viral infectivity. Despite taking the risk into consideration, the treatment technologies including disinfection methods that are currently employed at the different stages in the WWTPs are, thus, proved to reduce or remove the viral load considerably from wastewater. Furthermore, a higher dosage of disinfectant is established as an effective tool for eliminating the viruses in hospital wastewater. For the prevention of transmission, and reduction in the ecological and health risks, more in-depth research on the efficacy of commonly employed treatment techniques in the WWTPs with the assessment of parametric effect for the complete inactivation of viruses, especially SARS-CoV-2 is required.

6. Future perspectives

Currently, significant knowledge gaps persist in the viability of SARS-CoV-2 in the water matrices and the probable route of transmission of the infection via wastewater. Though the data pertaining to the frequency of viral shedding and viral load of coronavirus in wastewater is limited, the shedding of the virus through feces and urine is quite evident from the studies and needs to be explored further. A few studies have investigated the temperature sensitiveness of SARS-CoV-2 [93,104]. Nonetheless, the data related to the viability of viral nucleotide is still very scarce. On the other hand, the study investigating the effect of climatic conditions on the stability of SARS-CoV-2 can be inferred from the past knowledge of SARS-CoVs. Additionally, a standardized protocol needs to be developed to identify SARS-CoV-2 in water samples, pertaining to the analysis of the various studies which illustrated the use of different primers for the amplification of the viral particles. This might produce conflicting results.

Many uncertainties involved with the WBE technique affecting the wastewater surveillance data to quantitatively resemble the confirmed cases of COVID-19 at any sewerage catchment warrants further research. Further, the efficacy of the different conventional and advanced treatment techniques including disinfection methods commonly employed in the WWTPs for the removal or inactivation of SARS-CoV-2 should be comprehensively assessed with the parametric effects to understand the mechanism of removal or inactivation to minimize the environmental and health risks arising out of any future outbreak. Moreover, the risk of SARS-CoV-2 dissemination by the treated effluent intended to be reused in the urban water cycle needs to be assessed with respect to the extent of viral infectivity. Additionally, critical unit operation and treatment process are required to be realized with plausible implications on the upgradation and maintenance of WWTPs for minimizing viral dissemination. Risks associated with the processes and equipment in WWTPs including mechanical agitation likely to generate aerosols need to be ascertained to lessen the spread of infection. The analysis of the occupational risks posed by SARS-CoV-2 along with the persistence of the virus in the wastewater and receiving water bodies is also required.

7. Conclusions

This review critically highlights the occurrence, survival, detection, and fate of SARS-CoV-2 in wastewater along with its removal in various techniques and unit operations commonly employed at the different stages of the treatment viz. primary, secondary, and tertiary stages in the WWTPs. The viral load of SARS-CoV-2 in feces of individuals tested positive is highly variable in the range of 10^4–10^8 copies/L depending on the course of infection. This viral load decreases considerably with feces getting diluted in wastewater with a concentration in the range of 10^2–$10^{6.5}$ copies/L. SARS-CoVs are sensitive to the temperature owing to the higher instability of the enveloped viruses than of the non-enveloped viruses. Other factors that could influence the persistence of SARS-CoVs in wastewater include pH, organic matter, and suspended solids. The application of WBE based on the quantitative data on viral load in influent WWTP samples could supplement the real epidemiological data to assist in monitoring and preventing disease outbreaks in a sewerage catchment. However, the WBE technique is susceptible to many uncertainties like population normalization, differential shedding rate by infected individuals, the decay of viral biomarker in the sewer system, influent wastewater flow variations, albeit promising and requires further research for reliable and useful information. Considering limited research, the removal and inactivation of SARS-CoV-2 in WWTPs are inferred based on the removal mechanism of human enteric viruses and are severely affected by operational and environmental parameters like HRT, temperature, pH. Longer HRT in the WWTPs could be effective for the inactivation of SARS-CoV-2 in wastewater, and this effect is expected to be more pronounced at higher temperatures. Tertiary treatment and disinfection techniques with a higher dosage are generally effective for the inactivation of SARS-CoV-2 in WWTPs. The risk of SARS-CoV-2 dissemination via the direct contact or aerosolization of the treated effluent upon disposal or reuse cannot be neglected without the assessment of the extent of viral infectivity.

CRediT authorship contribution statement

Bhavini Saawarn: Investigation, Resources, Data curation, Visualization, Writing - original draft. **Subrata Hait**: Conceptualization,

Methodology, Supervision, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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