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SARS-CoV-2 in environmental perspective: Occurrence, persistence, surveillance, inactivation and challenges

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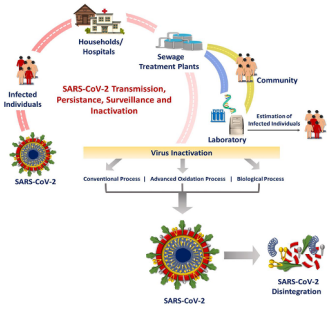
HIGHLIGHTS

- Virus-induced infections like SARS-CoV-2 is a serious threat to human health and economics.
- SARS-CoV-2 sheds out through stools making a possible faecal-oral route of transmission to environment matrix.
- Detection of enteric viruses in the environmental samples is extremely challenging.
- Integrating two or more disinfection strategies provides an effective inactivation method for viral pathogens.

GRAPHICAL ABSTRACT



Abbreviations: ACE2, Angiotensin-converting enzyme 2; AH, Absolute Humidity; AOPs, Advanced Oxidation Processes; ASP, Activate Sludge Process; A-WWTS, Algal-WWTS; BCoV, Bovine Enteric Coronavirus); BoRv, Bovine Rotavirus Group A; BSL, Biosafety Level; BVDV1, Bovine Viral Diarrhea Virus Type 1; BVDV2, Bovine Viral Diarrhea Virus Type 2; CCA, Carbon Covered Alumina; Cl^-, Chlorine; ClO_2, Chlorine dioxide; CNT, Carbon Nanotubes; COVID-19, Coronavirus Disease 2019; CRFK, Crandell Reese feline kidney cell line (CRFK); CVE, Coxsackievirus B5; Cys, Cysteine; DBP, Disinfection by-products; DBT, L2 and Delayed Brain Tumor Cell Cultures; DMEM, Dulbecco's Modified Eagle Medium; DNA, deoxyribose nucleic acid; dPCR, Digital PCR; ds, Double Stranded; dsDNA, Double Stranded DNA; E gene, Envelope protein gene; EV, Echovirus 11; FC, Free Chlorine; FFP3, Filtering Face Piece; FIPV, Feline infectious peritonitis virus; GI, Gastrointestinal tract; H_2O_2, Hydrogen Peroxide; H3N2, InfluenzaA; H6N2, Avian influenza virus; HAdV, Human Adenovirus; HAV, Hepatitis A virus (HAV); HCoV, Human CoV; HEV, Hepatitis E virus; HKU1, Human CoV1; ICC-PCR, Integrated Cell Culture with PCR; JCV, JCV polyomavirus; $\log_{10}$, logarithm with base 10; MALDI-TOF MS, Mass Spectrometry; MBR, Membrane Bioreactor (MBR); MERS-CoV, Middle East Respiratory Syndrome Coronavirus; Met, Methionine; MHV, Murine hepatitis virus; MNV-1, Murine Norovirus; MWCNTs, Multiwalled Carbon Nanotubes; N gene, Nucleocapsid protein gene; STP, Sewage Treatment Plant; NCoV, Novel coronavirus; NGS, Next generation sequencing; NTP, Non-Thermal Plasma; O_2, Singlet Oxygen; O_3, Ozone; ORF, Open Reading Frame; PAA, Para Acetic Acid; PCR, Polymerase Chain Reaction; PEC, Photoelectrocatalytical; PEG, Polyethylene Glycol; PFU, Plaque Forming Unit; PMMoV, Pepper Mild Mottle Virus; PMR, Photocatalytic Membrane Reactors; PPE, Personal Protective Equipment; PTAF, Photocatalytic Titanium Apatite Filter; PV-1, Polivirus-1; PV-3, Poliovirus 3; PVDF, Polyvinylidene Fluoride; qRT-PCR, quantitative RT-PCR; Qβ, bacteriophages; RH, Relative Humidity; RNA, Ribose nucleic acid; RONS, Reactive Oxygen and/or Nitrogen Species; RT-PCR, Real Time Polymerase Chain Reaction; RVA, Rotaviruses A; SARS-CoV-1, Severe Acute Respiratory Syndrome Coronavirus 1; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SBR, Sequential Batch Reactor; SODIS, Solar water disinfection; ss, Single Stranded; ssDNA, Single Stranded DNA; ssRNA, Single Stranded RNA; T_{90}, First order reaction time required for completion of 90%; $T_{99.9}$, First order reaction time required for completion of 99.9%; TGEV, Porcine Coronavirus Transmissible Gastroenteritis Virus; TGEV, Transmissible Gastroenteritis; Trp, Tryptophan; Tyr, Tyrosine; US-EPA, United States Environmental Protection Agency; UV, Ultraviolet; WBE, Wastewater-Based Epidemiology; WWT, Wastewater Treatment; WWTPs, Wastewater Treatment Plants; αCoV, Alphacoronavirus; βCoV, Betacoronavirus; (h+), Photoholes; +ssRNA, Positive Sense Single-Stranded RNA

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ABSTRACT

The unprecedented global spread of the severe acute respiratory syndrome (SARS) caused by SARS-CoV-2 is depicting the distressing pandemic consequence on human health, economy as well as ecosystem services. So far novel coronavirus (CoV) outbreaks were associated with SARS-CoV-2 (2019), middle east respiratory syndrome coronavirus (MERS-CoV, 2012), and SARS-CoV-1 (2003) events. CoV relates to the enveloped family of Betacoronavirus (βCoV) with positive-sense single-stranded RNA (+ssRNA). Knowing well the persistence, transmission, and spread of SARS-CoV-2 through proximity, the faecal-oral route is now emerging as a major environmental concern to community transmission. The replication and persistence of CoV in the gastrointestinal (GI) tract and shedding through stools is indicating a potential transmission route to the environment settings. Despite of the evidence, based on fewer reports on SARS-CoV-2 occurrence and persistence in wastewater/sewage/water, the transmission of the infective virus to the community is yet to be established. In this realm, this communication attempted to review the possible influx route of the enteric enveloped viral transmission in the environmental settings with reference to its occurrence, persistence, detection, and inactivation based on the published literature so far. The possibilities of airborne transmission through enteric virus-laden aerosols, environmental factors that may influence the viral transmission, and disinfection methods (conventional and emerging) as well as the inactivation mechanism with reference to the enveloped virus were reviewed. The need for wastewater epidemiology (WBE) studies for surveillance as well as for early warning signal was elaborated. This communication will provide a basis to understand the SARS-CoV-2 as well as other viruses in the context of the environmental engineering perspective to design effective strategies to counter the enteric virus transmission and also serves as a working paper for researchers, policy makers and regulators.

1. Genesis – virions and pandemic

The ongoing global spread of the pandemic due to novel coronavirus (CoV) called COVID-19 or SARS-CoV-2 causing severe acute respiratory syndrome (SARS) is posing unprecedented repercussion on human health and economy. A virus is an infectious agent (non-cellular parasite) with genetic material (either deoxyribose nucleic acid (DNA) or ribose nucleic acid (RNA); single (ss) or double-stranded (ds) or non-enveloped/enveloped (lipoproteins)) encompassed by a protein capsid. It can replicate (or make copies) only with a host cell (living) either of animal, plant, or bacteria. Viruses depend on the biochemical machinery of the host cell to inject genetic information and express through transcription (DNA to RNA) and translation (RNA to protein) [1]. Capsid made of similar sub-units called capsomere are arranged tightly together in a pattern and serves as an impenetrable shell protecting the nucleic acid giving it a defined structure (helical and icosahedral) [2].

CoV (60–220 nm size; first identified in 1960) belongs to a large family of the enveloped virus with a + ssRNA and crown-like spikes on their spherical surfaces. CoV virion is classified as highly pathogenic virus and associated with SARS-CoV-1 (2003), MERS-CoV (2012) and SARS-CoV-2 so far [3–6]. SARS-CoV and MERS-CoV belong to βCoV and are reported to have a high mortality rate [7]. The genome sequence of SARS-CoV-2 (25–32 kb) showed 82% of similarity with SARS-CoV-1 [8–10] and has two open reading frames (ORF1a and ORF1b, located at the 5′ end) coded for polyproteins and one-third of the genome encoding (terminal) for the proteins (spike, envelope, and capsid) [5]. The capsid (outer protein shell) confers specificity to the virus (Fig. 1) and the inner core confers to the infectivity and enveloped proteins associated with a virus life cycle (assembly, envelope formation and pathogenesis) [5]. Enveloped viruses are diverse with reference to genome, structure, replication, pathogenicities and persistence [11–14]. The lipid bilayer with glycoproteins (protein coded and carbohydrates are added by cellular glycosyl transferase) helps the virus to identify the host cell and fuses with its membrane. Peplomers (spike-like projections; glycoproteins) helps in virus attachment to the host [15]. Angiotensin-converting enzyme 2 (ACE2) is the host receptor that interacts with the spike protein to facilitate entry of SARS-CoV-2

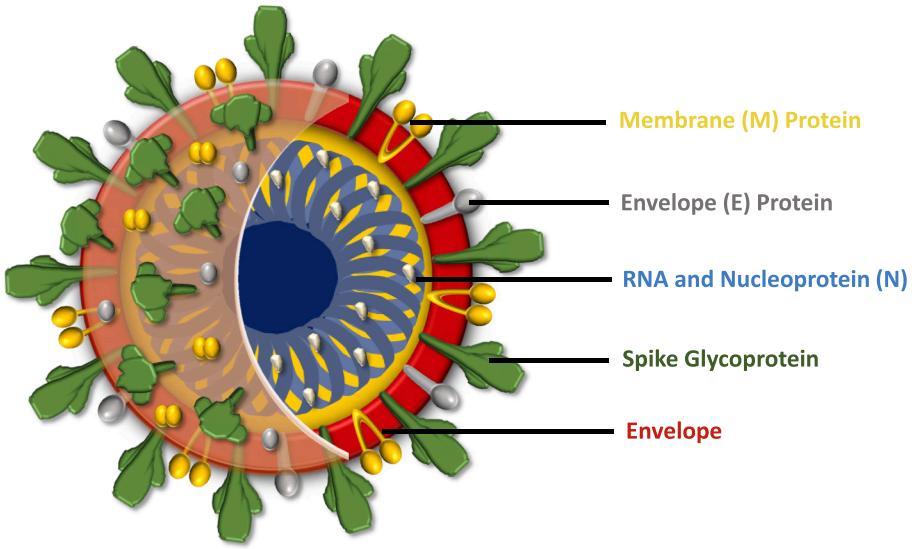

Fig. 1. Structure of Corona Virus (SARS-CoV-2).

Table 1
Persistence and occurrence of coronavirus in wastewater tested at varied temperature.

Virus	Cell line/Host/Media	Purification of virus particles	Environment matrix	Condition	Persisting Time		Findings	Reference
SARS-CoV-1	Detected using culture methods on Vero E6 cell. Cells were grown in Eagle's growth medium with 8% fetal bovine serum and 0.015 M DMEM buffer and kanamycin + gentamycin 50 μg/mL each.	Centrifugation	Stool sample	Incubation at 20 °C and 4 °C	**At 20** °C 3 days	**At 4** °C > 17 days	• SARS-CoV persisted in hospital wastewater, domestic sewage and tap water for 2 days at 20 °C and 14 days at 4 °C • SARS-CoV showed inactivation with chlorine (10 mg/L for 10 min – free residue chlorine of 0.4 mg/L) and chlorine dioxide (40 mg/L for 30 min – free residue chlorine of 2.19 mg/L) • SARS-CoV inactivated effectively than *E.coli* and f2 phage	[46]
			Urine samples		17 days	> 17 days		
			Hospital wastewater		2 days	14 days		
			Domestic sewage		2 days	14 days		
			Tap water		2 days	14 days		
E.coli and f2 phage	As an indicator microorganism for evaluating disinfection							
Transmissible gastroenteritis (TGEV)	Grown in swine testicular cell cultures (ST)	Cells propagated by infecting with confluent layer of host cell cultures followed by harvesting and clarifying by centrifugation. The supernatant is used as viral stock	Reagent-grade water	Incubation at 23-25 °C and 4 °C	**At 23–25** °C 33 days	**At 4** °C TGEV reduced after 49 days	• In all water types tested (reagent-grade water, lake water and settled sewage), the titer of infectious virus declined more rapidly at 25 °C than at 4 °C	[48]
			Lake water		13 days	14 days		
			Pasteurized settled human sewage (70 °C - 3h)		14 days	73 days		
Mouse hepatitis (MHV)	Grown in delayed brain tumor cell cultures (DBT)		Reagent-grade water		26 days	MHV reduced after 49 days		
			Lake water		10 days	No decline		
			Pasteurized settled human sewage (70 °C - 3h)		10 days	105 days		
Feline infectious peritonitis virus (FIPV)	Propagated and assayed in Crandell Reese feline kidney cell line (CRFK)	Centrifugation and addition of 9% PEG and 0.5 M NaCl followed by overnight stirring at 4 °C. The suspension was centrifuged and the resultant pellet was resuspended in 0.01 M PBS and stored. Whereas, poliovirus was purified by extraction with Vertrel XF. The resultant was emulsified, centrifuged and stored	Filtered tap water	Incubation at23 °C and 4 °C	**At 23** °C 10.1 days	**At 4** °C > 100 days	• PV-1 survived six times longer than coronavirus in both filtered and unfiltered tap water at 23 °C • PV-1 survived 2-3 times longer than coronavirus in wastewater • Filtered tap water showed quicker reduction of coronavirus than unfiltered tap water	[47]
			Unfiltered tap water		12.5 days			
			Filtered Primary effluent		2.40 days			
			Unfiltered Primary effluent		2.56 days			
			Secondary effluent		2.42 days			
Human coronavirus 229E (HCoV)	Propagated and assayed in the fetal human lung fibroblast, MRC-5 cell line		Filtered tap water		10.1 days			
			Unfiltered tap water		12.1 days			
			Filtered Primary effluent		2.35 days			
			Unfiltered Primary effluent		3.54 days			
			Secondary effluent		2.77 days			
Poliovirus-1 (PV-1)	Propagated and assayed in Buffalo green monkey kidney cell (BGM)		Filtered tap water		64.9 days			
			Unfiltered tap water		71.3 days			
			Filtered Primary effluent		35.5 days			
			Unfiltered Primary effluent		10.9 days			
			Secondary effluent		5.74 days			

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Table 1 (*continued*)

Virus	Cell line/Host/Media	Purification of virus particles	Environment matrix	Condition	Persisting Time	Findings	Reference
Murine hepatitis virus, strain A59 (MHV) (enveloped)	L2 and delayed brain tumor cell culture (DBT) grown in Dulbecco's modified Eagle Medium (DMEM) + 10% new born calf serum + 1% L-glutamine + 1% penicillin/streptomycin incubated at 37 °C with 5% CO_2	Centrifugation followed by filtration using 0.22 μm poly-ether sulfone-(PES) membrane	Wastewater Pasteurized wastewater	Incubation at 25 °C and 10 °C	**At 25** °C 13 ± 1 h 19 ± 8 h **At 10** °C 36 ± 5 h 149 ± 103 h	• Two model enveloped viruses showed rapid inactivation in wastewater than non-enveloped (inactivation > 100 h)	[49]
Pseudomonas phage ϕ6 (enveloped)	Luria-Bertani medium with NaCl at 26 °C		Wastewater Pasteurized wastewater		7 ± 0.4 h 53 ± 8 h	28 ± 2 h 146 ± 103 h	
Enterobacteria phage MS2 and Enterobacteria phage T3 (non-enveloped)	MS2 and T3 propagated and assayed in *E.coli* hosts	Fast protein liquid chromatography (FPLC) using Sepharcryl S-400 HR column followed by filtration using 0.22 μm poly-ether sulfone-(PES) membrane	Wastewater Pasteurized wastewater		121 ± 36 h 121 ± 55 h	175 ± 33 h 212 ± 88 h	

into the host cell and replicate mostly in the lungs apart from or besides heart, intestines, blood vessels and muscles [16–20]. Envelope allows the virus to exit using host cellular machinery and increases the virus particle packaging capacity with the additional viral proteins, hides the capsid antigen from freely circulating antibodies and has more structural flexibility and persistence [21]. Most of the zoonotic infecting human relate to the enveloped virus and their host choice vary depending on their surface proteins [22].

Outbreaks of SARS-CoV-2 evidenced the global vulnerability to emerging and infectious diseases [23]. The anthropogenic activities and unprecedented resource consumption manifested by the population explosion is causing ecological imbalance creating stress on the ecosystem and is being considered as one of the causing factors for this kind of epidemics/pandemics. According to an estimate, 1.67 million viral species exist on Earth and among them nearly 40% can infect humans due to the increased frequency of human interactions with the pristine nature [23,24]. The rate of zoonotic viral transmission among humans is accelerating due to increment in the global footprint leading to a non-linear rise in pandemic risk [25–27].

SARS-CoV-2 transmits via contact through human-to-human (infected patients or incubation/asymptomatic individuals) or contact to infected surfaces (fomites or skin-to-skin) or mediated through the mouth, nose, eyes or through inhalation of the exhaled virus in the respiratory droplets (coughs or sneezes from an infected person) [7,28], which eventually recommends the need for 'social distancing' to reduce the virus spread. The replication and persistence of SARS-CoV-2 in gastrointestinal (GI) tract and shedding through stools is now evidenced as a new potential transmission route to the environmental matrix, which is a major concern. Virions that persist in water, wastewater or air contacts host for further onward indirect transmission [12]. In this context, this communication attempts to review the virus influx in the environmental settings regarding its occurrence, persistence, detection, and inactivation based on the published literature so far.

2. Enteric virus – an emerging concern to the environment

Shedding of CoV in faeces was reported before the outbreak of SARS-CoV-2 pandemic [29,30–32]. The viral genetic material detected in the stool does not necessarily indicate the viable infectious virions [18,33]. The replication of SARS-CoV-1 was detected in the GI tract of the patients along with stools [29]. Cultivable SARS-CoV-1 virus was also detected in urine along with stool samples for a longer period (29–36 days) indicating its persistence in the patient excreta [30]. SARS-CoV-1 shares 82% genetic homology with SARS-CoV-2 in a subset of patients [18,30,34]. Patients infected with SARS-CoV-2 reported GI symptoms such as abdominal discomfort, GI bleeding, nausea/vomiting, and diarrhea apart from the respiratory infection [8,18,35]. Gastric, duodenal and rectal epithelia showed positive for ACE2 receptor and nucleocapsid protein providing evidence for GI infection of SARS-CoV-2 [36]. The expression of ACE2 was observed high in the upper oesophagus and stratified epithelial cells and absorptive enterocytes of ileum and colon, which enumerates the digestive system as a potential transmission route for SARS-CoV-2 infection [37]. Biopsy (colonoscopy/autopsy) studies on small and large intestine samples showed the presence of the virus and further their persistence in the faeces samples for more than 70 days after the symptom onset [38]. SARS-CoV-2 RNA was detected in faeces, respiratory specimens or blood from 6 patients, while 7 patients excreted virus in respiratory tract specimens and faeces or blood [35]. Viable SARS-CoV-2 occurrence was observed in the stool samples of 2 patients who did not suffer from diarrhea. High frequency of virus (83.3%) in faeces of mild patients was observed along with prolonged virus RNA shedding in faeces for one month [39]. Patients (18 no) samples with a mild respiratory tract infection diagnosed with SARS-CoV-2 resulted in the presence of virus in stools but not in urine [40]. SARS-CoV-2 genomic RNA was detected on the seventh day of illness in stool specimen [41]. The stool

Table 2
Occurrence of Coronavirus in wastewater.

Virus	Bench-mark	Environmental Matrix	Concentration of virus particles	Detection Method	Primers/Probe used	Findings	Reference
SARS-CoV-1	Stool from symptomatic patients from two hospitals	Sewage water from two hospitals receiving SARS patients	3X nutrient broth (pH 7.2) was used to elute the adsorbed viruses, followed by reconcentration by PEG	RT-qPCR	• Cor-p-F2/Cor-p-R1 • Cor-p-F3/Cor-p-R1	• SARS-CoV RNA was found in the sewage before disinfection in both hospitals, and from one hospital after disinfection • Infectious SARS-CoV-1 was not detected in the hospital sewage either before or after disinfection • 7/11 samples detected SARS-CoV-1 RNA • No infectious virus detected in any of the stool samples	[51]
Human Coronavirus 229E Human Coronavirus HKU1	Virome	Class B biosolids from wastewater treatment	US EPA Method	PCR	• *In-silico*	• Nine sequences of Human conronvirus 229E and one sequence of Human Coronavirus HKU1 was detected • Human pathogenic viruses like Coronavirus and Parechovirus detected in biosolid samples • Detected viruses were both environmentally transmitted pathogens (Parechovirus, Coronavirus, Adenovirus and Aichi virus), and viruses associated with chronic human infections (Herpesvirus and Hepatitis C virus)	[53]
Human Coronavirus HKU1 Human Coronavirus 229E	Virome	Influent and effluent sludge	250 ml liquid sludge mixed with 0.25 M glycine (pH-9) and centrifuged followed by filtration using 5 μm and 0.45 μm sterile membrane filter	PCR	• Adenovirus Forward • Adenovirus Reverse • Enterovirus Forward Primer • Enterovirus Reverse Primer • Enterovirus Probe • BHQ Norovirus GII Forward Primer • Norovirus GII Reverse Primer • Norovirus GII Probe • Parechovirus Forward Primer • Parechovirus Reverse Primer • Parechovirus Probe	• Coronavirus detected in 83% of the samples • HKU1 was the most prevalent RNA virus • Higher relative abundance of coronavirus was observed in influent samples than effluent • 26 DNA and 17 RNA viruses were identified in sewage sludge • Viral pathogen identification included both DNA and RNA viruses	[52]
Coronaviridae	Virome	Surface water	Bacteriophages precipitated by ultracentrifugation and isolated nucleic acid was concentrated using absolute ethanol	qPCR	• Kit assay	• 37 families with dsDNA, ssDNA, ssRNA were identified • dsDNA samples were mostly bacteriophages • Other viruses belonging to poxviridae, Herpesviridae, adenoviridae, coronaviridae, reoviridae and picornaviridae were detected	[54]
Alphacoronavirus Betacoronavirus	Hepatitis A virus	Surface water	Modified glass wool filtration method (increasing pH, contact time of beef extract buffer with glass wool, addition of detergent, recirulation of the buffer) and reconcentration of viruses using PEG	Semi-nested RT-PCR RT-qPCR	• HAV240 and HAV68, probe HAV150(-) labelled at the 5′ with 6-carboxyfluorescein (FAM)	• 1/ 21 was positive for Coronavirus by broad-range semi-nested RT-PCR • The detected virus belonged to lineage A of Alphacoronavirus and related to a rodent clade • Eight samples out of 21 were positive for Hepatitis A virus by real-time RT-qPCR	[55]
SARS-CoV-2	-	Wastewater	RNA extraction from electronegative membranes followed by ultrafiltration	RT-qPCR	• N_Sarbeco • NIID_2019-nCOV_N	• SARS-CoV-2 detected in wastewater. • The model with the given input parameters estimated a median range of 171 to 1,090 infected persons in the selected catchment • Wastewater based epidemiology (WBE) was recommended	[56]
	-	Raw sewage from wastewater treatment plant	Filtration followed by PEG 8000 (8% w/v) precipitation and addition of 0.9 g NaCl to the filtrate followed by its centrifugation to get a pellet	RT-qPCR	• CDC primers for the nucleocapsid N gene	• Demonstrated the feasibility of measuring SARS-CoV-2 in wastewater. • WBE can able to detect number of population prevalence of SARS-CoV-2	[57]

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Table 2 (*continued*)

Virus	Bench-mark	Environmental Matrix	Concentration of virus particles	Detection Method	Primers/Probe used	Findings	Reference
	-	Raw wastewater Sample	Samples were homogenised, centrifuged to get a pellet. The pellet was resuspended in 1X PBS buffer and concentrated	RT-qPCR	• E_Sarbeco_F • E_Sarbeco_P1 • E_Sarbeco_R	• Sample scored positive for SARS-CoV-2 genomes assessed by RT-qPCR on viral E gene. • Positive samples confirmed by RT-qPCR on the viral RdRp gene	[58]
	-	12 raw sewage sample	Using two phase PEG-dextran method as detailed in WHO, 2003	Broad range PCR targeting ORF1ab and semi-nested PCR PCR followed by Nested PCR PCR followed by Nested PCR	• Primer targeting highly conserved nsp12 region among all coronavirus • Bat-CoV pol 15197 • Bat-CoV pol 15635 • Bat-CoV pol 15419 • Bat-CoV pol 15635 • Novel nested primers, amplifying a 332 bp fragment of ORF1ab • 2274-CO-FW1 • 2275-CO-REV1 • 2276-CO-FW2 • 2277-CO-REV2 PCR • Wuhan CoV-spk2-r and • WuhanCoV-spk1-f Nested PCR • NIID_WH-1_F24381 • NIID_WH-1_R24873	• Detection of RNA of SARS-CoV-2 in sewage • Nested PCR assay used to detect SARS-CoV-2	[59]
	Fragments of the nucleocapsid protein gene (N1-3). One fragment of the envelope protein gene (E)	Sewage sample collected from 7 sites	Ultracentrifugation	RT-PCR	• 2019-nCoV_N1-F • 2019-nCoV_N1-R • 2019-nCoV_N1-P	• SARS-CoV-2 was detected before three weeks of first confirmed case • N1, N3 and E fragment were detected • Recommended sewage surveillance as tool to monitor the community spread of virus.	[60]
	-	WWTPs	PEG precipitation followed by centrifugation and ultrafiltration	RT-PCR	• Fast Start Universal Probe Master, forward and reverse primers including TaqMan probe	• Detected SARS-CoV-2 RNA in sewage	[61]
	-	18 Grab samples in 3 WWTPs (both influent and effluent)	Ultrafiltration	RT-PCR	• N gene • ORF1ab gene • E gene	• SARS-CoV-2 RNA was detected in the influent.	[63]
	-	Influent, Secondary and tertiary treated samples from WWTPs serving major municipalities	Concentration using Al(OH)3 absorption followed by precipitation, centrifugation, resuspension of pellet in PBS	RT-PCR	• CDC primers	• 11% secondary treated water samples tested positive for at least one gene. • None of the tertiary effluent samples (n = 12) tested positive for SARS-CoV-2.	[64]
	-	WWTPs	Filtration followed by PEG precipitation	RT-PCR	• ORF1ab • N-gene • S-gene	• Conventional treatment plant showed removal of genetic materials of SARS-CoV-2	[65]
	-	7 WWTPs and 2 Manholes	Filtration and PEG precipitation	RT-PCR	• Primers TaqMan probe sets targeting SARS-CoV-2 RdRp gene	• 5/7 WWTPs and 2/2 manhole samples tested positive for SARS-CoV-2 genome	[66]
	-	6 WWTPs	Filtration and PEG precipitation and UV treatment	RT-PCR	• FDA and ICMR approved Allplex™ 2019-nCoV Assay kit	• Detected COVID-19 genome at ambient temperature (above 40°C)	[67]
	-	Wastewater Sewage Sludge	Wastewater-ultrafiltrationSewage sludge-PEG precipitation	RT-PCR	• RdRp • N-gene • E-gene	• SARS-CoV-2 genome detected in secondary treated wastewater samples. • Biosolid in the sewage lines detected virus genome	[62]
	-	Five influent and five effluent grab samples from WWTPs and three river samples	Concentration using the electronegative membrane-vortex (EMV) followed by filtration, adsorption (direct RNA extraction) and filtration	qPCR Nested PCR	• ORF1ab • S-gene	• SARS-CoV-2 RNA was detected in 1/5 secondary-treated wastewater samples • SARS-CoV-2 RNA was not detected in three river water samples	[68]

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Table 2 (*continued*)

Virus	Bench-mark	Environmental Matrix	Concentration of virus particles	Detection Method	Primers/Probe used	Findings	Reference
			through a mixed cellulose-ester membrane				
	-	Fourteen influent and Fourteen treated effluent samples from 7 STPs (80% of STP capacity of Hyderabad, India) and two sewage samples from gated residential community	Filtration through 0.22 um followed by centrifugation (10 kDa cut-off)	RT-PCR	• N-gene • E-gene • ORF1ab	• SARS-CoV-2 RNA was detected in the all influent sewage samples. • As a testimony of efficient wastewater treatment, no viral RNA copies were detected in the treated outlet of the STPs that were sampled	[69]

samples of SARS-CoV-2-infected patients (73 no; hospitalized) showed positive to viral RNA (53.42%) and remained in feces (23.29%) even after the viral RNA decreased to an undetectable level in the respiratory tract [36]. The virological analysis of stool samples of nine SARS-CoV-2 cases showed a combination of high virus RNA concentrations and occasional detection of single guide (sg) RNA containing cells for longer periods indicating active replication in the GI tract [42]. SARS-CoV-2 recovered patients (66 no) after treatment were detected positive for viral RNA in both stool (16.7%) and urine (6.9%) samples [43]. Viral RNA persisted in faeces for 33 days after the patient was tested negative for viral RNA in the respiratory tract [44]. Viral shedding through the digestive system can last longer than shedding from the respiratory tract [19]. The presence of fragments of viral RNA was also detected in the faeces of infected patients [5,36,41]. The presence of both viable virion and viral RNA of SARS-CoV-2 (Supplementary Table 1) was detected in stool samples [Supplementary References 1-11].

3. Persistence of virus

To transfer via the water cycle, the virus should be able to persist in waste and retain its infectivity before contact [45]. The fate of CoV persistence in wastewater (Table 1) was tested at varying temperatures using bacteriophages as indicators [46–49]. Bacteriophages share morphological and biological properties with the enteric viruses and serve as surrogates [50]. Temperature is one of the critical factors that govern the virons survival in the environment. Various kinds of wastewaters were spiked with SARS-CoV-1, Human CoV (HCoV 229E) and surrogates of animal CoV as transmissible gastroenteritis (TGEV), Feline infectious peritonitis virus (FIPV) and Murine hepatitis virus A59 (MHV) to study their persistence at variable temperature conditions (4–25 °C) [46–49,51]. *In-vitro* studies on SARS-CoV-1 spiked in stool and urine sample, hospital and domestic wastewater and tap water showed virus persistence at relatively lower temperature (4 °C) [46]. SARS-CoV-1 persisted for 2 days at 20 °C, while for 14 days at 4 °C. On the contrary, urine samples showed longer persistence (17 days) at 20 °C. Salt content present in urine helped the virus to maintain osmotic pressure that is needed for their persistence [46]. Similar persistent period of HCoV and FIPV in filtered and unfiltered tap water, primary effluent and secondary effluent at 23 °C was observed contrary to 100 days at 4 °C [47]. The indicator organism (polivirus-1 (PV-1)) showed six times longer persistence than HCov and FIPV [47]. The persistence of the virus decreased with the increase in temperature. Surrogates of CoV was used to study the persistence [48,49]. Spiking water (reagent grade), lake water, and human sewage (pasteurized) with surrogates of CoV (TGEV and MHV) showed the decline in virulence rapidly at 25 °C than 4 °C [48]. Wastewater and pasteurized wastewater spiked with MHV A59 was incubated at 25 °C and 10 °C (typical summer and winter temperatures) [49]. MHV A59 persisted for 19 ± 8 h at 25 °C in pasteurized wastewater which was less when compared to *Pseudomonas* phage ϕ6 (53 ± 8 h), MS2 and T3 enterobacteria phage (indicator organism) (121 ± 55 h). The decline in the infectious virus was more rapid and effective at higher temperatures [46–49]. Enveloped viruses also showed rapid inactivation than the non-enveloped ones (> 100 h) [49]. CoV is more sensitive than PV-1 due to the presence of envelope and low transmission rate observed with the CoV due to rapid inactivation at ambient temperatures [47].

4. Occurrence of virus in environmental matrix

4.1. Wastewater

Earlier studies [51–60] showed the occurrence of CoV in wastewater samples (Table 2). Wastewater originating from the hospital during the SARS-CoV-1 outbreak was tested for its virulence/occurrence before and after disinfection using a symptomatic patient sample as a benchmark [51]. Infectious SARS-CoV-1 was found in the sewage of two

hospitals before disinfection [51]. Influent and effluent sludge samples studied for the occurrence of HCoV 229E and HKU1 using virome as a benchmark detected 83% of CoV with HKU1 prevalence [52]. The relative abundance of CoV was higher in the influent sample than the effluent sample. Reports around the globe demonstrated the occurrence of SARS-CoV-2 in wastewater [56–69] (Table 2). Sewage samples collected from seven sites detected nucleocapsid protein gene (N1-3) and E gene [60]. SARS-CoV-2 was detected before three weeks of the first confirmed case, which is very important as early warning signal. N1 and N3 fragments were detected in five sites and E fragment in 4 sites [60]. In another study, SARS-CoV-2 RNA fragments were detected in raw sewage sample [59] and showed positive for SARS-CoV-2 [58]. Bioinformatics with Monte Carlo simulation study detected SARS-CoV-2 from wastewater as inputs estimated the infected people in the selected catchment [38,56]. Increasing the circulation of the virus in the population will increase the virus load into the sewer systems [60]. The presence of solvents and detergents in wastewater can compromise the viral envelope and therefore affects its persistence [47,68,70].

4.2. Water

The presence of human enteric viruses were reported on surface and groundwater, public water supply, freshwater and sediments apart from sludge and wastewater samples [71]. Surface water (river, lake, and water reservoir) detected 37 families with a genetic material (dsDNA, ssDNA, and ssRNA) of which dsDNA samples were mostly related to bacteriophages and the majority of sequences related to families of Coronaviridae, Reoviridae and Herpesviridae [54]. Another study on the detection of CoV from surface water using hepatitis A virus (HAV) as a benchmark using semi-nested, real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and quantitative RT-PCR (qRT-PCR) found positive for CoV to the lineage A of alpha-coronavirus (αCoV) related to the rodent clade [55]. Water treatment plants are also susceptible to have viral contamination and pose the possibility to transmit through water distribution if not fully inactivated [45]. Viruses in surface waters are exposed to inactivating stresses such as sunlight and predations [45]. The time required for virus infectivity in pure water and pasteurized settled sewage was several days at ambient temperature, which adds another concern for this potential transmission route [7,48].

4.3. Engineered wastewater systems

Wastewater treatment plants (WWTPs), in general, have high density and diversity of viruses [72]. Human enteric viruses studied (rotavirus and enterovirus) in the final effluents of five WWTPs showed the persistence of Rotavirus (up to 10^5 genome copies (GC)/L in 41.7% of the samples) [73]. CoV persisted in primary wastewater for a longer period than secondary wastewater due to the presence of suspended solids [47]. The transmission of COV in the aqueous environment is less susceptible due to inactivation at ambient temperatures [47]. SARS-CoV-2 RNA was detected within six days from WWTPs indicating their persistence in water [56]. The indigenous microbial population generally affects virus survival in wastewater/water [74,75]. Avian influenza virus (H6N2) survived in methanogenic landfill leachates and water showed rapid inactivation at elevated temperature and non-neutral pH while conductivity did not show any influence on the survival [76]. Viruses abundance in wastewater streams also influenced the bacterial community structure and catalyzed virus-mediated horizontal gene transfer [72]. Bacteriophages associated with the human GI tract are commonly reported in wastewater and phages infecting Bacteroides are used as an indicator for assessing the wastewater quality and contamination [77]. The inactivation time of virus in landfill leachates varied between 30 and 600 days indicating the viable viral infection during and after the waste disposal [76].

Viruses survive in WWTPs absorb/attach to solid surfaces (activated sludge) resulting in virus-laden sludge/biosolids [71]. The affinity of SARS-CoV-2 with biosolids of the sewage line acts as an indicator of incidence as they act as concentrators of SARS-CoV-2 genetic material [62]. The enveloped virus was found to adsorb on the solid fraction (26%) of wastewater, which indicates the virus removal by solid settling [49]. Biosolids as per United States Environemntal Protecton Agency (US-EPA) classified into two-namely Class A or pathogen-free biosolids and Class B biosolids, which may have some pathogens [78]. Class A biosolids can be used for gardening, while Class B biosolids can be applied on agricultural and forest lands which showed the presence of relatively large numbers of viable viruses [79]. Both enveloped and non-enveloped viruses (CoV, Herpesvirus, Torque Teno virus, and Parechovirus) were detected in Class B biosolids [52,53]. Sewage sludge (influents and effluents) was detected about emerging viruses (CoV, Klassevirus, and cosavirus), wherein 83% of samples were detected for CoV followed by CoV-HKU1 [52]. WWTPs shed viruses through treated effluent discharge as well as biosolids [71]. HCoV inactivation was reported faster in filtered tap water than unfiltered tap water, suggesting that the suspended solids present in water infuse protection for viruses by adsorption [47]. The aerosolization potential of viruses in WWTPs evaluated with the partitioning of bacteriophages (MS2 and Phi6) with reference to sludge (synthetic and anaerobically digested) showed 94% of virions partition with the liquid phase [80]. The biofilm system (trickling filter) showed the abundances of viral sequences compared to the suspended growth system (activated sludge) [72]. Biofilm fosters multiplication of viruses due to the prevalence of higher density of microflora or due to the entrapment of viruses in the extracellular exopolysaccharide matrix that might contribute to viral abundance in the biofilm system. The sediment can function as enteric virus reservoir and viruses can survive for several months in soil and groundwater when temperatures are low and soils are moist, increasing risk because of water contamination [71]. The treatment capacity of WWTPs in the elimination of SARS-CoV-2 genome were reported by few studies (Table 2). SARS-CoV-2 RNA was detected in the sewage influent samples and secondary treated samples, whereas it was not detected in effluent samples of WWTPs stating that the treatment plants are capable of removing the genetic material of SARS-CoV-2 [61–69].

5. Environmental exposure routes – virus transmission

It is evident from studies reported so far that the GI tract is another site of SARS-CoV-2 replication and facilitates shedding through stools [18,19] (Supplementary Table 1). The transmissibility of SARS-CoV-2 in the community is a major concern that cannot be ignored knowing the ability of other viruses spread to cause waterborne transmission through faecal-oral route [5,12,18] which resulted in many sporadic cases and outbreaks that are severe or sometimes fatal [81]. Enteric viruses infect GI tract, multiply and excrete through faecal-route contaminating water, food, and air through sewage discharges, septic tanks, water supplies, and runoff [82]. The range of severity depends on the virus survival and replication capability and most of the time an asymptotic individual unknowingly can infect and thus spreads [69,83]. So far low SARS-CoV-2 loads in stool samples were reported [84]. The persistence of viral RNA in faeces for 33 days after the patient was tested negative [44] cannot be ignored. In general, enteric viruses facilitate transmission at a very low infectious dose (< 20 particles) required to cause illness [153]. In some reports, high titers of virus in the faeces and occasionally, at lower concentrations in urines were detected in infected humans [5,85].

Enteric virus transmission to the community with wastewater streams is now a major point of focus and concern [12,70] even though SARS-CoV-2 transmission via the faecal-oral route is still unclear with the limited information [19,44,86,87]. The possibility of community transmissibility spread cannot be ignored based on the previous experience [5,12,18,81,88]. Viruses excreted through faecal-oral route usually link with the existing water infrastructure connected with

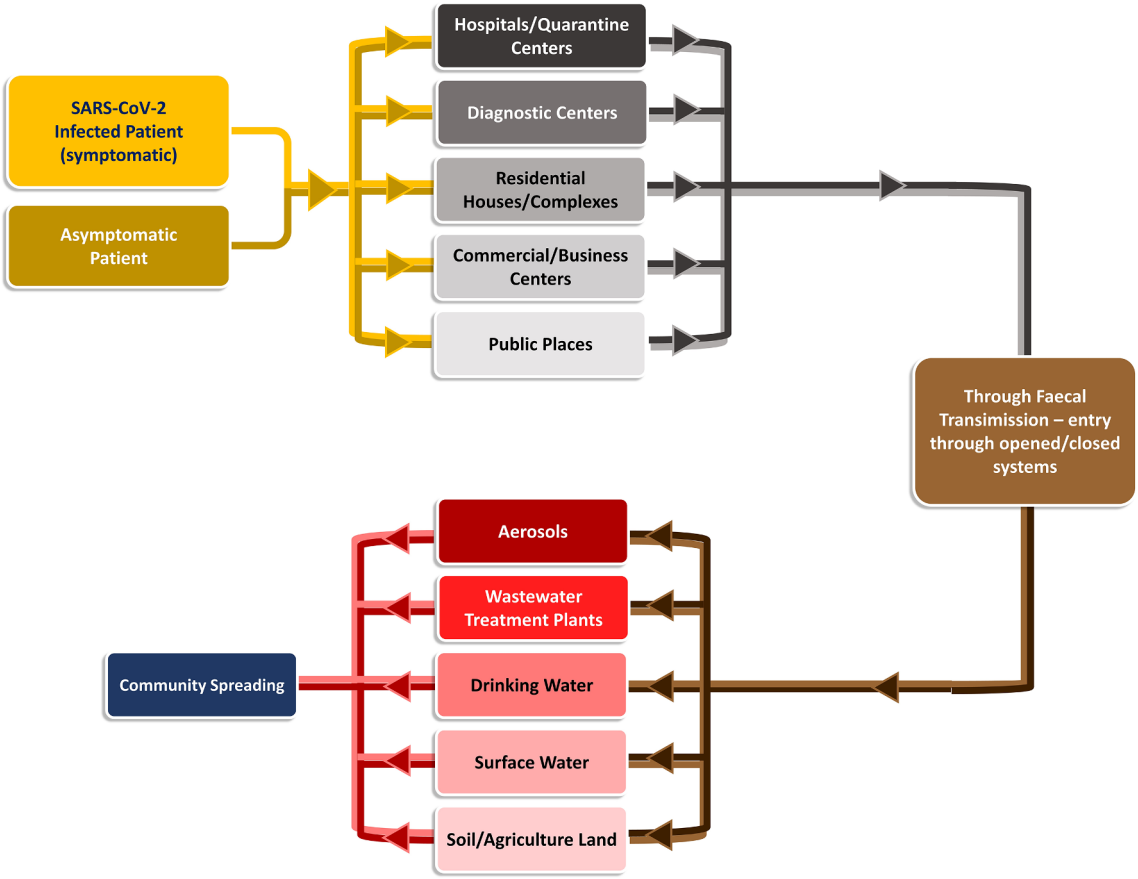

Fig. 2. Possible Transmission Route of SARS-CoV-2.

drains, sewage, water, treatment plants, hospitals/clinics, diagnostic centres, etc. (Fig. 2) and manifest the transmission. Enteric virus occurrence was reported in surface water, sewage, WWTP discharges, septic tank outflow, sewer overflows, runoffs, and infiltration during rainfall which eventually leads to contaminate surface and groundwater [71]. The majority of the population will exhibit mild symptoms or carry viruses asymptomatically and are still capable of shedding the virus through faeces [69,70,89,90], which eventually spread the virus through sewerage infrastructure [6,91].

5.1. *Airborne transmission – virus laden aerosols*

Given, the spread of SARS-CoV-2, the interconnectedness of the wastewater infrastructure, the concentrations of infected people pose a greater threat for airborne transmission due to aerosolization of the virus [92,93]. Infectious viruses, including CoV, can survive for long periods outside of its host organism [94]. Contaminated water/wastewater will also function as a potential vehicle for human exposure if virus-laden aerosols are formed [48]. Infected stools further transmit through virus-laden aerosols during flushing operation [7]. The faulty sewage-drainage system was linked to the SARS-CoV-1 outbreak among the residents living in the surrounding buildings [95] wherein aerosolized particles play a major role in virus spreading [96]. The transportation of CoV in water potentially increases the virus spread through the aerosol formation [97] particularly during wastewater discharge and flow in the drainage system, pumping stations and WWTPs.

Airborne dust is another transmission route linked to infectious diseases [7,96]. Although SARS-CoV-2 is not an airborne virus, adsorption of the virus on airborne dust and particulate matters (PM) contributes to long-range transport of the virus and their inhalation could pass the virus into deeper alveolar and tracheobronchial regions, which could increase the chance of infection [7,98]. The high levels of PM concentration in air pollution might increase the susceptibility of the population to more serious symptoms of the disease [7]. High COVID-19 infection rate was linked to the air pollution in the Italian cities that exceeds the PM10 levels [99] and the cities with > 100 days of air pollution had shown a high number of infection rate which was further associated with low wind speed. In other air quality studies with reference to two regions in Italy namely Lombardy and Emilia Romagna reported for their highest level of virus lethality in the world was among the most polluted areas in Europe [100]. Larger respiratory droplets (> 5 μm) in general stay in the air for a short period of time [101,102], while small aerosolized droplets (< 5 μm) can persist for a longer time in the air and also could travel for long distances (> 1 m) [103]. Transmission via the inhalation of small, exhaled respiratory aerosol droplets remain airborne for prolonged periods, mediating long-range human via air movement [7]. The aerosols with PM2.5 transmit to the respiratory tract more easily [104–106], which can be correlated with the SARS outbreak in Hong Kong and MERS outbreak in South Korea [107]. Air transmission dynamics of COVID-19 suggests possible 'pollution-to-human transmission' associated with the airborne viral infectivity [98].

Atmospheric loading of CoV as aerosols from wastewater provides a more direct route for human exposure [47] which is so far less understood. The virus that enters the sewer systems forms virus-laden aerosols [7] through mechanisms of cross-contamination [108,109]. Unit operation in WWTP such as biological oxidation normally employed for treatment of sewage/wastewater influences the aerosol formation due to aeration operation. Particulate matter emissions from aeration basins demonstrated that wastewater material is getting aerosolized and transported beyond the facilities [97]. The potential for viruses to be aerosolized was studied and monitored by spiking Ebola virus

surrogates (MS2 and Phi6) in wastewater systems (toilets, lab-scale aeration basin, and lab-scale model of converging sewer pipes) [110]. Emission rates of MS2 and Phi6 were 547 PFU/min and 3.8 PFU/min, respectively, for the aeration basin and 79 PFU/min and 0.3 PFU/min for the sewer pipes. Rotavirus and Norovirus were detected above the ASP from WWTP higher than the threshold values recommended [111]. Even though no concrete evidence is reported so far on the spread of SARS-CoV-2 through air, it is important to investigate and understand [93].

6. Environmental factors influence on the virus transmission

Environmental (meteorological) factors such as temperature, humidity, precipitation, and air-flow will have a significant influence on virus transmission/infection [102,112].

6.1. Temperature

Temperatures are prone to inactivation of enteric viruses under specified conditions [47,49,74]. Two surrogate CoV namely TGEV and MHV survival were studied in water (reagent-grade), lake water, and settled human sewage at room temperature (23–25 ˚C) and 4 ˚C for 6 weeks [48]. The virus infection declined rapidly at 25 ˚C, indicating the regulatory role of temperature on the viral inactivation in the water matrix. At 25 ˚C, 99.9% reduction ($T_{99.9}$) in reagent-grade water was observed for 33 days (TGEV) and 26 days (MHV), while in settled sewage (pasteurized) between 10 and 14 days. The fate of a HCoV-229E and animal FIPV CoV was evaluated in tap water (filtered and non-filtered) and wastewater (primary and activated sludge effluents) [47]. HCoV inactivated rapidly in wastewater ($T_{99.9}$ of 2.77; 3.54 days] and tap water [$T_{99.9}$ of 12.1; 12.5 days] while FIPV survived 2 to 3 times longer for a comparable reduction in wastewater (10.9 days) and in secondary effluents (5.7 days). Wastewater (unpasteurized and pasteurized) spiked with the viral stocks [enveloped viruses (MHV and Pseudomonas phage φ6) and non-enveloped viruses (bacteriophages MS2 and T3) incubated at 25 ˚C (summer) or 10 ˚C (winter) showed rapid inactivation of enveloped viruses in unpasteurized wastewaters at 25 ˚C [T_{90} of 13 h (MHV) and 7 h (phage φ6)], compared to non-enveloped phage MS2 [121 h] [49]. The inactivation kinetics of both MHV and φ6 at 10 ˚C was relatively slower [T_{90} of 28–36 h]. In pasteurized wastewater, both MHV and phage φ6 lost infectivity at a significantly slower rate compared to unpasteurized wastewater (T_{90} of 19 h (MHV) and 53 h (phage φ6)) at 25 ˚C [49].

6.2. Humidity and precipitation

The relationship between relative humidity and disease transmission depends on the persistence of viral viability before infecting susceptible individuals [110]. The absolute/relative humidity (AH/RH) role in mediating virus infectivity still needs investigation. Lower RH than 33% showed good viability than the corresponding intermediate RHs [110]. The aerosols (submicron) and droplets (1 μL) showed the transmission of infectious diseases [110]. Enveloped bacteriophage (Phi6) survived good at both high (> 85%) and low (< 60%) RHs with a significant decrease in infectivity at mid-range RHs (~60 to 85%) [113] and suggested that RH is the important factor in controlling virus infectivity in droplets. The non-linear nature of the relationship of influenza A virus with temperature and influenza B virus with AH, RH, and temperature explained the system complexity on virulence [112]. Temperature (air/water) and RH showed marked influence in the inactivate rates of the enveloped virus [48,110,114]. Viral transmissibility also significantly depends on precipitation [102]. Rainfall events manifest the mixing of untreated sewage and wastewater and get discharged into surface water pruning to increased exposure risk [115]. The risk of exposure via the faecal-oral route is also of particular concern where open defecation or non-sewered sanitation is being

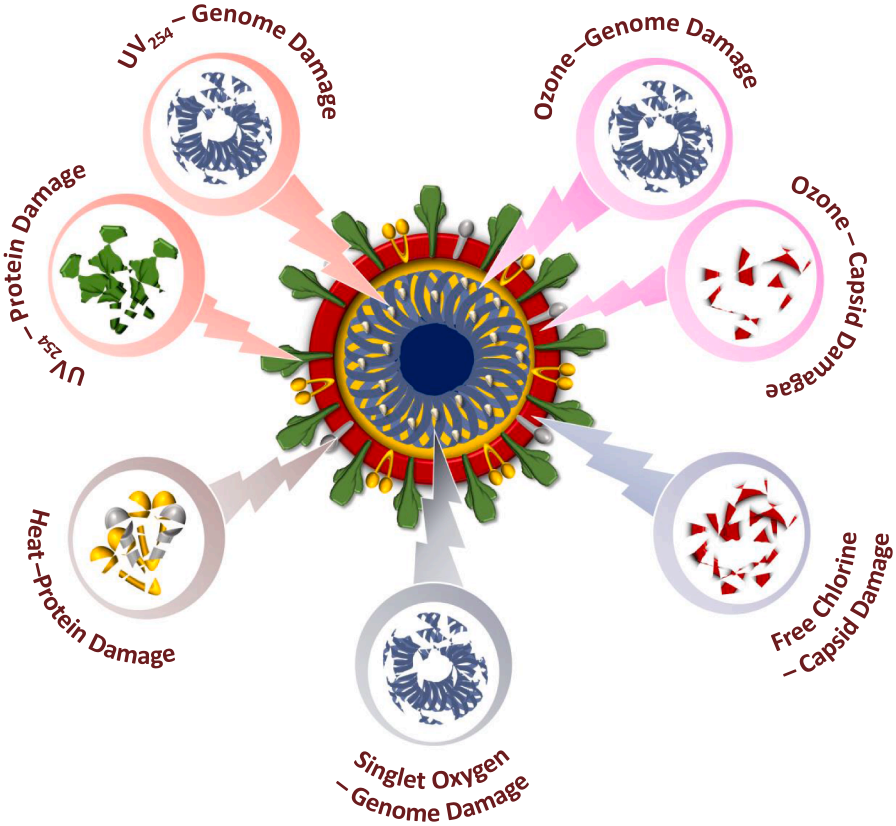

Fig. 3. Conventional disinfections methods of virus inactivation.

Table 3
Methods of Virus Inactivation.

Type	Method	Process/Mechanism	Action on virus	Reference
Conventional methods	• Chlorine/Chlorine dioxide (ClO_2) • Ozone (O_3) • Sodium Hypochlorite (NaOCl) • Hydrogen Peroxide (H_2O_2) • Peracetic acid (PAA) • Solar • Non-ionizing (UV) • Ionizing (Gamma ray)	Inactivates by oxidizing the structure of DNA/RNA and protein layer of enveloped and non-enveloped viruses. Excess dosages may lead to the formation of residual by-products.	Inactivates upto 99% of viruses	[51,69,71,171–174,175–182,184–186,189]
Filtration methods	• Slow sand and Ceramic • Ultra-filtration • Nano-filtration • Reverse osmosis • Membrane	Allows separation through a usage of physical barrier from water.	Remove virus and bacteriophages upto 0.001 m size	[6,71,172,191]
Advanced oxidation	• Photocatalysis • Ozone-based Oxidation • Cold plasma • Fenton reagent • Photo- Fenton • Electro Fenton • Ultrasound • Wet-Air Oxidation Technology • Supercritical Wet-Air Oxidation Technology (SCWO) • Nano-catalyst based advanced oxidation process	Rapid release of high reactive species (OH* radicals) that oxidizes or destroys enteric viruses and nucleic acids, cell membrane/ capsid of bacteriophages without the release of byproducts.	Inactivates enveloped viruses upto 99.99%	[6,142,159,171,193,195,198–206,214,217–220,237,238]
Other Processes	• Stabilization/algal ponds • Constructed wetland and water bodies	Synergic functions of sunlight	Bacteriophages also gets inactivated	[188,190]

practised [70] and will further intensify when rainfall events occur.

6.3. Animals and plants

Reports indicate that the CoV infects pets, livestock, and wildlife [116,117]. Due to prevailing environmental conditions, the transmission possibility through animals might happen but still needs to be investigated. Several viral families (highly stable pathogens) remain infective and transport long distances in water [118], which increased the potential impact when reclaimed wastewater was used for irrigation and animal husbandry. Studies on water-mediated plant virus transmission with reference to WWTPs, virus inactivation need to be investigated thoroughly along with enteric virus-plant association [118,119].

7. Detection of virus in environmental matrices

Detection of enteric viruses in wastewater, ground/surface water, sludge samples, and drinking water is challenging due to the presence of low virus concentration (traces of viral particles or virions) in huge volumes of water which also depends on the severity of the infection. For accurate detection environmental samples need to be concentrated using microbiological and molecular methods prior to the detection [77,120]. However, virus recovery by concentrating methods are always challenging [77].

7.1. Concentration

Concentrating viruses by ultracentrifugation, ultrafiltration, adsorption and elution (VIRADEL), coagulation, size-exclusion and flocculation are often used either in alone or in combination [120,121]. Wastewater spiked with bovine enteric coronavirus (BCoV) was recovered using glass powder adsorption with 0.05 M glycine solution and a 3% beef extract solution varied with acidic (pH 3.3) and alkaline (pH 9) pH solution yielded in the recovery of 24% (pH 3.3) and 28% (pH 9) [122]. Glass columns equipped with electropositive filter particles (silica gel with $Al(OH)_3$)) were used for concentrating SARS-CoV-1 and f2 phage and their mixture from sewage [46]. The spiked sewage samples concentrated using glass column followed by polyethylene glycol (PEG) precipitation showed recovery efficiencies of 21.4% (SARS-CoV-1)) and > 100% (f2 phage) [46]. The concentration of a variety of waterborne viruses was reported using glass wool filtration from runoff of agricultural fields [123]. Bovine origin viruses like bovine viral diarrhea virus type 1 and 2 (BVDV1 and BVDV2), bovine rotavirus group A (BoRv) and poliovirus 3 were spiked to the water samples prior to glass filtration followed by elution (3% beef extract-glycine buffer; pH 9.5) and PEG flocculation before qPCR analysis. A higher recovery of 57.9% was observed with non-enveloped poliovirus 3 [123]. The recovery of enveloped (murine hepatitis virus ((MHV)–rodent coronavirus) and non-enveloped (phage MS2) viruses spiked in untreated sewage was evaluated employing PEG precipitation, ultracentrifugation and ultrafiltration [49]. Ultrafiltration resulted in the highest recoveries of both MHV (25.1%) and MS2 (55.6%). HAV and TGEV spiked water samples were concentrated using glass wool filtration followed by PEG precipitation [55]. The pH of elution buffer (pH 11) showed an increase in recovery from 2.6% to 28.8% when 5 L sample is used. Elution buffer with pH 11 with the addition of tween 80 resulted in the recovery of 23.9% (HAV) and 18% (TGEV). Secondary concentration by PEG precipitation showed a recovery of 51.3% (TGEV) and 47.2% (HAV) [55]. SARS-CoV-2 and MHV [124] were also concentrated to observe their existence in wastewater samples employing electronegative membrane filtration followed by ultrafiltration (100–200 mL concentrated to ~250 μL) [56,124], PEG precipitation followed by centrifugation (11 mL concentrated to 400 μL) [57,124], homogenization (40 mL) followed by centrifugation to achieve a pellet which was resuspended in 1X PBS buffer [58,124], and also two phase PEG-dextran method [59,124] using ultrafiltration and ultracentrifugation was also adopted [60,69].

7.2. Detection

Various methods like isothermal amplification of target genes [51], biosensors [125], microarrays [126,127], culture-based assays [128], metagenomics [129] and polymerase chain reaction (PCR) and its types [130,131] are employed for the detection and quantification of the virus in the environmental samples (Table 2). *E. coli* and f2 phage [51], Poliovirus-1 (PV-1) Virome [47,52–54], and HAV [55] were used as indicators for the evaluation of persistence and occurrence of CoV in environment wastewaters (Tables 1 & 2). PCR and its types like quantitative PCR (qPCR), real-time RT-PCR, RT-qPCR, nested PCR and digital PCR (dPCR) have been implemented for detecting enteric virus contamination in the water/wastewater [132]. qPCR can able to multiplex and detect viral targets [80,133]. dPCR quantifies the viral genome and its population count in wastewaters without depending on known and calibrated standards [134,135]. RT-PCR, RT-qPCR, and nested PCR use specific primers and probes for detection and

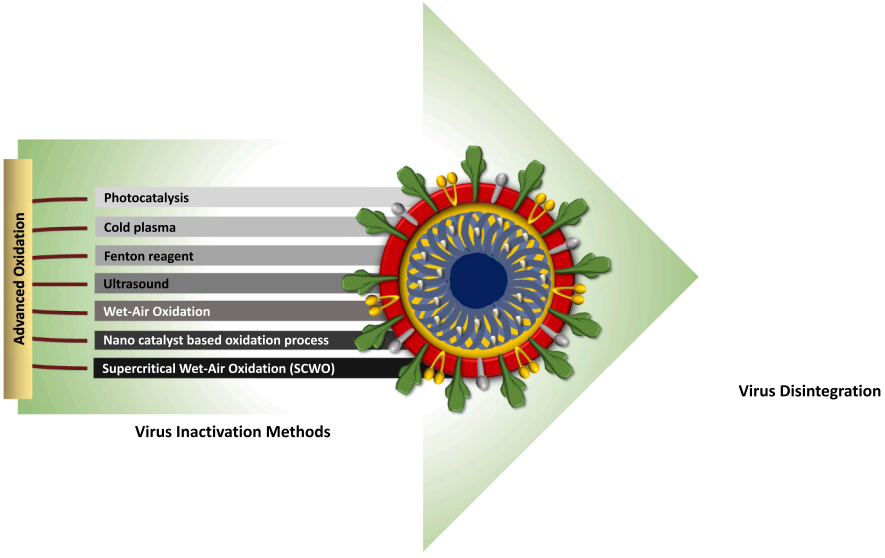

Fig. 4. Advanced oxidation methods used for virus inactivation

quantification [136]. The detection rates depend on the primer and probes used to detect target virus which should be revised frequently to assure the novel strain detection [77]. An integrated method, where integrated cell culture (ICC) is combined with PCR called ICC-PCR was used to monitor the viruses in wastewater matrices [137]. Molecular techniques possess the potential to detect the low concentration of viruses in a large volume of wastewater either individually or with integrity [138].

A model enveloped virus Murine hepatitis strain A59 (MHV) grown in L2 and delayed brain tumor cell cultures (DBT) were used to detect the persistence of CoV in waste environmental matrices [49]. Cell lines of swine testicular cell cultures [48], Crandell Reese feline kidney cell line (CRFK), fetal human lung fibroblast, MRC-5 cell line, Buffalo green monkey kidney cell line [47] and Vero E6 [46] were also used to detect CoV. The occurrence of CoV (SARS-CoV-1 and 2) was detected majorly using PCR based techniques as RT-PCR [55,60,69], RT-qPCR [46,55–58], qPCR [54], semi-nested PCR [49,58], nested PCR [59] (Table 1).

Next-generation sequencing (NGS) facilitates to study of virus diversity using amplicon sequencing [139]. Data achieved from NGS has the potential to increase knowledge of the viral community and its diversity in the environment [140]. Apart from whole virus detection, genome, and capsid integrity of the viruses can also be detected using molecular techniques and markers [141]. The wastewater disinfection using chemical or physical treatments causes virus inactivation modifying viral genome and proteins. The quantitative data for their integrity can be detected using RT-qPCR and protein mass spectrometry (MALDI-TOF MS) [142]. These methods help to identify the point changes that occurred rather than observing the complete modification caused [142]. Inconsistency was most often observed due to inhibitory substances present in a stool sample (bile salts, polysaccharides, lipids, and urate) and other samples (debris, humic acids, and polyphenols) as they co-precipitates or adsorbs with nucleic acid during the detection [143]. Cloning, metagenomic analysis (virus), and RT-qPCR with the use of process controls help to detect viruses accurately by knowing the virus recovery rate and the level of inhibition [121,144–147]. The selectivity and choice of process controls depend on the molecular and biochemical structure of the virus and their route of infection [148,149].

Although PCR based techniques are more sensitive, specific, consumes less time (in hours), can also detect virus which cannot be culturable providing quantitative data of viral genome and is sometimes not conducive and effective [121,150]. Rapid detection methods using dry samples [151] and paper analytical devices [150], were reported as a diagnostic tool to detect SARS-CoV-2 from a patient sample. These modifications and developments in methods and use of portable devices detects virus on-site, track virus carriers and serves as an early warning signal to the community to prevent the spread of the outbreak [150]. Paper-based devices are portable, easy to store, transport and incinerate [150]. A pilot air sampling study conducted to detect SARS-CoV-2 RNA (0.87 virus genomes/L air) and the approach illustrate the feasibility of tracking the progression of the outbreak using environmental aerosol samples instead of human specimens [152].

8. Fate of enveloped virus

Outbreaks of enveloped viruses, such as CoV (MERS, SARS-COV-1, and SARS-CoV-2), Ebola, Hantavirus, Measles, Zika, Avian influenzas, and Lassa virus, persisted in the water environment warranted effective management of the infectious waste and wastewater [153,162,163]. Enveloped viruses are structurally diverse to the non-enveloped viruses, and behave differently in water environment [154]. The survivability of the viruses also varies based on the presence and absence of envelope [155]. Reports on the enveloped virus with reference to their fate, transport, and inactivation are limited [11]. The persistence of enveloped viruses in aqueous medium was studied with the enveloped bacteriophage Phi6 as a surrogate (influenza viruses and coronaviruses) and observed that T_{90} (time for 90% inactivation) of Phi6 varied between 24 min and 117 days [49] which depends on the temperature, biological activity, and aqueous media composition [12]. The capsid protein of non-enveloped viruses is less susceptible to lipid solvents, temperature, and pH [156]. On the contrary, enveloped viruses are more sensitive to disinfectants, heat, and solvents [156,157]. Non-enveloped viruses displayed higher resistance in water environments compared to the enveloped viruses [47]. CoV is much more sensitive to temperature than Poliovirus 1 LSc-2ab (PV-1) and that there is a considerable difference in survivability between PV-1 and the CoV in wastewater attributed due to the fact that enveloped virus is less stable in the environment than non-enveloped viruses [47].

9. Inactivation of virus

The survivability of viruses in nature as well as engineered systems impacts the public health [158] and therefore, enteric viruses must be removed or inactivated by 4 logs (99.99%) during water treatment [158]. For enteric viruses, WWTPs will function as an important and key barrier [71,159–161]. The viral pathogens load discharging to wastewater treatment plants through domestic wastewater is typically in the range of 10^6–10^8 GC/L [159]. The occurrence of human adenovirus (HAdV), JC polyomavirus (JCV), and A rotaviruses (RVA) was reported in the range of 10^6–10^8 GC/L [162]. Human viruses (adenovirus, polyomavirus, and torque teno virus) were detected in the influent (10^5–10^6 GC/L) [163].

9.1. Wastewater treatment plants

WWTPs functions as the main barriers to terminate the virus transmission. Unit operation in WWTPs typically includes primary (screening, equalization (optional), coagulation, or settling (primary and secondary), main or biological ASP or sequential batch reactor (SBR) or membrane bioreactors operated with aerobic, anoxic and/or anaerobic microenvironments) and tertiary treatment (filtration (sand/membrane), adsorption (activated carbon or disinfection (UV irradiation, chlorination, ozonation, etc.), constructed wetlands or stabilization ponds in addition to the anaerobic digestion (for stabilization of sludge). Unit operations play a crucial role in virus removal [159]. The placement of unit operations in a defined sequence will mainly depend on the inflow wastewater quality, treated water quality required for discharge, and intended use of the treated water. Four WWTPs were evaluated for the presence of HAdV, JCV, and RVA for one year [162]. In the secondary effluent, HAdV was detected in all the analyzed samples followed by JCV (85.4%) and RVA (97.9%) [162]. HAdV was the more persistent virus detected in the tertiary effluent (62.2%), while membrane bioreactor (MBR) showed relatively better virus removal. The fate of three human viruses (adenovirus, polyomavirus, and torque teno virus) in three WWTPs was evaluated mainly operating with ASP [161]. All three human viruses were consistently persisted in the secondary treated effluent (10^2–10^3 GC/L). ASP in sub-tropical climate condition which could function as an effective treatment barrier with > 3 $\log_{10}$ removal efficiency of enteric virus and tertiary treatment, in addition, is essentially required prior to reuse for non-potable purposes or discharge [161]. A comprehensive assessment of the fate of eight viruses during multiple steps of full-scale WWTPs was also reported [160]. Viral communities in WWT include large numbers of bacteriophage specific to bacterial hosts that influence the bacterial community structure and dynamics [72,163].

Enveloped virus partition to solids is more compared to non-enveloped viruses [49], wherein the virus gets absorbed to suspended particles in wastewater as well as sludge/biosolids [71]. Solids separation and activated sludge flocs majorly contribute to virus removal. Sedimentation and sand filtration also contributed to virus removal before disinfection [164,165]. Coagulation followed by sand filtration

showed improved virus removal capability [166]. Physical process (sedimentation and filtration) and biological process (ASP-Sludge) separates/transfer the infective viruses from liquid to solid phase without inactivating and therefore exists a potential risk of transmission through other pathways [159]. Application of sludge stabilization processes, such as heat treatment (50–75 °C), mechanical dehydration/desiccation (virus capsid rupture), liming process, composting process (temperature and antagonistic organisms) and anaerobic digestion (mesophile) showed variable viral inactivation capability [159,167–169]. During the treatment process, the virus gets inactivated due to the prevailing environmental conditions as well as microbial antagonism [170].

9.2. Disinfection process & technologies

The conventional WWTPs at present are not specifically designed to remove viruses. Disinfection is the unit operation designed in existing WWTPs for intended inactivation of pathogens including viruses.

9.2.1. Conventional process

Disinfectants agents/Chemical oxidants (chlorine (Cl^-), chlorine dioxide (ClO_2), ozone (O_3), hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl) and paracetic acid (PAA)) and UV-irradiation are most commonly used for inactivation of viruses (Fig. 3) (Table 3). Solar irradiation, filtration (slow sand, porous ceramic), membrane (micro/ultra/nano and reverse osmosis), etc. along with stabilization ponds and constructed wetlands also reported inactivating virus to some extent depending on the operational conditions and wastewater nature. Chlorine (ClO_2, free chlorine (FC)) is well established and achieves inactivation by oxidizing the cellular material of the virus [71]. Contact time, dose, pH, temperature, and wastewater/water composition are critical factors that influence the virus inactivation efficiency. Chlorine (dose, 10/20 mg/L; contact time, 10/1 min) documented complete inactivation of SARS-CoV-1 in spite of in-effective inactivation of f2 phage [51]. Free chlorine was reported effective in inactivating SARS-CoV-1 and f2 phage than chlorine dioxide. The free residual chlorine (0.5 mg/L from Cl^- or 2.19 mg/L from ClO_2) ensured near complete inactivation of SARS-CoV-1 in wastewater [51]. However, the requirement of high doses of Cl^- and the possibility of formation of disinfection by-products (DBPs) warrants its usage [171,172]. PAA is considered as an alternative to the chlorination process, wherein the H_2O_2 and acetic acid (CH_3COOH) generates reactive hydroxyl radicals [173,174]. PAA application as an inactivation agent eventually increases the carbon concentration in final treated effluent due to the presence of acetic acid [172]. In a study conducted to estimate the SARS-CoV-2 infected individuals real field influent samples from STPs were collected and subjected to various concentration of NaOCl (0.1%, 0.5%, 1%, 2%, 3% and 4%) [69]. The concentration ≥2% NaOCl did not show any detection of SARS-CoV-2 genome indicating its complete inactivation [69]. 0.5 mg/L of free chlorine with 30 min of exposure time at 22 ± 3 °C would inactivates SARS CoV-2 viruses completely [44].

Ozone (oxidation-potential, 2.07 V) leads to the formation of secondary oxidants (hydroxyl radicals) with higher reactivity which can inactivate in shorter reaction times [171,175,176], in spite of its reactive/corrosive nature, and the cost. The susceptibility of the selected viruses towards ozone application in the increasing order: bacteriophages (Qβ) > coxsackievirus B5 (CVEnv2) > echovirus 11 (EV) > bacteriophages (MS2) > bacteriophages (Φ174 & T4) > human adenovirus (HAdV) > coxsackievirus B5 (CVF, CVEnv1) [176]. The UV irradiation (220–320 nm) is also one of the widely used methods to inactivate pathogens including viruses. Inactivation of enteric viruses (noroviruses, rotavirus, reovirus, sapovirus, astrovirus, enteroviruses, adenoviruses, and JC virus) was evaluated with UV during WWTPs operation over two years period [177]. Both pre-UV and post-UV samples showed a relatively higher load of selected viruses. Log_{10} reduction of total infectious viruses (1.46 and 1.67 log) by UV irradiation was comparable to reovirus reduction (1.23 and 1.75 log). UV_{254} irradiation primary damaged the nucleic acid while inactivating human Norovirus surrogate (HuNoV) [178]. MS2 (with ssRNA) showed resistant to $UV_{253.7}$ inactivation (7 log; 1800 J/m^2) compared to other ssRNA viruses [179]. Heterogeneous sensitivities were observed in the genome regions after UV_{254} treatment [180]. The efficiency of UV disinfection depends on the time of exposure, irradiation intensity along with suspended particles/colour/turbidity of water/wastewater. UV functions with few seconds of contact time without chemical addition (no residual or chemical formation). Viral aggregation enhances genome recombination (damaged viruses inside their host cells) and therefore, reduces viral inactivation during the UV treatment [181]. The inactivation of bacteriophages (MS2, fr, and GA) by UV_{254}, singlet oxygen (O_2), free chlorine (FC), and chlorine dioxide (ClO_2) was also studied [182].

Solar water disinfection (SODIS) on the inactivation of HAV and HNoV, murine Norovirus (MNV-1) indicated that the sunlight radiation associated with temperature play a major role in the inactivation [183,184]. Wastewater temperature manifest higher inactivation rates specifically at higher values [47,49]. Six hours of exposure to sun inactivated enteric viruses [184,185]. Sunlight inactivates viruses via endogenous inactivation (absorption of solar light in the UVB range) and exogenous processes (adsorption of sunlight by external chromophores/sensitizers, which generate reactive species) [186]. Irradiation of water with solar simulator showed inactivation of human enteric viruses (MS2 coliphage) [187]. Viruses that are not susceptible to exogenous inactivation use UVB wavelengths (280–320 nm) for inactivation [188]. Endogenous sunlight inhibits viral RNA synthesis [189]. Extended solar exposure time ensured both bacterial and viral inactivation and discharge of the solar treated wastewater could allow the viral replication when the host is present [190]. Sunlight irradiation in natural/engineered treatment systems (drains, stabilization/algal ponds, constructed wetlands, water bodies, etc.) also functions to inactivate pathogens to a certain extent [188].

The membrane-based process allows separation of the pathogen by a physical barrier. Microfiltration (0.1–10 m) enables removal of bacteria/protozoan cysts and ultrafiltration (0.01–0.1 m) and nanofiltration (0.001–0.01 m) allows removal of viruses [172]. Reverse osmosis (< 0.001 m) reject viruses from water along with virus surrogates such as bacteriophage MS2 [191]. MBR is a hybrid system with the integration of ASP with membrane filtration for biomass separation in a submerged or side-stream configuration [6,71]. Full-scale MBR (pore size 0.04 μm) showed good log removal of four pathogenic viruses [adenovirus (3.9–5.5), norovirus GII (4.6–5.7), F^+ coliphage (5.4–7.1)] [192].

9.2.2. Emerging processes

Although virus removal was achieved by conventional treatment processes to some extent, there is still emerging scope and need for effective virus inactivation technologies [6,159]. The multibarrier wastewater and drinking water treatment systems are likely effective in protecting against SARS-CoV-2 [12]. The advanced and integrated process specifically targetting functional genome damage will aid in developing better methods for diverse viral pathogens [142]. UV-based advanced oxidation processes (AOPs) (Fig. 4) integrating with H_2O_2, Cl_2, O_3 and functional material facilitate enhanced reactive radical generation by photolysis [159]. AOPs employing UVC, UVC-H_2O_2, and UV-Fenton for inactivation of enteric surrogates (MS2 bacteriophage) in ultrapure water and synthetic matrices (wastewater and urine) was studied [193]. The occurrence of MS2 or *E. coli* notably decreased the antagonist microorganism inactivation kinetics, whereas, the addition of H_2O_2 improves the overall inactivation performance. The presence of H_2O_2 and Fe improved the inactivation rate significantly. Pulsed UV irradiation and low-pressure UV irradiation were applied for the inactivation of NoV and FRNA bacteriophage (GA) in secondary treated

wastewaters [194]. At a high dose of UV (6.9 J/cm^2) GA showed significant inactivation. Suspended solids concentration showed a direct impact on the process efficiency and settlement processes played an important role. The integration of UV irradiation with PAA yielded effective inactivation [195]. Ozone integrated with H_2O_2 or persulfate/monopersulfate promoted the production of hydroxyl radicals [196]. Ozone and photo-assisted integrated disinfection facilitated higher quality of water despite of cost [171].

Photocatalytic disinfection due to its potential oxidative capability and ability to utilize solar energy is attracting significant attention in the disinfection domain [197]. Photocatalysis induces catalyst (semiconductor) function in the presence of irradiation source, sunlight or UV lamp [168,198,199]. Magnetic metals (iron (Fe), cobalt (Co) and nickel (Ni)), semiconductor nanoparticles (NP) such as metal oxides and doped structures (TiO_2, ZnO, CuO, MgO, SnO_2, WO_3, SiO_2, Fe_2O_3, Nb_2O_3, TiO_2/CuO, Ag/TiO_2, TiO_2/Pt, Fe_2O_3/TiO_2, Au/TiO_2 and TiO_2 doped with N-, C-, S-), noble metals (gold (Au), silver (Ag) and platinum (Pt)) based inorganic NPs and engineered carbon NPs (carbon nanotubes (CNTs), C_{60}, and C_{70} fullerenes) [200]. TiO_2 is a potential photocatalyst semiconductor material due to its strong oxidizing power [201], prolonged stability high reactivity and faster disinfection kinetics. Illumination of UV light at a wavelength (< 385 nm), TiO_2 generates an electron-hole on the surface pair by photon energy and generated hydroxyl radicals (*OH), and the electrons present in the conduction band produced superoxide ions (O_2^-).*OH radicals are highly reactive species upon contacting the virus surface and enable inactivation rapidly [171,198,202–207]. The generated oxidative species penetrate into the lipid layer of the virus which leads to inhibition or lysis [208]. Photocatalytic viral inactivation processes follow mainly three steps i) shape distortion, ii) protein oxidation, and iii) gene damage [209]. Photocatalytic membrane reactors (PMRs), using nano-TiO_2–membrane coupled system inactivation of phage F2 model for a human enteric virus-like poliovirus, hepatitis A viruses, coxsackie virus, Norwalk, Rotavirus, Adriano virus, Herpes virus, and influenza virus was studied in flat membranes showed 1.8–5.9 log units of disinfection efficiency in wastewater [197,210]. PMR (Nano-TiO_2 P_{25} (10–25 mg/L); Flat-sheet polyvinylidene fluoride (PVDF) membrane (pore size of 0.15 μm), UV intensity, 0.16 mW/cm^2) showed bacteriophage f2 (25 ± 1 nm) inactivation (5 log) primarily by photocatalysis where membrane functioned as a barrier [197].

Composite SiO_2-TiO_2 and silver-doped TiO_2 NPs showed 2.7 times higher photocatalytic inactivation rates with bacteriophage MS2 than the unmodified TiO_2 [211,212]. TiO_2 and ZnO showed efficient inactivation in the following order: viruses > prions > Gram-negative bacteria > Gram-positive bacteria > yeasts > molds [204]. TiO_2 membrane with UV irradiation of airborne pathogens was noticed between photocatalyst sterilization and UV treatment process and the combination/impregnation of these TiO_2 with various active metals such as Pt, Ag, and Cu. The TiO_2/Ag nano-anti microbial materials used to study growth inhibition rates and found 97.9% inhibition [213]. Photocatalytic oxidation using undoped TiO_2 and platinized sulfated TiO_2 (Pt/TiO_2) yielded 90% inactivation within 30 min using UV-irradiation on TiO2 and 90% to 99.8% with Pt/TiO_2 over the viral and bacterial aerosols of influenza A (H3N2) virus, vaccinia virus, *Mycobacterium smegmatis* and *Bacillus thuringiensis* [214]. Photocatalytic titanium apatite filter (PTAF) absorbs and inactivates SARS-CoV-1 up to 99.99% within 6 h interaction under non-UV irradiation [203]. Photoelectrocatalytical (PEC) reactors perform the photocatalytic reactions enhancing the viral inactivation significantly (> 90%) by applying a negative potential lead to damage of the virus due to the manifested electrostatic attraction between negatively charged viral capsid and catalyst surface [208,215]. The virucidal activity in the presence of low concentrations of halides with reference to adenovirus inactivation by PEC systems showed positive influence by halide oxidation with a prolonged lifetime of photoholes (h^+) [216].

Filters based on PVDF membranes (5 μm) coated with multiwalled carbon nanotubes (MWCNTs) layer showed effective removal of viral bacteriophages [217]. The main limitation of using MWCNTs for virus filtrations is the retention of the virus after the filtration [218]. MWCNT fictionalization with metals and metal oxides with anti-microbial silver, copper, and copper oxides would help an additional virus inactivation [219]. MWCNTs functionalized with silver-based filters showed complete removal of poliovirus, norovirus, and coxsackie virus [32]. Silver NPs deposition by electrochemical means over carbon covered alumina (CCA) showed effective inactivation of pathogens in water [220]. MWCNTs coated with copper(I) oxide (Cu_2O) yielded good virus (MS2 bacteriophages) efficiency for specific water applications at relatively low cost [219]. Coagulation followed by ultrafiltration in pilot-scale wastewater reclamation plants (using secondary treated effluent as feed) using F-specific RNA bacteriophage MS2 showed stable at pH 5.5 with high virus removal rate [221].

Cold plasma (CP) is one strategy that mediated virus inactivation (Table 3). Plasma (partially or fully ionized gas) facilitates the emission of UV radiation along with reactive oxygen and/or nitrogen species (RONS) which eventually damage the nucleic acids and oxidize nucleic acids, proteins and lipids respectively [237]. The formation of ROS and/or RNS is the main limiting factor that contributes to virus inactivation. Viral aerosols inactivation in an air-stream with non-thermal plasma (NTP) exposure showed exponentially inactivation of aerosolized MS2 phage by increasing the applied voltage using a packed-bed dielectric barrier discharge reactor [239].

The removal of Enterovirus and Norovirus in algal-WWTS (A-WWTS) demonstrated feasibility in producing pathogenically-safe effluent with minimal post-disinfectant [240]. The virus community in the chlorinated-WWTS effluent showed ~250 diverse species while only 14 discrete non-pathogenic virus species were found in the non-chlorinated effluent of A-WWTS [240]. The fate of human enteric viruses (i.e. norovirus, adenovirus, Aichi virus 1, polyomaviruses, and enterovirus) and plant virus (pepper mild mottle virus (PMMoV)) were studied with reference to two surface flow wetlands [238]. Adenovirus and Aichi virus 1 was found in abundance in treated wastewater (an inlet of the wetlands) at concentrations of 10^2–10^5 GC/L [238]. Viral removal efficiencies by wetlands ranged between 1 and 3 $\log_{10}$. Polyomaviruses were removed to below detection limits while PMMoV was detected in a greater concentration in the inlet of both (10^4–10^7 GC/L), but exhibited little or no removal. Photosynthetic induced biological activity and temperature played a key role in virus removal in the wetlands [238].

9.3. Inactivation mechanism of virus

The extent and type of damage a virus can sustain before losing its ability to infect is important to understand in terms of the applied inactivation strategy. The ssRNA of SARS-CoV-2 likely renders it more susceptible to inactivation than enteric ssRNA viruses [12]. A virus to be infective, it must bind to the host, inject its genome inside the host, and replicate its genome within the host [142]. Oxidants and radiation cause damage to the viral proteins and nucleic acids, which is non-specific, however, the loss of specific virus functions (host recognition/binding, genome injection/replication) is important to understand [142]. UV_{254}, singlet oxygen, and hypochlorous acid inhibit genome reliability, whereas, ClO_2 and heat applications inhibit host-cell recognition/binding [142]. ClO_2 inactivation induce damage to the proteins without inhibiting the genome function. On the protein level, ClO_2 is a selective oxidant, which reacts mainly with Cys, Trp, and Tyr [222]. UV irradiation and chlorine treatment cause site-specific capsid protein backbone cleavage with the inhibition of both genome replication and injection [142]. UV_{254} application showed similar susceptibility for human norovirus and enteric (+) ssRNA viruses (Echovirus 12 and Feline calicivirus) with loss in genome functionality accounting for 60% of virus inactivation due to the protein damage [223]. Compared to ozone, inactivation by FC caused little or no loss of genome

functionality, indicating its specific role in protein damage [223]. Ozone application to the E11 virus caused genome functionality loss proportionally to the infectivity [223]. Singlet oxygen impaired genome replication and host binding with a minor impairment associated with significant genome decay [142]. OH* radical oxidative action altered permeability and damage the capsid proteins [184].

The inactivation of three related bacteriophages (MS2, fr and GA) employing UV_{254}, singlet oxygen, FC and ClO_2 showed some interesting observations [182]. ClO_2 did not induce genome damage in spite of variable inactivation kinetics, while all other inactivation processes showed more or less similar inactivation kinetics with marked genome damage [180]. On the protein level, UV_{254} damaged MS2 and capsid proteins, whereas, GA's capsid remained intact. FC and ClO_2 rapidly impaired the capsid proteins of all three viruses. Heat treatment to the E11 virus resulted in capsid protein denaturation rather than inhibiting genome functionality in spite of a high degree of infectivity loss [223]. MS2 inactivation by heat (72 °C) showed a loss inability to bind to the host cell and binding function decay rate correlated with inactivation rate, while injection and replication function rates were almost negligible [142]. Lack of replication function loss is in agreement with the lack of RNA degradation without peptide modifications. Heat induces structural changes due to attack on protease might cause inactivation by disrupting the specific structures needed to recognize and bind the host cells.

9.3.1. Enveloped virus inactivation

Enveloped viruses are less resistant to environmental conditions and inactivation compared to non-enveloped viruses [161,207] and get inactivated at faster rates [47,49,224–226]. The inactivation/disinfection practices currently used are effective against non-enveloped viruses will also be effective for enveloped viruses also [99]. Enveloped viruses are more susceptible to oxidants than non-enveloped viruses [12,158,227]. Chemical-based disinfectants damage viral capsid, where UV irradiation affects nucleic acid [159]. Direct oxidation of reactive radicals damages the viral envelope manifesting the functional loss of the receptors [154]. The envelope does not impact the susceptibility of the virus to UV as the irradiation functionally impairs genomes [49]. To understand the enveloped virus inactivation mechanism of FC and UV irradiation was studied using model enveloped virus (*Pseudomonas* virus Phi6) [158]. FC reacts with proteins in the nucleocapsid and polymerase complex and Phi6 peptides which are more reactive when compared with the most reactive peptides with reference to non-enveloped coliphage MS2 [158]. Phi6 peptides contain a relatively large number of solvent-accessible Met and Cys residues which are responsible for rapid inactivation with FC [158]. UV_{254} photolysis kinetics of four model viral genomes (ssRNA, dsRNA, ssDNA, and dsDNA) showed high resistance of dsRNA [227]. UV and free chlorine applications cause protein backbone cleavage in MS2 in part inactivate the virus by inhibiting the genome injection function [142]. Inactivation of enveloped viruses in sewage by spiking bacteriophage Φ6 depicted that the enveloped viruses can undergo 6–7 log inactivation in sewage in 3–7 days, depending on the temperature [225]. Heat treatment induces denaturing of capsid protein rather than the genome functionality inhibition in spite of a high degree of infectivity loss [223].

10. Epidemiology Studies-Surveillance for early warning and spread

The detection of the virus in wastewater/sewage when the SARS-CoV-2 prevalence was low indicates the functional role of sewage for surveillance to monitor the circulation of the virus in the population [45,60,69] via WBE [56]. The wastewater will function both as a surveillance system and an early warning tool as shown previously for poliovirus [228], and Aichi virus [229]. The surveillance/monitoring of SARS-CoV-2 in wastewater can quantify the scale of infection prevailing within the community with a benefit of detecting virus for individuals who have not been tested, or are asymptomatic, potentially symptomatic, pre-symptomatic, or only have mild symptoms [69,73,188,232]. It could provide an unbiased method of evaluating the spread of infection in different areas, even where resources for clinical diagnosis are limited and when reporting systems are unavailable [38,230]. WBE approach will help to minimize the outbreak spread and also serve for future epidemics surveillance [150,231]. WBE ability to detect low levels of viruses especially at early stages of an outbreak or when infection levels are decreasing following intervention is also critical [38]. Virome analysis of wastewater will enable to detect novel viruses before their clinical recognition in a community, allowing for early preventative measures and allocation of resources to potentially affected areas [52,233–235]. The major limitations to WBE in establishing quantitative predictions from viral RNA results in over or underestimation of infected cases due to the complexity of wastewater, the dilute nature of biomarker in wastewater and inability to pinpoint specific locations [124,150,236]. Effective wastewater sampling techniques, effective virus concentration methods, robust and sensitive RT-qPCR assays asociated with source appropriation and transmission analysis through modelling approach are very much essential for successful WBE studies [150,236].

11. Summary, challenges and recommendations

Since ancient times, viral-induced infective diseases are persistently posing a serious threat to human health as well on the economics at epidemic and pandemic scales. The CoV induced outbreaks were observed in three widespread cases viz., SARS-CoV-2 (2019), MERS-CoV (2012), and SARS-CoV-1 (2003) in the last two decades. CoV belongs to the enveloped family of βCoV with + ssRNA having crown-like spikes on its surfaces. SARS-CoV-2 genomic sequence showed more than 80% similarity with the SARS-CoV-1. Spike protein bind to the host cell by means of ACE2 receptor and replicates in the lungs, heart, intestines, blood vessels, and muscles. Data so far reported evidenced the replication and persistence of CoV (including SARS-CoV-2) in the human GI tract and shedding through stools showing the possibility of transmission through the faecal-oral route to the environment matrix. Along with virus-infected patients, pre-symptomatic and asymptomatic individuals also shed the virus. The faeces showed the presence of viral RNA and infectious virions in a few cases. The enteric virus in faeces persisted for a longer period of time (for many days) retaining its infectivity which is a major concern. The occurrence of SARS-CoV-2 in the water/wastewater/sewage was reported based and their persistence in the aqueous phase was also reported for days. However, the transmission of SARS-CoV-2 through faecal-oral route to the community is yet to be established in spite of the available evidence on the SARS-CoV-1 community spread through aerosol. Virus in general retains the infective property and infuses genetic information in the proximity of host leading to their replication. The presence of SARS-CoV-2 at an infectious dose and the duration of persistence in stools/wastewater are significant aspects that need to be studied and elucidated with a larger number of samples from the public health point of view. Routine and regular monitoring of viruses in the inflow to WWTPs if practiced along with other quality parameters will help in early detection of the outbreak with a warning. In this realm, water scientists are advocating the need for WBE for surveillance which could possibly enumerate the virus circulating flux in the community. However, rapid, effective and simple detection methods need to be developed specific to the environmental samples and implemented through a regulatory framework.

Detection, quantification, and determination of virulence in the environmental samples are of the major challenge for the scientist in this domain. Detection of the enteric virus in the environmental matrices is one of the important prerequisites to assess the fate and virulence of the pathogen as well as to design appropriate inactivation/treatment strategy. Virus detection protocol in environmental matrices will involve sample collection, transport, storage, concentration and

detection, and inactivation which should ensure all the safety measures at each step of activity with Biosafety level (BSL) 2 (or above) procedure. Detection of enteric viruses in the environmental samples is extremely challenging because of their relatively low load (with reference to the quantity of wastewaters/water), interference caused due to the associated material in wastewater/water and sensitivity of the methods for precise quantification. The concentration of enveloped viruses from large volumes of environmental samples is an essential pre-requisite prior to detection. Electropositive filter particles, PEG precipitation and flocculation, ultrafiltration/ultracentrifugation are now practiced to concentrate to smaller volumes by simultaneously eliminating the associated interfaces. Culture (ICC-PCR) and molecular-based techniques (RT-PCR, qPCR, dPCR, Nested PCR, NGS and MALDI-TOF) were employed for the detection of virus from the concentrate solution which is considered to be sensitive and specific to provide quantitative information about the viral genome. Internal/external process controls are generally used to ensure accuracy in the detection. Rapid and cost-effective detection methods for virus monitoring with reference to environmental samples need to be developed.

Considering the fact that viruses pose a potential risk on human health, their inactivation in water and wastewater is an essential priority. Specific reports on the inactivation of SARS-CoV-2 in wastewater were not reported so far. Understanding the nature and fate of CoV are important to design an effective strategy for inactivation. An enveloped virus such as CoV is more unstable to oxidants and prevailing environmental conditions than the non-enveloped viruses. The primary and secondary unit operation in WWTPs only removes viruses to a marginal extent. Conventional disinfection methods such as chlorination, ozonation, H_2O_2, NaOCl and PAA along with solar and UV irradiation are the most frequently used unit operation in WWTPs to inactivate virus along with other pathogens. In general, reactive radicals produced during theses process damage either the envelope or/and capsid or/and viral RNA that ensures inactivation of virion in wastewater/water. However, the dosage of disinfectant agent/irradiation, contact time, pH and carbon of aqueous phase, presence of predators, etc. will have a regulatory role in the inactivation efficiency. The virus attachment to biofilm/sludge, interaction with a native microorganism (bacterial phases), plants, and animals is also a point of concern. The persistence of enveloped as well as non-enveloped viruses are detected after application of disinfection in treated water/wastewater, which warrants the necessity of advanced strategies such as AOP or integrated process to ensure virus free discharge. Optimized integration strategies of AOP and conventional disinfection process, design and development of large-scale disinfection unit operation, understanding the mechanism of viral inactivation, surveillance of discharges for the virus, the role of bacteriophages, etc. are some of the critical aspects to be investigated specifically on SARS-CoV-2 in diverse environmental settings. WWTPs need to be re-engineered keeping in the current and future pandemic situation by considering a multi-barrier approach. Integrating two or more disinfection strategies to strategically target to damage genome functional activity and envelope will able to provide an effective inactivation of the viral pathogens.

Enteric virus association with the water/wastewater infrastructure assuming the possible risk of transmission to the operators should take all the necessary protective measures against viral infection–wearing personal protective equipment (PPE) (protective clothing, gloves, boots, safety glasses, a face mask and/or respirator mask (FFP3)), face shield (to cover eyes, nose, and mouth), regular washing of common utility areas as well surfaces, frequent hand washing, virtual meetings, etc. According to the occupational health and safety protocols with safe working practices should be employed and should avoid direct contact with wastewater. Awareness needs to be created by the local governance by means of camps, leaflets, and social media by suggesting appropriate safety measures against the growing pandemics.

12. Conclusion

Past epidemiological experience showed frequent viral outbreaks and, in the future, there will also have epidemic/pandemic outbreaks due to the ever-changing anthropogenic activities affecting the ecological balance. In the environmental context, the possibility of transmission through water infrastructure is a major point of concern and its detection and inactivation will play a major role to curb their spread to the community. There is an imperative need to consider the virus as a regular parameter for routine monitoring along with other quality parameter with environmental samples to understand the early warning of the outbreaks and to effectively inactivate before discharge. Accordingly, a policy in the framework of regulation by appending to the environmental systems will help to safeguard the global community with future outbreak and transmissions.

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

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