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Survivability of Viruses, Especially Coronaviruses, in Sewage and Sludge: A Narrative Review (2000–2025)

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Abstract

Introduction: Conventional wastewater treatment processes significantly reduce but do not completely remove viral genetic material. This narrative review (2000–2025) synthesizes evidence on the presence and persistence of viral genomes, particularly RNA from coronaviruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in sewage and sludge, distinguishing these detectable genetic signals from infectious viruses.

Methods: A literature search was conducted in PubMed, Scopus, Web of Science, Science Direct, and Google Scholar. Keywords included "virus," "coronavirus," "SARS-CoV-2," "sewage," "sludge," "concentration," "detection," and "quantification." After screening 1,350 records, 152 studies meeting the inclusion criteria (peer-reviewed studies on human viruses in municipal sewage/sludge, published between 2000 and 2025) were included.

Results: SARS-CoV-2 RNA was detected in raw sewage at concentrations up to 10^6 copies/L during the 2020 peak, declining to 10^3–10^4 copies/L by 2025. In sludge, SARS-CoV-2 RNA persisted at 10^3–10^5 copies/g dry weight after anaerobic digestion, with only a 1–2 $\log_{10}$ reduction. Viral genomes of other pathogens (adenoviruses [AdVs], noroviruses [NoVs], hepatitis E virus [HEV], and rotaviruses [RoVs]) were also frequently identified. Notably, the reviewed studies reported the detection of viral RNA, not the isolation of infectious virions.

Conclusion: Viral genomes, especially coronavirus RNA, are not fully eliminated during wastewater and sludge treatment and can persist in the environment. This persistence underscores the utility of wastewater-based epidemiology for surveillance. However, the detection of RNA does not indicate the presence of an infection risk, as standard treatment processes effectively inactivate viable viruses.

Keywords: Wastewater, Sewage, SARS-CoV-2, Coronavirus, Wastewater-based

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Introduction

Wastewater is a complex mixture that contains a wide array of pathogenic and commensal viruses. It provides crucial information about virus circulation, the emergence of novel viruses, and their transmission among the population (1). However, wastewater treatment processes are not entirely effective in reducing the concentration of most viral genomes. For instance, the severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), which is an enveloped virus, persists in raw sewage for 2 days at 20°C and 14 days at 4°C (2).

Adenoviruses (AdVs) and polyomaviruses (PyVs) serve as potential indicators of wastewater contamination. These viruses are usually carried asymptomatically in the human population, are frequently detected in wastewater at high concentrations, and resist wastewater treatment (3). The most common viruses dispersed in sewage worldwide include hepatitis A virus, hepatitis E virus, rotaviruses, adenoviruses, noroviruses, astroviruses, parvoviruses, coronaviruses, polioviruses, and other enteroviruses (4).

Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late 2019, fecal shedding from infected individuals has been the primary source of coronavirus entry into municipal sewage systems. Initial concentrations reached up to 10^6 copies/L in raw influent during 2020 peaks (5). By 2025, global concentrations have declined to 10^3–10^4 copies/L due to population immunity from vaccination and natural infection, alongside upgrades in disinfection processes, such as UV and advanced oxidation (6). Key factors influencing persistence include low temperature (<10°C), neutral pH (6-8), high total suspended solids (TSS >200 mg/L), and organic matter that shields viruses from inactivation (7). Variant-specific monitoring of SARS-CoV-2 (including

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Omicron sublineages) using next-generation sequencing (NGS) of wastewater samples has been widely implemented in national surveillance programs across many high-income countries by 2024–2025. These programs cover substantial portions of the population (for example, approximately 45% of the U.S. population through the CDC's National Wastewater Surveillance System) and enable early detection of community transmission and emerging variants (8).

A few studies have indicated that SARS coronaviruses have not been found in effluents from WWTPs (2). Research on continuous SARS-CoV-2 data from wastewater in various countries, including France, the USA, Qatar, Finland, Spain, Germany, the UK, and Sweden, has shown that tracking viruses in raw wastewater is an effective and timely method for monitoring the spread of community infections (9).

Sewage sludge is a semi-solid residue generated in WWTPs through biological processes or the separation of solids from chemically precipitated effluents. It represents a major by-product of the wastewater treatment process (10,11).

This sludge contains concentrated contaminants, such as heavy metals and pathogenic microorganisms, including bacteria, viruses, protozoa, and helminths (12).

Many pathogenic microorganisms present in raw sewage are identified in urban sludges. Due to the limited capacity of current treatment processes, wastewater sludge intensification from WWTPs has become a significant concern. Despite considerable viral reduction during treatment, viruses are still detected in the discharge (13).

Dewatered sewage sludge (DWS) is a biologically compatible product that contains nutrients for agricultural soil, making it a suitable replacement for chemical nitrogen and phosphorus fertilizers (14). However, DWS harbors microbial pollutants, including fecal coliforms, Salmonella, helminth ova, and viruses, which restrict its agricultural use. Human enteroviruses and hepatitis A virus may also be detected in significant concentrations in sewage (15).

Most microbial species in raw sewage concentrate in sludge during initial sedimentation. Intestinal viruses have low mass and do not settle alone, but strongly attach to particles and concentrate in sludge (16). Secondary sludge exhibits higher viral loads than primary sludge due to biosolid adsorption and microbial flocculation during biological treatment. Anaerobic digestion achieves only a 1–2 $\log_{10}$ reduction for enveloped viruses such as SARS-CoV-2, posing risks in land application (2).

Soares (1990) discovered that viral reduction in anaerobically processed sewage sludge varied significantly, ranging from 50% to over 99.9%. Treatment efficiency was also highly variable (17). Moreover, even at 99% efficiency, virus concentrations in released sludge could exceed 1000 viruses/L. In Florida, Wellings et al. documented 24 plaque-forming units (PFU) of virus per 250 g of sludge cake (15,18).

According to Goddard and Butler (1981), several variables affect virus survival during wastewater treatment. Based on virus recovery from effluent and sludge, viruses can survive for long periods, contaminating receiving water and soil (19).

This narrative review describes the impact of various pathogens on human and animal systems, including the respiratory, gastrointestinal, hepatic, and central nervous systems. These pathogens, namely SARS-CoV-2 and SARS-CoV, infect a wide range of species, including humans, livestock, avians, bats, mice, and other wild animals (20). SARS-CoV-2 and SARS-CoV belong to the *Sarbecovirus* subgenus of *Coronaviridae*, which includes enveloped viruses with single-stranded, positive-sense RNA genomes. Both viable SARS-CoV-2 and viral RNA have been found in excreta, including saliva, sputum, and feces, as well as in wastewater (21,22).

As infectious viruses can persist in wastewater and raw sewage sludge, treating these substances may not be enough to prevent the spread of disease. Therefore, it is crucial to identify the viruses present in sewage and sludge to prevent microbial transmission. Accordingly, the present review focuses on the quality of sewage and sludge based on the presence of viruses, especially coronaviruses.

Materials and Methods

A narrative review was conducted to synthesize the literature on viral survivability, concentration, detection, and quantification in sewage and sludge from 2000 to 2025. The search process is illustrated in Figure 1. The narrative literature search process across five databases using defined keywords resulted in 152 included studies. Databases searched included PubMed, Scopus, Web of Science, Science Direct, and Google Scholar. Keywords were "virus," "Coronavirus," "SARS-CoV-2," "COVID-19," "sewage," "sludge," "wastewater," "wastewater treatment," "urban sewage," "urban sewage sludge," "municipal sewage," and "municipal sewage sludge." Approximately 1,350 articles were retrieved; 285 full texts were reviewed based on relevance to human viruses in municipal systems, and 152 were included after excluding non-peer-reviewed, animal-only, or pre-2000 studies.

Concentration Methods

Viruses were concentrated using virus adsorption-elution (VIRADEL) with electronegative or electropositive filters, polyethylene glycol (PEG) precipitation, ultrafiltration, and skimmed-milk flocculation. VIRADEL relies on electrostatic attraction: at pH below the virus isoelectric point (pI), the surface becomes positively charged and binds to electronegative filters; conversely, electropositive filters capture negatively charged viruses at neutral pH (23,24). Adding salts such as magnesium chloride ($MgCl_2$) or aluminum chloride ($AlCl_3$) alters the virus surface charge from positive to negative via flocculation, enhancing adsorption to negatively charged membranes (24,25). In PEG precipitation, PEG dehydrates the solution, causing viruses to aggregate and precipitate for centrifugation (26).

The ultrafiltration method is based on the principle of size exclusion, employing membrane filters with pore dimensions that are smaller than viral particles (27).

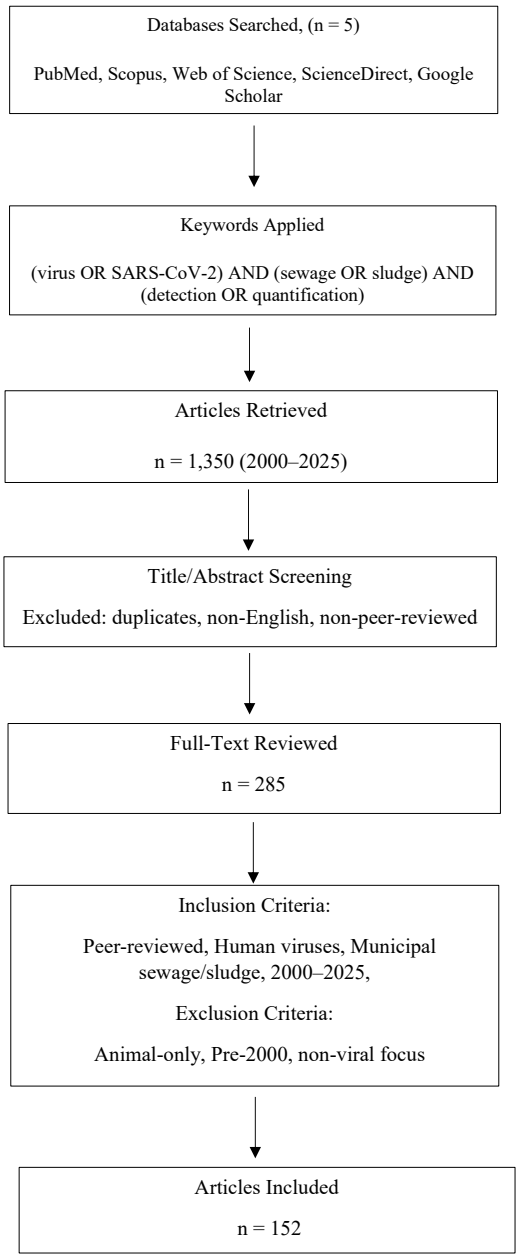


Figure 1. Search Study Flowchart

The technique of skimmed milk flocculation, initially devised for the extraction of adenoviruses from seawater, has also been adapted for the recovery of viruses from wastewater samples. This method is based on several physical processes: (i) the adsorption of viruses onto preflocculated skimmed milk proteins, (ii) the sedimentation of the resulting flocs containing the adsorbed viruses, and (iii) the dissolution of these sediments using a phosphate buffer solution (28,29).

Detection and Quantification Methods

Techniques employed for the identification and measurement of viruses present in wastewater encompass epifluorescence microscopy, transmission electron microscopy, pulsed-field gel electrophoresis, immunofluorescence assays, flow cytometry, traditional cell culture, and various molecular approaches. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was the primary method, targeting RNA from coronaviruses (30,31). RT-qPCR involves RNA extraction, reverse transcription to cDNA, and amplification with specific primers. Digital PCR (dPCR) and NGS were used in recent studies for absolute quantification and variant detection (32). Figure 2 illustrates the sequential steps from sample collection to quantification, including VIRADEL (electrostatic-based), PEG precipitation (dehydration), and RT-qPCR/dPCR detection. Key mechanisms include pH-dependent charge reversal in VIRADEL, PEG-induced aggregation, and turbidity fouling in ultrafiltration.

Results

Detection, Concentration, and Quantification of Viral Genomes in Sewage and Sludge

Accurate detection and quantification of viral RNA in sewage and sludge require a three-step workflow: (i) virus concentration from large volumes of complex matrices, (ii) nucleic acid extraction with inhibitor removal, and (iii) sensitive molecular detection and quantification. The most widely adopted primary concentration methods are VIRADEL, using electronegative or electropositive membranes, PEG precipitation, ultrafiltration, and skimmed-milk flocculation (5,34). Electronegative filters (e.g., 0.45 µm HA membranes) combined with $MgCl_2$ or $AlCl_3$ pre-treatment achieve recoveries of 30–80% for both enveloped and non-enveloped viruses in sewage (35). Electropositive 1MDS filters eliminate the need for pH adjustment and are particularly effective for SARS-CoV-2 RNA recovery in neutral-to-alkaline influent (mean recovery 56 ± 18%) (36). PEG precipitation (8–12% PEG 8000/6000 + 0.3–0.5 M NaCl) is inexpensive and yields 40–70% recovery with minimal equipment, making it suitable for resource-limited settings (37). Ultrafiltration with 10–100 kDa hollow-fiber or centrifugal devices routinely concentrates 10–100 L samples to 500–1000 µL with recoveries of 50–90% for SARS-CoV-2 and enteric viruses (38,39). Skimmed-milk flocculation (1% pre-flocculated skimmed milk at pH 3.5–4.5) has been widely applied since 2020 because it is low-cost, chemical-free, and achieves 45–85% recovery even in highly turbid samples (40).

Nucleic acid extraction from concentrated samples typically employs magnetic bead-based kits (e.g., QIAamp Viral RNA Mini Kit, MagMAX, and NucliSENS) with added inhibitor-removal steps (OneStep PCR Inhibitor Removal Kit or Zymo columns) to remove humic acids, heavy metals, and PCR inhibitors abundant in sewage and sludge (41). For sludge, bead-beating or freeze-boil cycles are required to disrupt viral particles adsorbed to solids (42).

Quantification is almost exclusively performed by RT-qPCR targeting conserved regions of SARS-CoV-2 (N, E, RdRp, and ORF1ab genes) and enteric viruses (43). DPCR and droplet digital PCR (ddPCR) have become increasingly common since 2022 because they provide absolute quantification without standard curves and are less affected by inhibitors, achieving limits of detection (LODs) as low as 1–5 genome copies/µL. NGS and metagenomic approaches (e.g., Illumina or Oxford Nanopore) are now routinely

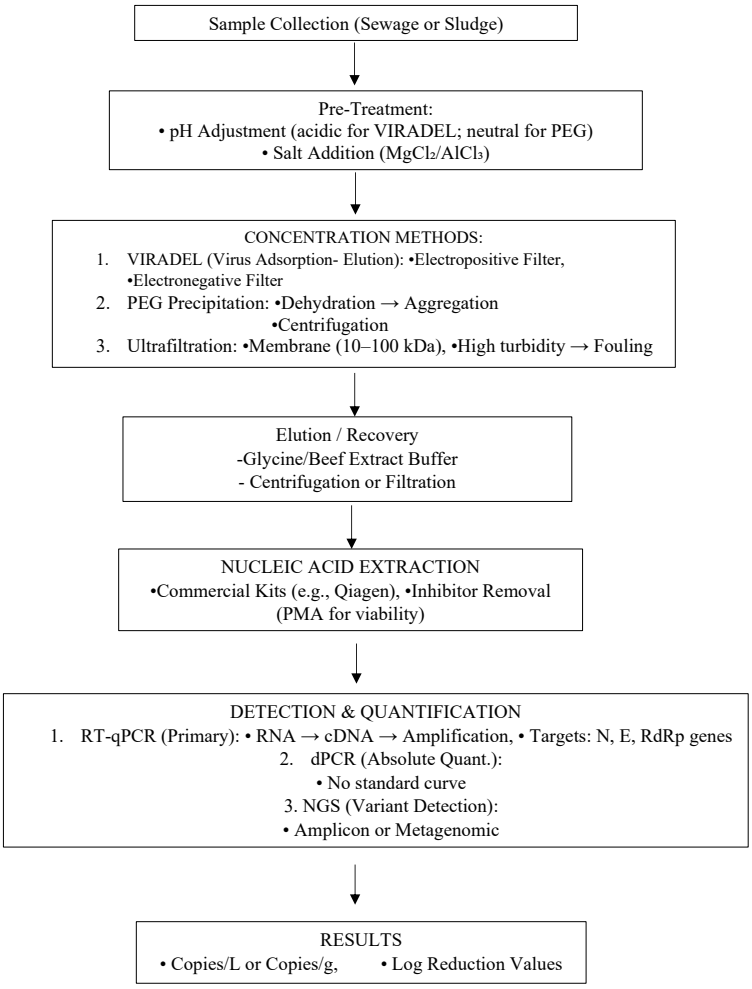


Figure 2. Flowchart of Virus Concentration and Detection Methods (23,25,26,33)

applied for variant surveillance and multi-pathogen profiling in both sewage and digested sludge (44,45).

In sludge, viral RNA persists at higher concentrations than in liquid phases because of adsorption to solids and protection from inactivation processes. Mesophilic anaerobic digestion reduces SARS-CoV-2 RNA by only 0.5–2.0 $\log_{10}$, whereas thermophilic digestion or pasteurisation achieves >3 log reduction (46). Recent studies have confirmed that combining ultrafiltration or skimmed-milk flocculation with ddPCR/NGS yields the highest sensitivity for monitoring low-abundance genomes in digested sludge (47).

Viral RNA (genomic material) from a range of human pathogenic viruses, including both enveloped and non-enveloped types, was detected in raw sewage, treated effluent, and sewage sludge across diverse concentrations and geographic locations using various concentration, recovery, and molecular quantification techniques. The key findings on virus type, concentration, study location and year, detection methods, and corresponding references are summarized in Tables 1–4 and described below.

Table 1 presents the detection and quantification of viral RNA from pathogenic viruses in raw sewage and treated effluent worldwide (2000–2025). SARS-CoV-2 RNA concentrations ranged from 10^4–10^6 copies/L in raw influent and 10^2–10^4 copies/L in effluent and were detected primarily by RT-qPCR targeting the N1/N2 genes following electronegative/electropositive filtration or PEG precipitation. Other viral genomes reported include hepatitis E virus (10^3–10^5 copies/L), rotavirus (10^2–10^4 copies/L), human adenovirus (10^3–10^5 copies/L), and norovirus GII (10^2–10^4 copies/L), quantified using dPCR, RT-qPCR, and NGS after VIRADEL-based concentration methods.

Table 2 summarizes the detection and quantification of viral RNA in sewage sludge (secondary and digested) from global studies conducted between 2020 and 2025. SARS-CoV-2 RNA was detected at 10^3–10^5 copies/g dry weight in secondary sludge and at 4.8×10^4 copies/g in digested sludge using RT-qPCR after ultrafiltration or skimmed-milk flocculation. Additional viral genomes quantified include parechovirus (10^2–10^4 copies/g), human adenovirus (10^3–10^5 copies/g), and human herpesviruses, primarily detected through metagenomic sequencing and dPCR following concentration by electronegative membrane filtration or ultracentrifugation.

Table 3 shows SARS-CoV-2 RNA concentrations in Iranian municipal WWTPs from 2020 to 2025. Raw influent RNA levels ranged from 10^5–10^6 copies/L in 2020 (Tehran) to $<10^3$ copies/L in 2025 (southwest Iran). Effluent RNA concentrations decreased from 10^3–10^4 copies/L in the early years to non-detectable levels by

Table 1. Detection, Concentration, and Quantification of Viruses in Municipal Wastewater Samples

Author (date of publication)	Type of virus	Type of sample	Detection results		Virus concentration and detection methods	
			Positive rate	Concentration (genome copies/L)	Concentration method	detection method
Kitajima et al. (2020) (48)	SARS-CoV-2	Untreated wastewater	2/9 (22%)	1.2×10^2	Virus adsorption-elution method (Electronegative membrane)-direct RNA extraction; ultrafiltration	quantitative PCR (qPCR)
Kitajima et al. (2020) (48)	SARS-CoV-2	Untreated wastewater	10/14 (71%)	$>2 \times 10^5$	PEG precipitation	quantitative PCR (qPCR)
Kitajima et al. (2020) (48)	SARS-CoV-2	Untreated wastewater	23/23 (100%)	$>10^{6.5}$	Ultracentrifugation	quantitative PCR (qPCR)
Kitajima et al. (2020) (48)	SARS-CoV-2	treated wastewater	6/8 (75%)	$\sim 10^5$	Ultracentrifugation	quantitative PCR (qPCR)
Lapolla et al. (2020) (49)	SARS-CoV-2	raw wastewater treated wastewater	23/23 (100%) 6/8 (75%)	Not mentioned	Not mentioned	RT-qPCR
Corpuz et al. (2020) (2)	-HEV -RoV -HAdV	MWWTP Effluent Effluent pre-UV Effluent post-UV Secondary effluent	30/30 (100%) 3/51(5.88%) 4/50 (8%) 4/12 (33.33%)	-4.00×10^3 to 1.00×10^4 - 1.01×10^3 to 4.47×10^6 - 5.50×10^4 to 7.59×10^{5a} (a= Secondary Treatment Effluent)	N. A	N. A
Silva-Sales et al. (2020) (50)	Salivirus belongs to the Picornaviridae family	Raw sewage	37/52 (71.1%)	7.56×10^4 to 7.20×10^6	organic flocculation	RT-qPCR
Wang et al. (2005c) (51)	- SARS-CoV - Phage f2 SARS-CoV	hospital sewage samples hospital sewage samples	N. A	N. A	N. A Virus adsorption-elution method (Electropositive filters)	Infectivity assay onto Vero E6 cell line Both infectivity assay onto Vero E6 cell line and RT-PCR
Carducci et al. (2020) (52)	- HCoV strain 229E - FIPV	wastewaters (primary and secondary effluents)	N. A	N. A	N. A	Infectivity assay on MRC-5 cell line for HCoV and CRFK cell line for FIPV
Ahmed et al. (2020) (53)	SARS-CoV-2	Raw sewages (n^0 9).	N. A	N. A	concentration based on different methods: - Electronegative membranes; - Ultrafiltration (cut-off 10 kDa)	RT-qPCR and sequencing
Wang et al. (2020) (54)	SARS-CoV-2	Wastewater at different step of the treatment in a disinfection pool: untreated (n^0 3), partially treated (n^0 1), treated (n^0 1).	N. A	N. A	Not specified	RT-qPCR and infectivity assay onto Vero E6 cell line
Medema et al. (2020) (55)	SARS-CoV-2	Raw sewages (n^0 24).	N. A	N. A	ultrafiltration (cut-off 100 kDa)	RT-PCR
Nemudryi et al. (2020) (56)	SARS-CoV-2	Raw sewages (n^0 7 in triplicate)	N. A	N. A	ultrafiltration (cut-off 10 kDa)	RT-qPCR and sequencing
Wu et al. , (2020) (57)	SARS-CoV-2	Raw sewages (n^0 14).	N. A	N. A	filtration on 0.2 mm membrane and centrifugation with polyethylene glycol 8000	RT-qPCR and sequencing
Wurtzer et al. , (2020) (58)	SARS-CoV-2	Wastewater samples both raw (n^0 23) and treated (n^0 8)	N. A	N.A	Ultracentrifugation (details not provided)	RT-qPCR

Table 1. Continued.

Author (date of publication)	Type of virus	Type of sample	Detection results		Virus concentration and detection methods	
			Positive rate	Concentration (genome copies/L)	Concentration method	detection method
Fumian et al., (2019) (59)	Norovirus GI	-raw sewage -primary effluent -final effluent	N. A	4.2 to 6.2 $\log_{10}$ 4.1 to 5.7 $\log_{10}$ N. A	centrifugation at 8000×g	RT-qPCR
	Norovirus GII	-raw sewage -primary effluent -final effluent		4.4 to 7.1 $\log_{10}$ 4.5 to 7.3 $\log_{10}$ N. A	centrifugation at 8000×g	RT-qPCR
Haramoto et al., (2020) (60)	SARS-CoV-2	secondary-treated wastewater	1/5 (20%)	2.4×10^3	electronegative membrane-vortex (EMV)	N_Sarbeco qPCR assay
Wu et al., (2020) (57)	SARS-CoV-2	Untreated municipal wastewater	71%	10- ~100 copies/mL	-Filtration through 0.22 µm membrane to remove bacterial cells and debris (initial tests revealed little to no viral RNA on filters) -PEG precipitation, centrifugation at 12,000g for 2 h or until a pellet was visible	US CDC rRT-PCR primer
Ahmed et al., (2020) (5)	SARS-CoV-2	Untreated municipal wastewater	22%	1.9 and 12 copies/100 mL	- Method A: Direct RNA extraction from the electronegative 0.45 µm filter 90 mm diameter (Ahmed et al., 2015) - Method B: Centrifugation at 4750g for 30 min. Supernatant centrifuged at 3500g for 15 min through a centrifugal filter with a cut-off of 10 kDa	RT-qPCR
Medema et al., (2020) (61)	SARS-CoV-2	Untreated municipal wastewater	58%	N.A	- Centrifugation step at 4654g for 30 min to remove large particles (debris, bacteria) - Supernatant filtered with centrifugal ultrafiltration with a cut-off of 10 kDa at 1500 g for 15 min	PCR Assay with Four primer sets were selected: the N1, N2, N3 sets (US CDC) targetings different regions of the nucleocapsid (N) gene and the set against the envelope protein (E) gene (Corman et al., 2020)
Di Bonito et al., 2017) (62)	HPV, HPyV, HHV, MMTV	Raw sewage	N. A	N. A	Elution with glycine	Combined multiplex PCR and bead-based Luminex technology
prado et al. (2019) (63)	HAdV JCPyV RoV-A	Influent and Effluent of Secondary wastewater treatment	N. A	N. A	Concentration with Celite	RT-qPCR
Calgua et al. (2011) (64)	HAdV JCPyV	Influent wastewater	N. A	N. A	Concentration with Phosphate-buffered saline (PBS) solution	(a) Immunofluorescence assay (b) Plaque assay (c)Tissue culture infectious dose-50 (d) qPCR
Ali et al. (2021) (65)	SaV	N. A	87/218 (39.9%)	(4.3 × 103 to 5.3 × 108)	Beef extract & AlCl3 method followed by PEG	Tunisia SaV RNA extracted from (150 µL) of concentrated wastewater samples using the Nucleo Spin RNA Virus Kit (MachereyNagel; Germany). SaV quantification performed through real-time RT-qPCR using SaV124F and SaV1245R primers and the SaV124TP probe, which mainly target the polymerase-capsid junction in ORF-1. Beef extract & AlCl3 method followed by PEG Total of 218 wastewater samples analysed, SaV 87/218 (39.9%) (4.3 × 103 to 5.3 × 108) (Varela et al., 2018)

Table 1. Continued.

Author (date of publication)	Type of virus	Type of sample	Detection results		Virus concentration and detection methods	
			Positive rate	Concentration (genome copies/L)	Concentration method	detection method
Ali et al. (2021) (65)	HAdV, NoV	N. A	Total of 12 wastewater samples analysed, HAdV and NoV GII 12/12 (100%) samples.	HAdV and NoV GII (1.98 × 105) and (5.17 × 106), respectively	Skimmed milk flocculation	Viral nucleic acids extracted from concentrated wastewater samples using the QIAmp Viral RNA kit (Qiagen, Inc., Valencia, CA). HAdV and NoV GII quantitated using real-time qPCR and RT-qPCR.
Gurda et al. (2022) (9)	SARS-CoV-2	Untreated municipal wastewater	N. A	2.27×10^7 / ml	PEG(polyethylene glycol)- precipitation	RT-qPCR
Ahmad et al. (2021) (66)	SARS-CoV-2	WWTPs	**6/13 (46%)**	N. A	N. A	RT-qPCR
Ahmad et al. (2021) (66)	SARS-CoV-2	Sewage samples	71% positive detected	N1: 10^3 –2×10^4 (gc/100 ml) N2: 3×10^3 – 10^4 (gc/100 ml) N3: 10^3 –10^4 (gc/100 ml)	N. A	RT-qPCR
Mainardi and Bidoia (2025) (67)	SARS-CoV-2	Untreated Sewage (Valencia)	N. A	5.22 and 5.99 $\log_{10}$ /L	N. A	RT-qPCR
Mainardi and Bidoia (2025) (67)	SARS-CoV-2	Untreated Sewage (Gothenburg)	N. A	0.14 to 1.87 $\log_{10}$ / L	N. A	RT-qPCR
Mainardi and Bidoia (2025) (67)	SARS-CoV-2	Untreated Sewage (Massachusetts)	N. A	~ 50/ml	N. A	RT-qPCR
Mainardi and Bidoia (2025) (67)	SARS-CoV-2	Treated Sewage (Santiago)	N. A	10 and 20 per mL	N. A	RT-qPCR
Mainardi and Bidoia (2025) (67)	SARS-CoV-2	Treated Sewage (Pris)	N. A	-	N. A	RT-qPCR
Mainardi and Bidoia (2025) (67)	SARS-CoV-2	Treated Sewage (South of Tehran)	N. A	-	N. A	RT-qPCR
Coralia et al. (2024) (68)	SARS-CoV-2	Primary clarified sludge (PCS), Influent post grit solids (PGS)	PCS samples: 92.7% and 90.6% . PGS samples: 79.2 and 82.3%, With RT-qPCR to detect the N1 and N2 genes . PGS samples: 92.7 and 90.6%.	N. A	N. A	RT-qPCR , RT-ddPCR.
Sadhana et.al (2023) (69)	SARS-CoV-2	Untreated Wastewater	67% (88/132)	N. A	N. A	RT-PCR
Dos Santos (2024) (70)	SARS-CoV-2	Untreated Wastewater	22% (2/9)	N. A	N. A	RT-PCR

Note: N.A: No available quantitative (concentration) or viability data of virus in wastewater or sludge

Table 2. Detection, concentration and quantification of viruses in sewage sludge

Author and date of publication	Type of virus	Type of sample	Detection Results		Virus concentration and detection methods	
			Positive rate	Concentration (copies/Kg of dry solids)	Virus Concentration	Virus detection
Bibby et al. (2011) (71)	human viral pathogens (parechovirus, coronavirus, adenovirus and aichi virus, human herpes, Tanapox virus, Torque Teno Virus-like minivirus,Orf virus and hepatitis C viruses)	Treated sewage sludge (Class B biosolid) Class B biosolids were sampled from an anonymous US wastewater treatment facility that collected solid residuals by primary sedimentation and secondary activated sludge clarification and treated by mesophilic anaerobic digestion (35–37°C, 15 d solid retention time).	Within the 51 925 annotated sequences, 94 DNA and 19 RNA sequences were identified as human viruses.	N. A	Sample concentration according to standardized US procedure for virus concentration in sludge	Shotgun sequencing techniques
Bibby et al. (2013) (72)	human viral pathogens	Untreated sewage sludge (n0 5) and treated sewage sludge (n0 5) The influent and effluent sludge from mesophilic anaerobic digesters was sampled from five domestic wastewater treatment plants within the continental United States. All influent samples were mixtures of primary and secondary sludge. Effluent sewage sludge samples were of a class B product, prior to dewatering.	N. A	N. A	polyethylene glycol 6000 precipitation	Shotgun sequencing techniques
la Rosa et al. 2020)(73)	NoV GI	Anaerobically digested sludge (of municipal wastewater)	N. A	5.00×10^{7} (Anaerobically digested sludge)	N. A	N. A
He et al. 2011)(74)	RoV	Primary sludge of municipal wastewater	N. A	8.00×10^{3} to 8.00×10^{5}b (primary sludge)	N. A	N. A
Corpus et al. (2020) (2)	HAV	Activated sludge of municipal wastewater	N. A	3.10×10^{5} to 5.20×10^{5}b	N. A	N. A
Wong et al., 2010) (75)	EV	Primary sludge Activated Sludge Anaerobically digested sludge Anaerobically digested sludge	N. A	5.47×10^{7} to 1.15×10^{8} (Primary sludge) 5.66×10^{6} to 1.44×10^{7} (Activated Sludge) 1.44×10^{7} to 2.40×10^{8} (Thickened Sludge) 2.60×10^{7} to 7.60×10^{7} (Anaerobically digested sludge)	N. A	N. A
De Gascun & Carr, 2013 (76)	HPyV	Anaerobically digested sludge	N. A	7.40×10^{7} to 2.5×10^{8} (Anaerobically digested sludge)	N. A	N. A
La Rosa et al.,2020; Wong et al., 2010 (73,77)	Nov GI	Anaerobically digested sludge	N. A	5.00×10^{7} (Anaerobically digested sludge)	N. A	N. A
La Rosa et al., 2020; Prado et al., 2014; Wong et al., 2010, (73,75,78)	Nov GII	Primary sludge Anaerobically digested sludge of municipal WWTP	N. A	1.60×10^{4} to 4.90×10^{5}b (Primary sludge) 1.50×10^{8} (Anaerobically digested sludge)	N. A	N. A
Olijve et al., 2018; * Bibby and Peccia, 2013) (72,79)	Parechovirus	Anaerobically digested sludge of municipal WWTP	N. A	2.51×10^{7} to 2.51×10^{8} (Anaerobically digested sludge)	N. A	N. A
La Rosa et al., 2020; Prado et al., 2019; Sidhu et al., 2018; Prado et al., 2014; (63,73,78,80)	HADV	Primary sludge, Thickened sludge, Anaerobically digested sludge of municipal WWTP	N. A	1.80×10^{4} to 1.20×10^{9}b (Primary sludge) 9.40×10^{9} to 3.90×10^{11} (Thickened sludge) 9.10×10^{7} to 6.90×10^{9} (Anaerobically digested sludge)	N. A	N. A
Zhu et al. , 2020 (81)	SARS-Cov-2 Activated Sludge	Primary sludge	N. A	1.25×10^{4} to 4.60×10^{8} (primary sludge) 1.17×10^{4} to 4.02×10^{8} (Waste Activated Sludge	N. A	N. A

Table 2. Continued.

Author and date of publication	Type of virus	Type of sample	Detection Results		Virus concentration and detection methods	
			Positive rate	Concentration (copies/Kg of dry solids)	Virus Concentration	Virus detection
Bibby and Peccia, 2013 (72)	Enteroviruses Coronavirus HKU1, Klassevirus, Cosavirus Parechoviru	Sludge samples (influent and effluent of anaerobic digesters)	N. A	N. A	Precipitation with PEG	PCR; High-throughput Sequencing
Sano et al., ,2003,(82)	Enteric viruses	Sludge sewage samples	N. A	N. A	EVE	RT-PCR
Prado et al., (2014) (78)	HAdV, RoV-A, NoV GII, HAV	Sludge sewage samples	N. A	N. A	Ultracentrifugation; Beef Extract Elution	RT-qPCR qPCR
Monpoeho et al., (2000) (83)	Enteric Virus	Primary sludge samples Activated sludge samples Thickened sludge samples	N. A	N. A	EV Beef Extract elution with sonication; Precipitation with PEG	RT-PCR
Gallagher and Margolin, (2007)(84)	Mammalian orthoreovirus	-Raw sludge samples -Treated sludge samples	N. A	N. A	-Adsorption elution; -Organic Flocculation	ICC-RT-qPCR; Plaque Assay
D'Aoust et al. (2021) (85)	SARS-CoV-2	Post grit sludge Primary clarified sludge	N. A	N. A	Filtration and PEG concentration	PCR
Kittigul, L., and Pombubpa, K., 2021 (2021)(86)	Rotavirus	Lime stabilized sludge	N. A	N. A	Adsorption-elution with glycine & arginine/ vacuum centrifuge	PCR
Balboa et al. (2021)(87)	SARS-CoV-2	Primary sludge Biological sludge Thickened sludge Digested sludge	N. A	N. A	Elution with glycine and beef extract/ PEG 8000 precipitation	PCR
(Serra-Compte et al., 2021)(88)	SARS-CoV-2	Non-treated sludge (a) PCR Treated sludge (b)	N. A	N. A	Elution with glycine and beef extract/ PEG 8000 precipitation	PCR
(Pourakbar et al., 2022) (89)	SARS-CoV-2	Primary sludge Activated sludge Anaerobically digested sludge	N. A	N. A	Elution with glycine and beef extract/ PEG 8000 precipitation	PCR
Mainardi and Bidoia (2025) (67)	SARS-CoV-2 (New Haven, Connecticut)	Primary sludge	N. A	1.7×10^3 to 4.6×10^5 /mL	N. A	RT-qPCR
Mainardi and Bidoia (2025) (67)	SARS-CoV-2 (Orense)	Primary sludge	N. A	1.3 /mL	N. A	RT-qPCR
Mainardi and Bidoia (2025) (67)	SARS-CoV-2 (Santiago de Queretaro)	Activated sludge	N. A	3.5 $\log_{10}$ / mL	N. A	RT-qPCR
Mainardi and Bidoia (2025) (67)	SARS-CoV-2 (Istanbul)	Activated sludge	N. A	1.17×10^4/ L to 4.02×10^4 / L	N. A	RT-qPCR
Espinosa et al. (2022) (90)	SARS-CoV-2	N. A	AllPrep PowerViral DNA/RNA kit	copies/mL 4/6 positive samples (66,66 %)	Adsorption- extraction method	RT-qPCR
Yanac et al. (2022) (91)	SARS-CoV-2	N. A	RNeasy PowerMicrobiome Kit	78 copies/mL	Ultrafiltration and Organic flocculation	RT-qPCR
Zhao et al. (2022) (92)	SARS-CoV-2	N. A	Direct-zol RNA Kit	$1{,}33\times10^5$ copies/mL 4/39 positive samples (10,25 %	PEG 8000	RT- PCR

Note: (a) Primary and secondary sludge.
(b) Sludge thickening, anaerobic digestion and anaerobic digestion plus thermal hydrolysis.
N.A: No available quantitative (concentration) or viability data of virus in wastewater or sludge

Table 3. SARS-CoV-2 Concentrations in Iranian WWTPs

City / WWTP Location	Raw Influent Concentration (copies/L)	Treated Effluent Concentration (copies/L)	Removal Efficiency (log_{10})	Detection Method	Reference
Tehran (4 plants)	10^5–10^6	10^3–10^4	2.0–2.5	RT-qPCR (N gene)	(93,94)
Qom & Anzali	10^4–10^6	10^2–10^4	1.8–2.8	RT-qPCR	(94)
Ardabil (5 plants)	10^4–10^5	10^2–10^3	1.5–2.0	RT-qPCR	(95)
Southern Tehran	10^4–10^5	10^2–10^3	2.0–2.5	RT-qPCR	(96)
Tehran (after MBR upgrade)	10^3–10^4	$<10^2$	2.5–3.2	RT-qPCR + dPCR	(95)
Southwest Iran (Khuzestan & Hormozgan)	$<10^3$ – N. D	N. D	>3.0	RT-qPCR + UV monitoring	(97)

Table 4. Removal Efficiency by WWTP Processes for Coronaviruses

Process	Log_{10} Removal Range	Key Mechanism / Notes	Reference
Primary sedimentation	0.4 – 1.0	Mainly solids-associated removal; limited efficacy for enveloped viruses	(98,99)
Conventional activated sludge	2.0 – 3.0	Adsorption to biomass flocs and biodegradation; higher removal for enveloped viruses	(49,99,100)
Membrane Bioreactor (MBR)	3.5 – 5.0	Physical retention by 0.04–0.1 µm membrane plus enhanced biological activity	(49,99)
Anaerobic digestion (mesophilic)	1.0 – 2.0	Temperature 31–37 °C and long HRT; partial inactivation of enveloped viruses	(49)
Chlorination (free chlorine)	0.5 – 2.0	Limited by organic matter shielding and viral aggregation	(98,100)
UV disinfection	1.5 – 3.0	Lower UV dose required compared to non-enveloped viruses	(98)
Ozonation + UV	3.0 – 5.0	Synergistic inactivation highly effective for enveloped viruses	(98)

2025. Removal efficiencies ranged from 1.5–2.5 log in 2020 to >3.0 log in 2025, determined by RT-qPCR and, in later studies, combined with dPCR. New 2024–2025 data indicate a significant decline and improved removal due to technology upgrades and lower community prevalence.

Table 4 summarizes the reported log_{10} removal of SARS-CoV-2 RNA and other coronavirus genomes by different wastewater and sludge treatment processes. Removal efficiencies ranged from 2.0–3.0 log for conventional activated sludge, 3.5–5.0 log for membrane bioreactors, 1.0–2.0 log for mesophilic anaerobic digestion, 0.5–2.0 log for chlorination, 1.5–3.0 log for UV disinfection, and 3.0–5.0 log for ozonation combined with UV.

Discussion

The present narrative review synthesizes evidence from 2000 to 2025 on the occurrence, concentration, detection, and quantification of viral nucleic acids, particularly those of enveloped coronaviruses, in municipal wastewater and sewage sludge. Although infectious SARS-CoV-2 has never been reliably cultured from properly treated secondary or tertiary effluents or anaerobically digested biosolids, viral RNA is frequently detected in both liquid and solid phases despite substantial reductions (typically >3–6 log_{10}) achieved by conventional and advanced treatment processes (5,101). This persistent detection of genetic material, rather than viable virus, highlights the exceptional sensitivity of molecular methods while underscoring the critical distinction between RNA persistence and actual infectivity risk, especially in regions practicing wastewater reuse or biosolid land application (7,102).

As summarized in Tables 1 and 2, viral RNA exhibits significantly greater environmental stability in the solid phase than in the liquid phase. During mesophilic anaerobic digestion (35–37°C, 15–30 days), SARS-CoV-2 RNA decay rarely exceeds 0.5–2.0 log_{10}, whereas liquid-phase decay under comparable conditions typically ranges from 3 to >5 log_{10} (46,103). This difference is attributed to electrostatic adsorption of viral particles and RNA to extracellular polymeric substances and organic solids, which protect nucleic acids from thermal inactivation and RNase activity (35,104). Comparable behavior has been documented for other enveloped surrogates (murine hepatitis virus, porcine epidemic diarrhea virus) and non-enveloped enteric viruses (adenovirus, norovirus GII), indicating that solid-phase protection is a general phenomenon rather than SARS-CoV-2-specific (38,39).

Methodological advances between 2020 and 2025 have dramatically improved the recovery and quantification of viral RNA from complex matrices (Table 1). Hollow-fiber ultrafiltration and electropositive/electronegative membrane adsorption–elution routinely achieve 50–90% recovery across 10–100 L influent volumes, while skimmed-milk flocculation has emerged as a robust, low-cost, and inhibitor-tolerant alternative, particularly suited to low-resource settings (33,102,103). In sludge, ddPCR and RT-ddPCR have largely replaced conventional RT-qPCR because of their superior tolerance to humic substances and heavy metals, as well as their ability to provide absolute quantification without external standards (LOD 1–5 copies μL^{-1} versus 10^2–10^3 copies μL^{-1} for RT-qPCR) (104,105). These improvements explain the consistent detection of SARS-CoV-2 RNA at 10^3–10^5 copies g^{-1} dry weight in digested biosolids long after clinical cases declined in many communities.The prolonged persistence of viral RNA in biosolids challenges early assumptions that enveloped coronaviruses are inherently degradable in the environment. Liquid-phase decay models used in 2020 significantly underestimated solid-phase stability (106-109).

Recent full-scale and mesocosm studies confirm that

RNA from SARS-CoV-2, HCoV-229E, and surrogate coronaviruses remains detectable in dewatered and stored sludge for weeks to months at ambient temperatures (110). While current World Health Organization (WHO) and European Union (EU) biosolids guidelines rely primarily on bacterial indicators, the data presented here suggest that molecular viral signals may warrant consideration in risk assessment frameworks, particularly when Class A biosolids are intended for unrestricted agricultural use (111).

From a surveillance perspective, sewage sludge offers distinct advantages over influent monitoring. Its higher solids content concentrates viral RNA, providing greater analytical sensitivity during periods of low community prevalence and enabling the detection of minority variants that may be diluted below the LOD in influent (44,45). The combination of ultrafiltration or skimmed-milk flocculation with ddPCR and metagenomic sequencing now permits simultaneous surveillance of SARS-CoV-2, influenza A, mpox, norovirus, and multiple enteric pathogens in a single biosolid sample, an approach increasingly adopted in high-income countries (46).

Limitations of the current evidence base should be acknowledged. Most persistence data are derived from RNA quantification rather than infectivity assays, and surrogate coronaviruses (MHV, phi6) may not perfectly replicate SARS-CoV-2 envelope stability. Long-term field studies on stored and land-applied biosolids remain scarce outside Europe and North America. Nevertheless, the convergence of independent datasets from diverse climates and treatment configurations supports the core conclusions.

Complementing the global overview, the longitudinal data from Iranian WWTPs (Table 3) provide critical insights into the temporal evolution of SARS-CoV-2 RNA concentrations and removal efficiencies in a real-world setting. The marked decline in raw influent viral load, from peaks of 10^5-10^6 copies/L in 2020 to non-detectable levels by 2025 in some regions, strongly correlates with the progression of the pandemic, encompassing increased population immunity and advancements in public health measures. Concurrently, the enhancement of removal efficiency from 1.5–2.5 $\log_{10}$ in the early stages to over 3.0 $\log_{10}$ following technological upgrades, such as the implementation of membrane bioreactors (MBR), underscores the pivotal role of modernizing treatment infrastructure in mitigating environmental viral loads. Furthermore, the comparative analysis of treatment processes in Table 4 elucidates the spectrum of SARS-CoV-2 RNA removal efficacies. While conventional activated sludge and mesophilic anaerobic digestion offer moderate removal (2.0–3.0 $\log_{10}$ and 1.0–2.0 $\log_{10}$, respectively), advanced processes such as MBR and combined ozonation with UV demonstrate superior performance, achieving 3.5–5.0 $\log_{10}$ reduction. This hierarchy of efficacy is primarily governed by the key mechanisms of each process: MBR combines physical membrane retention with biological activity, whereas ozonation with UV provides synergistic oxidative and genomic damage. These findings from Tables 3 and 4 collectively affirm that while SARS-CoV-2 RNA persists through many conventional treatments, significant reductions are achievable, and the trajectory of viral concentration in wastewater serves as a robust indicator of both community infection dynamics and the effectiveness of treatment interventions (93,95,97,99).

Conclusion

This narrative review demonstrates that, despite substantial log reductions during conventional and advanced wastewater and sludge treatment processes, viral nucleic acids, particularly from enveloped coronaviruses such as SARS-CoV-2, are not completely removed and frequently persist in both treated effluent and digested biosolids. However, infectious SARS-CoV-2 has never been reliably isolated from properly treated secondary or tertiary effluents or anaerobically digested sludge in any peer-reviewed study worldwide up to 2025, indicating that standard treatment effectively inactivates viable virions while residual RNA remains detectable.

The most crucial finding is the remarkably higher environmental persistence of viral genomes in the solid phase compared with the liquid phase. In digested sewage sludge, SARS-CoV-2 RNA typically undergoes only a 0.5–2.0 $\log_{10}$ reduction during mesophilic anaerobic digestion and can remain detectable at 10^3–10^5 copies g^{-1} dry weight for weeks to months, owing to adsorption onto biosolids and encapsulation within extracellular polymeric substances. This solid-phase protection applies to both enveloped and non-enveloped enteric viruses and explains why sludge represents a more stable and concentrated reservoir of community viral shedding than influent wastewater.

Methodological progress since 2020 has transformed challenging environmental matrices into reliable sentinels for public health surveillance. Hollow-fiber ultrafiltration, electropositive filtration, and skimmed-milk flocculation now achieve 50–90% recovery of viral RNA, whereas ddPCR and metagenomic sequencing offer superior inhibitor tolerance and absolute quantification in high-organic-content sludge. These advances enable sensitive, variant-aware monitoring even during low-prevalence periods and in resource-limited settings.

From a risk-management perspective, the absence of culturable virus in treated outputs supports the continued safety of regulated effluent reuse and biosolids land application when current treatment and storage requirements are met. Nevertheless, the prolonged detection of viral RNA in biosolids highlights the need for cautious interpretation of molecular signals as proxies for infectivity and suggests that RNA-based thresholds could complement traditional bacterial indicators in future biosolids guidelines.

In summary, while infectious coronaviruses and other enteric viruses are effectively inactivated by standard municipal wastewater and sludge treatment, their genetic material exhibits remarkable persistence, especially in biosolids. Integrating optimized concentration and dPCR-

based sludge monitoring into national wastewater-based epidemiology programs offers an effective complementary tool for early detection, variant tracking, and community-level surveillance of both current and future viral threats.

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Competing Interests

The authors declare that there are no known financial conflicts of interest or personal relationships that could have influenced the findings presented in this paper.

Ethical Approval

The study is approved by the Ethics Committee of Mazandaran University of Medical Sciences (ethics code: IR.MAZUMS.REC.1399.701).

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