CHAPTER 5

A comprehensive study of COVID-19 in wastewater: occurrence, surveillance, and viewpoints on its remedy

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5.1 Introduction

Wastewater is a multifarious compound containing an extensive series of physical, chemical, and microbial pollutants released due to human activities. Most of the infectious disease spread through inappropriate management of wastewater causing precarious threats to worldwide public health. The crisis is expected to be exaggerated in the near future due to rapid population increases, change of climate, natural disasters, immigration, globalization, and so forth.[1]

Most infectious diseases are related to microbial agents—principally to viruses. The ongoing episode of novel coronavirus (SARS-CoV-2) has represented a huge worldwide general health threat and caused intense social and financial waves. It was declared as a Public Health Emergency of International Concern (PHEIC), officially, by the World Health Organization (WHO). As of November 29, 2020, more than 61,654,661 infected persons and over 1,444,596 deaths was reported globally.[2] The reaction of the global communal health experts demonstrated an unavoidable delay because of absence of understanding of the COVID-19 infection.[3]

The development of wastewater-based epidemiology (WBE) tools are represented as a harmonizing method for current infectious disease surveillance system.[4]

The present chapter provides a comprehensive overview on various aspects of wastewater, mainly municipal and hospital wastewater. It further strengthens our knowledge on the existence, tenacity, and possibility of the public health threat coupled with the spread of SARS-CoV-2 in aquatic

Environmental Management of COVID-19
ISBN 978-0-323-85780-2
https://doi.org/10.1016/B978-0-323-85780-2.00015-9

waste. Finally, the capability to quickly scrutinize the extent of disease for prevention, intervention, and control, while having several limitations, were also discussed.

5.2 Wastewater: characterization and classification

The amalgamation of different squanders in an aquatic system, which were released from households, organizations, commercial, and industrialized institutions, together with groundwater, surface water, and storm water has been termed "wastewater." They comprised of 99.9% water and 0.1% solids and are organic in nature. In a community, the principal sources of wastewater are the human being and animal wastes. Other important sources include industrial wastes, household wastes, groundwater and stormwater. Corresponding sources producing wastewater have precise properties.[5]

Wastewater has been additionally categorized depending on the sources, i.e., municipal, industrial, sanitary, storm water combined, and health care wastewater as described in Fig. 5.1.

Different types of wastewater contain varied substances, which were accustomed for explicit categorization. The varieties of different substances with their amounts or concentration are source dependent.

5.2.1 Physical characterization of wastewater

The physical properties of aquatic waste body depend on solid content, color, turbidity, taste, and odor, along with temperature.[6] With the exception of gases, the entire mass of pollutants of water belongs to the **solid** content. The characterization is based on their form, their chemical properties, and their quantitative distribution. The amount of suspended solids in wastewater treatment is a notable aquatic characteristic parameter,

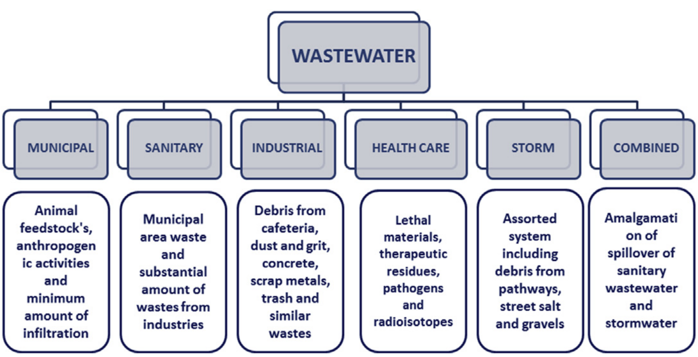

Figure 5.1 Classification of wastewater.

which needs to be utilized for assessing the effluent nature and for screening their performance with regard to numerous processes. **Color** is one additional important physical parameter to use when ruling on the features of water. For the treatment of wastewater, the question is not necessarily the appearance or color; the alternative suggested its use as an indicator to predict the wastewater condition. At the incipient the color of fluid of wastewater is light brownish-gray. The dissolved oxygen (DO) containing wastewater is normally gray in color. With the associated foul odor of wastewater, the color turns black, having no DO and thus depicted as septic in nature. The increase of travel time of the water in the pool and absence of oxygen turns the wastewater from gray to dark gray and ultimately to black. **Turbidity** is yet another measure. It is the degree where light absorption or scattering by suspended material in water takes place. Microbes, vegetable resources, detergents, and soaps, along with various other blending agents, contributed to turbidity. For the management of wastewater, measurements of turbidity are vital when ultraviolet (UV) irradiation is employed during the decontamination processes. **Odors** are generally produced by gases due to putrefaction of biological stuff or by constituents added to the wastewater. Also the **temperature** of wastewater found to be much higher compared to the supply of water. It occurs due to the mixing of warm aquatic wastes from human activities and industrial effluents released; nevertheless, significant amounts temperature fluctuations occurs due to infiltration or storm water flow. During the course of the day, there is variation in the **flow rates** of the wastewater. The phenomenon of variation is known as the diurnal flow variation.[6]

5.2.2 Chemical characterization of wastewater

Generally, the aquatic waste released from different sectors depicted versatile chemical properties. Primarily they were divided into three categories: organic and inorganic matter, along with gases. In the medium strength wastewater, almost three-fourths of the suspended solids along with 40% of solids are filterable and are organic in nature. Proteins, carbohydrates, oil, and grease, along with detergents (surfactants) are included under the organic substances. About 15%−30% of organic matter are **proteinaceous** in nature. Urea with protein are the foremost nitrogenous sources in wastewater. The products released from the breakdown of **carbohydrate** molecules during anaerobic condition are alcohols and organic acids, as well as gases such as hydrogen sulfide and carbon dioxide. Production of huge amounts of organic acid influences the process of treatment by overstraining the protecting capability of the aquatic waste, which causes a drip in pH with the stoppage of biological motion. Categorization of grease includes **oils and fats**, along with waxes and other associated constituents present in aquatic waste. Presence of grease in wastewater is the main reason for obstruction of filters, spigots, and sand beds. **Detergents** are considered to be one of the largest organic molecules. They are soluble in water, and in the wastewater treatment plants they create foams.

Apart from these, there are several other inorganic characters for wastewater, which include determination of pH, alkaline nature, and nitrogenous properties of the aquatic system along with presence of chlorides, phosphates, sulfur, toxic inorganic compounds, and heavy metals, of which quantities vary depending on the type of wastewater.[6]

5.2.3 Biological characterization of wastewater

Organisms that are competent of contaminating or spreading the disease in humans and animals are known as pathogens. The wastewater comprises species of different bacteria, protozoa and parasitic insects (helminths). Viruses were also found, which cause severe infectious diseases.[6] Among the viruses, different types of coronaviruses including SARS-CoV-2 were also found in the wastewater.[7] The existence of these viruses in wastewater is governed by several physicochemical factors such as variations in temperature and pH; the presence of organic matter and oxidants; and plenty of hostile bacteria.[8] The details are discussed in the later part of the chapter.

There are numerous categories of wastewater, but keeping in mind the purview of the topic, the present chapter principally focuses only on different aspects of municipal and health care wastewaters.

5.3 Study of municipal wastewater: characterization and classification

Wastewater created due to "human activities in households" is termed as municipal wastewater. These wastes are discharged from residential areas, workplaces, and factories. They are originated from showers, toilets, and washing machines.[6] They are classified as blackwater and graywater (Fig. 5.2).

1. **Blackwater**: In this case, dual modes of collection of effluents were considered. One is classic and the other one is selective collection. The former consists of entire aquatic collection and excretions discharged from toilets, whereas selective collections are usually from source-separating toilets and are composed of a fecal bin and urine

Figure 5.2 Classification of municipal wastewater.

diverter. Such selective collection allows compartmentalization of yellow water (urine and water from flush) with brown water (feces and toilet paper).

2. **Graywater**: Various emission sources were considered under this category. Effluent consists of kitchen sink and dishwasher discharge, and wash basins in bathrooms, baths, and showers.

Tables 5.1 and 5.2 show the overall physical and chemical characteristics of untreated municipal wastewaters. In many developing countries, municipal wastewater generation has superseded the other types of wastewater generation and has become the major cause of water pollution and eutrophication.

The microbial load of municipal wastewater was found to be versatile in nature, having bacteria, alga, protozoa, and viruses. The pathogenic bacteria include fecal coliform, fecal *Streptococci*, and *Clostridium perfingens*, along with some anaerobic bacteria such as *Clostridium sporogens*, *Bifidobacterium*, and Methanogenic bacteria like *Methanobacterium*. Algal composition includes *Chlorella phormidum*, *Ulothrix*, etc. Different viruses such as Poliovirus, Rotavirus, etc. were found in sewage which acquires access through stools of patients.[6,9] Presence of SARS-CoV-2 virus was also reported by Randazzo et al.[10], Wu et al.[11] and many more.

Table 5.1 Physical characteristics of municipal wastewater.[86]

Parameter	Remarks
Solid	Upended and dissolved organic solids
Color	Dark gray to black
Odor	Foul order due to hydrogen gas decomposition
Temperature	Presence of microbe and difference in the solubility of gases increase the temperature
Turbidity	High turbidity
Flow rate	Leer than pure water because of presence of upended solids

Table 5.2 Chemical characteristics of municipal wastewater.

Parameter	Description of the content	References
pH	6.8–8.3	87,88
Chloride	30–100 mg/L	89
Alkalinity	50–200 mg/L	88
Oil and grease	50–150 mg/L	87
Heavy metals	Ni, Cu, Cr, Cd, Pb, and Hg	87
BOD_5	110–400 mg/L	87,89
COD	250–1000 mg/L	88
Total nitrogen	20–85 mg/L	89
Total phosphorous	4–15 mg/L	87
Total sulfur	20–50 mg/L	89

5.4 Study of health care wastewater: characterization and classification

Hospital wastewater (HWW) usually generates from various types of medical care and its related activities. It comprises a variety of toxic substances, which include pharmaceutical products, radioisotopes, pathogens, solvents, and disinfectants. The characteristics of HWWs are comparable with municipal wastewater to some extent, but individually it also contains some other toxic pollutants.[12]

In the waste released from hospitals, approximately 85% of the waste are equivalent with municipal waste and termed as nonhazardous. The remaining 15% are hazardous.

5.4.1 Hazardous health care waste

These wastes were classified as infectious waste, sharps, pathological waste, pharmaceutical and cytotoxic waste, chemical waste, and waste from radioactive sources, as described in Fig. 5.3.

5.4.2 Nonhazardous health care waste

The wastes other than physical, chemical, biological, or radioactive are known as nonhazardous hospital waste.

Although different physical, chemical, and biological pollutants encompass hospital wastewater, the effluents for both conventional and nonconventional parameters were characterized and summarized in Table 5.3.

The hospital effluents are sources of inorganic/organic loads and were demonstrated by their different concentration ranges of both macro- and micropollutants.[13] Gadolinium (Gd), platinum (Pt), and mercury (Hg) were

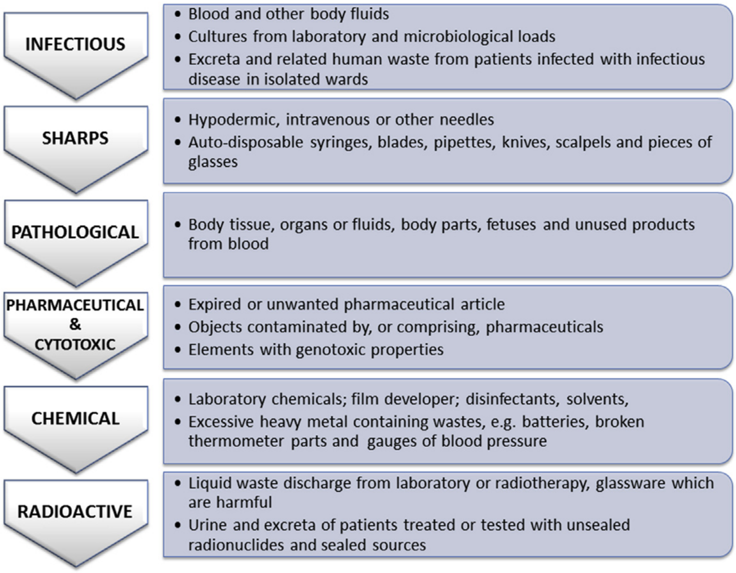

Figure 5.3 Classification of hazardous health care waste.

Table 5.3 Different characteristic properties of hospital wastewater.

Parameter	Concentration	References
pH	6–9	90
Electrical conductivity	300–2700 μS/cm	16
Total suspended solid	116–3260 mg/L	18
Chlorides	80–400 mg/L	90
Fats and oil	50–210 mg/L	91
Total Nitrogen	60–230 mg/L	22
Total phosphate	6–19 mg/L	16
COD	39–7764 mg/L	18
BOD	16–2575 mg/L	90
Heavy toxic metals	Zn, Pb, Ni, Ag, Hg, Gd	91
Total surfactant	4–8 mg/L	16
Microbial load	*E. coli*, enterococcus, adenovirus, rotavirus, norovirus, hepatitis A	22

found to be the main heavy metals in hospital runoffs.[14] Apart from these, Cd, Cu, Fe, Ni, Pb, Zn, and other heavy metals are similar in concentrations as reported in municipal discharge.[15] Various pollutants such as Gd, Hg, Pt, and pharmaceutical products along with acetaminophen, caffeine, ciprofloxacin, and gabapentin are released as hospital waste.[16–21] Along with the other microbes it also shows the presence of the SARS-CoV-2 virus.[22,23]

Hence, it could be predicted that when an infected individual's excreta containing pathogens gets flushed down the toilet or washed down the drain, it travels through the sewage system of a community. Exposure to such pathogens in a wastewater system could potentially cause serious health consequences in the community. Therefore, assessing the potentiality of infection and transmission of the disease through the wastewater system is desired.

5.5 Characteristics of coronavirus

Coronaviruses (CoVs) are a type of enteric virus that contain single-stranded positive RNA genome of the length of 27–32 Kb.[24] They belong to the order *Nidovirales*, family *Coronaviridae*, and subfamily *Coronavirinae*, and are the main cause of the present pandemic.[1] Spikelike projections on their exterior surface seen under the electron microscope are the glycoproteins, imitated as crownlike, hence the name coronavirus (CoV).[25]

Coronavirinae are comprised of four genera, Alpha-, Beta-, Gamma-, and Delta-, among which infection in *Homo sapiens* was instigated by the first two (Alpha- and Beta-) host viruses.[26] Among them, a novel virus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the beta- CoVs category. They cause the disease COVID-19 (CO means Corona, VI is Virus, D is disease, 19 is the year it was first discovered).[1] In December 2019, the virus spread from Wuhan, China, to the rest of the world. They showed high affinity for binding to the human angiotensin-converting enzyme 2 (ACE2) receptors.[1] Principally, SARS-CoV-2, along with ACE2 receptors, penetrate inside the host's system, where the proteins and the viral RNA are synthesized into multiple copies inside the cytoplasm to congregate the new virion gene.[27]

Though the primary modes of the viral transmission are through respiratory droplets and direct or indirect contact, SARS-CoV-2 virus might get incorporated into the wastewater (municipal and hospital) through numerous sources, such as hand washing, sputum and vomiting, fecal waste, etc.[28] Hence, the potentiality of wastewater and its effect on

SARS-CoV-2 was investigated for further understanding the epidemiology of COVID-19.

Existence of the virus in wastewater is highly influenced by subsequent factors, both intrinsic and extrinsic, depending on the wastewater or ecological environment:

- **Structure of the virus**: Enveloped viruses similar to coronavirus have shorter survival periods compared to nonenveloped viruses.[8] This was owing to the activity of detergents and proteolytic enzymes on the peripheral wall of the virus, which is lipid in nature.[29]
- **Composition of the wastewater**: The component of the wastewater likewise plays a vital function in the persistence of viruses. The rapid inactivation of coronaviruses in wastewater is credited to the bacterial proteolytic enzymes and existence of chemicals that are antiviral in nature, along with predation of protozoa and metazoa in the wastewater.[29]
- **Temperature**: Temperature has significant influence on the survival of coronaviruses in wastewater, similar to other microbes. The virus may survive longer in temperate or colder regions compared to tropical regions. With the increasing temperature, there is reduction in the persistence of the virus, which is attributed to the nucleic acid and protein denaturation, along with intensification of extracellular enzyme activity.[30,31]
- **pH**: Significant modifications in the configuration of proteins was achieved by alteration of pH. A pH variation toward its isoelectric point causes precipitation;[32] the steadiness of SARS-CoV-2 in different pH values ranges from 3 to 10 at room temperature.[32]
- **Suspended solid**: The suspended solids act as a shield to protect coronaviruses from inactivation.[8] Studies have shown the effect of suspended solids and organic matter on the survival of coronaviruses where it was reported that coronaviruses can survive longer in primary wastewater than in activated sludge wastewater. Likewise, Zhang et al.[23] reported the presence of organic matter in the patient's feces, which protect the SARS-CoV-2 virus from the disinfection process of medical wastewater.

Thus, the persistence of coronaviruses in wastewater is intensely reliant on the properties of the individual aquatic medium.

5.6 Occurrence and persistence of COVID-19 in wastewater

SARS-CoV-2 strains have also been found to persist inside human beings, such as feces, sputum, and serum, but are unstable in urine, due to the reduction of pH values and the presence of urea. Conversely, saliva showed SARS-CoV-2 positivity and is responsible for the spread of COVID-19 with the help of air. Also, contamination of water occurs when infectious saliva is mistakenly touched by a clinically fit individual. Depending on excessive load of SARS-CoV-2 on saliva as well as in stool samples of COVID-19 patients, contaminated aquatic waste released from the health sector, isolation centers, and municipal areas and from the areas with cases of positive COVID-19[33] finally indicated transmission of SARS-CoV-2 through the fecal route. From feces, the virus gets diluted in toilet water and then in other municipal wastewater, including graywater, to the wider environment. Graywater is anticipated to not be ideal for SARS-CoV-2 transmission, though it contains high viral titers of body fluids.[34] The transport of the viral RNA through a complex sewage system compel them to be exposed to different chemicals and fluctuating temperatures. Diagrammatic route of transmission of the SARS-CoV-2 virus has been presented in Fig. 5.4[35–37]

On the basis of the decaying nature of the pathogens, that is, SARS-CoV-2, the degree of the health threat varies in the aquatic environment. There are possibilities to improve the control measures and

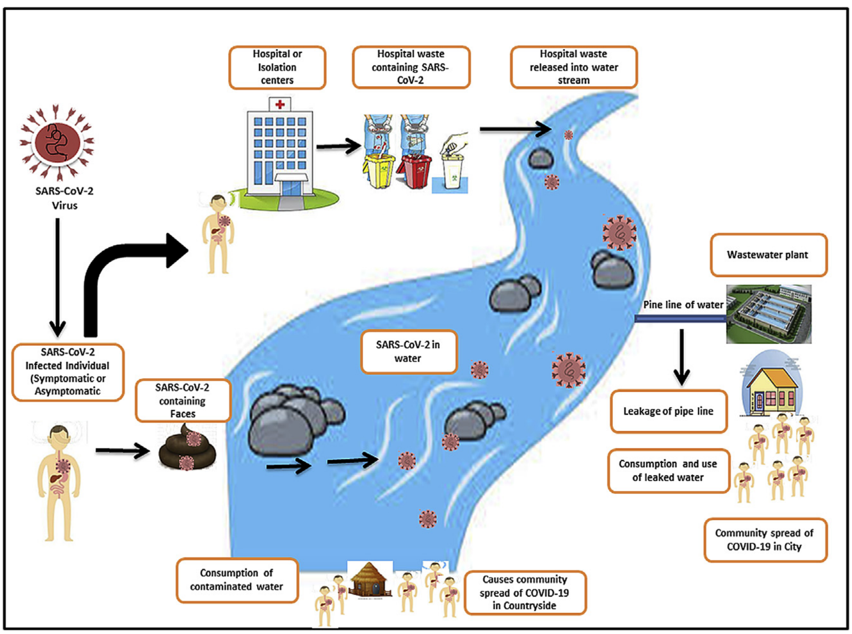

Figure 5.4 Representation of SARS-CoV-2 transmission in wastewater.

wastewater treatment with the analysis of the decomposition of viral RNA. It has been found that the average duration of life of SARS-CoV-2 in the feces was up to 22 days, which was longer than that of SARS-CoV-1. It has also indicated that SARS-CoV-2 can survive longer in the fecal specimens than in respiratory (18 days) and serum (16 days).[38] Van Doremalen et al.[39] confirmed the variation of the SARS-CoV-2 half-life from 0.8 h on copper to 6.8 h on plastic.

Limited data are available on SARS-CoV-2 infection related to its occurrence and its molecular nature in feces and wastewater. Viral shedding in feces has been reported[23,40,41] and summarized in Table 5.4.

After the feces is released into the wastewater, the presence of SARS-CoV-2 in wastewater endorses the fecal transmission route.

Table 5.4 Study on occurrence of SARS-CoV-2 in the fecal sample.

References	Findings
41	In feces, the SARS-CoV-2 positive specimens were 44 out of 153 patients (29%). For the stool specimens, the mean series of threshold value was 31.4, which indicates a viral load of less than 2.6×10^4 copies/mL. In nasal swabs the load was 1.4×10^6 copies/mL.
92	The researchers tested on 10 pediatric SARS-CoV-2 infection cases highlighted that some patients were positive on rectal swabs even after their nasopharyngeal testing had become negative.
93	Fecal samples of nine hospitalized patients with COVID-19 during the course of the disease showed high viral RNA concentrations in initial samples, with a peak during the first week of symptoms. The viral content declined gradually. The viral load in feces varies in the range 10^3-10^7 RNA copies/g feces.
94	The viral RNA in the stool was tested on the day of hospitalization. 15.3% of the patients detected the presence of SARS-CoV-2 RNA. In nine positive patients an average viral load of 104.7 copies/mL was found in the stool.
11	While investigating 74 fecal samples from patients, it was observed that over 50% of patients showed presence of SARS-CoV-2 RNA though the respiratory tract samples became negative
95	The feces of 73 hospitalized patients were examined for SARS-CoV-2 infection and it was evaluated that 53.4% of the patients showed positive results for viral RNA in stool. Additionally, greater than 20% of the patients having SARS-CoV-2 remain positive in feces, even after showing negative results in respiratory samples

Continued

Table 5.4 Study on occurrence of SARS-CoV-2 in the fecal sample.—cont'd

References	Findings
46	The researchers investigated 23 fecal samples, showing 83% positivity for SARS-CoV-2 infection. The average period of virus shedding was 22 days for feces when the titer value for the virus in feces was 5623 copies/mL, but the highest titer at the peak reached 105.8 copies/mL.
40	65 feces samples of hospitalized patients were tested for the presence of SARS-CoV-2, of which 22 (52.4%) were positive. Among them, 6 having GI symptoms that showed esophageal bleeding when subjected to endoscopy. SARS-CoV-2 RNA was detected in esophagus, stomach, duodenum, and rectum specimens for both two severe patients. Whereas, in four nonsevere patients only duodenum showed SARS-CoV-2 positivity.
96	Among the 42 hospitalized patients, 28 showed SARS-CoV-2 infection. Among them, 64.3% of patients remained positive after the pharyngeal swabs turned negative.
97	From 204 patients, 35 confirmed cases of SARS-CoV-2 infected samples were found. The viral loads were less than the respiratory samples (range 550 copies per mL to $1 \times 21 \times 10^5$ copies/mL

One of the most important studies was performed at the Ahmedabad wastewater treatment plant (WWTP) at Gujarat. Kumar et al. in 2020 reported 106 million liters per day wastewater, which receives effluent from the civil hospital treating COVID-19 patients.[42]

Ahmed et al.[43] confirmed the presence of SARS-CoV-2 in untreated wastewater samples collected from pumping stations and wastewater treatment plants in Queensland, Australia.

Alternatively, samples collected from influent and secondary-treated wastewater were investigated for the existence of SARS-CoV-2 virus in Japan.[44] SARS-CoV-2 presence was confirmed in five secondary-treated wastewater samples with a load of 2.4×10^3 copies/L. In contrast, the same was not detected in the influent wastewater samples. The authors explained the result on the basis of the limit of detection (LOD). The LOD value for the secondary-treated wastewater (1.4×10^2–2.5×10^3 copies/L) was unusually lowered compared to influent (4.0×10^3–8.2×10^4 copies/L). This is because the former (200 mL) had a lower filtration volume than the latter (5000 mL).

Likewise, there are many more cases in different parts of the world that corroborate the fact of viral transmission from feces to wastewater[23,45,46] and treated wastewater[10,47] in the recent worldwide outbreak, which was summarized in Table 5.5.

Overall, from the above studies it could be anticipated that the routine wastewater monitoring can help to isolate a noninvasive warning sign to alert the community for new SARS-CoV-2 infections, as a large number of the viral carriers are asymptomatic and are present in a community which might intensify the epidemic abruptly.

Table 5.5 Details of occurrence of SARS-CoV-2 in wastewater environment in different countries.

Name of the place	Findings	References
Australia	Concentrated SARS-CoV-2 RNA copies were classified using RT-qPCR. Two positive cases were detected within a six-day period from the wastewater treatment plant. The observed viral RNA copy numbers are then simulated using Monte Carlo model which estimated a median range of 171—1090 infected persons.	43
China	Three hospitals wastewater samples were used for detection of SARS-CoV-2 viral RNA. It detected 255 copies/L of SARS-CoV-2 viral RNA in adjusting tank of first hospital wastewater. The second hospital wastewater was treated by a series of treatment steps in which SARS-CoV-2 viral RNA was detected in the adjusting tank (633 copies/L), but not detected in MBBR and sedimentation tank. In the third hospital treatment unit, SARS-CoV-2 viral RNA was detected in the range of 557—18,744 copies/L, only in septic tank where 800 mg/L sodium hypochlorite was used. It was not detected with 6700 mg/L of sodium hypochlorite.	23,45
Czech Republic	Concentrated SARS-CoV-2 RNA was detected in 11.6% of samples and more than 27.3% of WWTPs; in many places, SARS-CoV-2 was detected repeatedly.	98

Continued

Table 5.5 Details of occurrence of SARS-CoV-2 in wastewater environment in different countries.—cont'd

Name of the place	Findings	References
France	A time-course quantitative analysis of SARS-CoV-2 by RT-qPCR showed rise of genomic units; six out of eight samples of treated wastewater were positive in crude wastewaters precisely tracked the escalation of human COVID-19 cases observed at the provincial level. The viral genomes were identified before the commencement of the exponential progress of the epidemic. An obvious reduction in the extents of genomic units was observed concurrently with the decrease in the number of new COVID-19 cases.	47
India	All three genes of SARS-CoV-2 were discriminated in the influents with none of the genes were spotted in the effluent. Temporal difference between different samples was of 10× in gene copy loading with corresponding change of 2× in the number active COVID-19 patients in the city. Samples from different local municipal WWTPs and hospital wastewater showed the occurrence of SARS-CoV-2 viral genome at an ambient temperature of 40°C.	3,42
Israel	SARS-CoV-2 RNA was systematically detected in the influent of the primary settler suggesting that, the virus particles have a higher affinity for the sludge. However, in the digested sludge genetic material is not detected. The results further confirm the safety of the sludge after thermal treatment and anaerobic digestion. The combined treatment of thermal hydrolysis and anaerobic digestion also prevented the detection of SARS-CoV-2 in the sludge leaving the plant.	54
Istanbul	Five samples out of seven from wastewater and all samples from manholes were tested positive. SARS-CoV-2 virus titers of manhole were higher than those of inlet WWTPs. Quantitative measurements of SARS-CoV-2 in wastewater were carried out using WBE which showed positive tested viral titer ranges from 1.17×10^4 to 4.02×10^4/L.	99

Table 5.5 Details of occurrence of SARS-CoV-2 in wastewater environment in different countries.—cont'd

Name of the place	Findings	References
Italy	Molecular analysis was undertaken with three nested protocols. SARS-CoV-2 RNA detection was accomplished in volumes of 250 mL of wastewaters collected in areas of high and low epidemic circulation, according to clinical data. Overall, 6 out of 12 samples were positive. One of the positive results was obtained in a Milan wastewater sample collected a few days after the first notified Italian case of autochthonous SARS-CoV-2.	1,55
Japan	SARS-CoV-2 RNA was detected in secondary-treated wastewater samples collected of 2.4 × 103 copies/L by qPCR. SARS-CoV-2 RNA was detected in a secondary-treated wastewater sample when the weekly reported cases in the community were high.	44
Netherlands	RT-PCR performed against three fragments of the nucleocapsid protein gene and one fragment of the envelope protein gene. The fragments were detected in sewage sites, even when the COVID-19 prevalence is low.	56
Spain	By using the real-time RT-PCR three regions of the virus nucleocapsid (N) gene were targeted. Quantification of SARS-CoV-2 RNA was performed. The titers reported for wastewater samples as 5.4 ± 0.2 $\log_{10}$ genomic copies/L on average. Two secondary water samples were positive and all tertiary water samples tested as negative. This environmental surveillance reveals that members of the community were shedding SARS-CoV-2 RNA in their stool even before the first cases were reported by local or national authorities.	10
USA	RT-qPCR technique was operated, which confirmed the presence of SARS-CoV-2 at high titers approximately of 100 viral particles per mL of sewage. Monitoring of wastewater demonstrated that SARS-CoV-2 RNA by RT-qPCR correlated with prevalence of viral infections in the community. Data indicated that symptom onset precedes detection of SARS-CoV-2 RNA in wastewater by five to eight days.	11,100

5.7 Detection of coronavirus in wastewater

Because the virus is novel and has fatal outbreak, detection of SARS-CoV-2 in various environmental matrices is a major bottleneck at present. There is an urgency to identify highly specific and sensitive diagnostic measures for infected areas or people to avoid its further spread. It was observed that the testing rates for COVID-19 were quite low.[48,49] Ever since the feces or urine showed early detection of the virus,[50] such sample testing from a community on a regular basis would minimize the spread of the virus. At present, various other diagnostic and detection methods are needed.

For prompt, regular governing of viral outbreaks, WBE holds greater potential by regularly monitoring the variety and concentration of the virus in wastewater. The approach of WBE is thought to be effective for underdeveloped countries to scrutinize the predominance of the COVID-19 infectious spread in the community. Another advantage of WBE is that with the help of phylogenetic studies it supported the detection of variations in the viral strains.[51] Since many cases of SARS-CoV-2 infection are asymptomatic, the recognition of the inhabitants, which act as vectors to avert dissemination of the virus, is highly needed.

For accurate detection, the samples need to be processed for measuring SARS-CoV-2 appropriately. This involves preparation of the sample, concentration, RNA extraction, and finally genomic analysis. Proper biosafety protocols for processing the wastewater samples that may contain SARS-CoV-2 should be followed as recommended by CDC.[52]

The process of concentration uses microbiological and molecular approaches prior to the detection process.[53] The recovery of virus by concentrating methods is way more challenging. There are various methods for concentration, such as PEG-based separation methods, applied electronegative membranes filtration methods, and applied ultrafiltration methods,[10,43,54–56] among them, concentration using PEG is the most prevalent method used for the COVID-19 WBE.[57]

Followed by concentration is the method of detection or analysis. The most common methods are the reverse transcription-polymerase chain reaction (RT-PCR) and the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). For example, Ahmed et al.[43] applied RT-qPCR to detect SARS-CoV-2 infected individuals in the catchment basin. Similar findings have been reported elsewhere.[4,23,44,56]

Although these PCR techniques have been applied as a yardstick for detection of SARS-CoV-2, they are unable to distinguish between infectious and inactive fractions.[58] Due to such inadequacies, another complementary method was adopted where direct absolute quantification of virus genome copy numbers in a sample without the necessity of external calibration can be performed. Digital PCR (dPCR) has recently been used for SARS-CoV-2 detection.[59]

Owing to the rapid growth of confirmed cases of COVID-19, there is a serious need to develop a robust tool to address the challenge.

The biosensing technique has emerged with an interdisciplinary approach, which enables point-of-care diagnosis for swift and fast viral detection and prevents epidemics at an initial stage. In this context, Bhalla et al.[60] and Samson et al.[61] has reviewed the opportunities and technical challenges related to biosensors and analytical tools.

In a biosensor-based method, as reported,[62] a paper-based analytical device was constructed for the speedy determination of the pathogens. The small analytical tool has different functional areas with a wax printer that integrates all the processes required for nucleic acid testing into an inexpensive paper material. In a way, a paper-based device has the potential for the diagnosis of COVID-19 in aquatic waste in real-time for tracking the viral carriers in the community.[32,63]

Yet another technology, the CRISPR-based DETECTR, has been recognized to reconfigure SARS-CoV-2 detection within a few days. The assay was performed by simultaneous reverse transcription and isothermal amplification using loop-mediated amplification (RT—LAMP). The extracted RNA was taken in universal transport medium (UTM), followed by Cas12 detection of predefined coronavirus sequences, after which detection of the virus was confirmed by the cleavage of a reporter molecule.[64] The CRISPR-based DETECTR innovation is discovered to be very adaptable to reconfigure within a short time to distinguish SARS-CoV-2. The future improvement of different versatile microfluidic based cartridges and lyophilized reagents could empower purpose of care testing outside the indicative research facility.[64]

For inactivating SARS-CoV-2 viral genome, photocatalytic nanoparticles (NPs) were also considered where titanium dioxide (TiO_2) showed photocatalytic properties under UV light. They remain dormant and are harmless in nature. If effective, the TiO_2 photocatalysis could be used for the deactivation of COVID-19.[65,66]

Electrospun nanofiber membranes were also found to be effective monitoring tools for screening disease causing pathogens. They play vital role in sieving intestinal *Enterococci*, *E. coli*, and coliform microorganisms.[67]

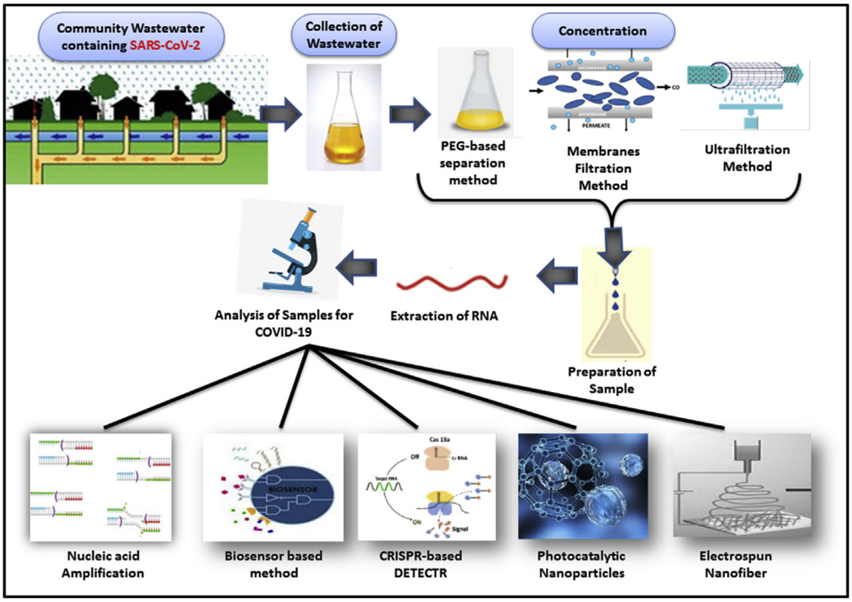

Figure 5.5 Promising methodologies for SARS-CoV-2 detection in wastewater.

These bioengineering-based nanofibers would especially increase the binding sites explicitness toward its objective, in this case COVID-19 viral RNA.

Numerous methods/devices have been applied for SARS-CoV-2 detection in different types of waste aquatic body as summarized in Fig. 5.5, but the standardized protocols and authentication for such methods/devices are yet to be accessible in the public domain.

5.8 Inactivation mechanism of COVID-19 in wastewater

The emergence of SARS-CoV-2 has become a global health concern. from the perspective of virologists, the intestinal tropism of SARS-CoV-2 is extraordinary. As reported by an ongoing study,[68] the intestinal tropism demonstrated the hostile nature of SARS-CoV-2 compared to other viruses. Initially specific treatments were unavailable, until Chan et al.[69] and Yeo et al.[31] deciphered that there is a phylogenetic relationship between the genome of both SARS-CoV-2 and other bat-related SARS coronaviral genome. Also, the nucleotides of the spike protein show 78% similarity with the human SARS-CoV-1.[31,69] With this assumption, scientists all over the world primarily hypothesized that SARS-CoV-2 would be reactive against various environmental aspects or disinfectants. Disinfectants typically act on the viral genome, capsid, or the protein layer, in a way destroying COVID-19.[7]

Sporadic reports show the use of different disinfectants in this field. Initially, a study of hospital wastewater[23] indicated nonappearance of SARS-CoV-2 viral RNA in the influent, whereas the effluent was verified positive for the presence of viral RNA. Afterward, Zhang and his group investigated another study where three clinics of China were considered for the identification of SARS-CoV-2 virus by RT-qPCR.[45] The clinical study reported the presence of 255 copies/L of viral titers in the adjusting tank. The aquatic waste of the second hospital followed a series of treatment steps in the order of adjusting tank—septic tank adjusting tank—moving bed biofilm reactor (MBBR)—sedimentation—disinfection, in which SARS-CoV-2 viral RNA was detected in three units: the adjusting tank (633 copies/L), MBBR (not detected-505 copies/L), and sedimentation tank (not detected-2208 copies/L). In the third clinical treatment unit, it consists of two units (preliminary disinfection tank followed by septic tank), SARS-CoV-2 was detected in the range of 557—18,744 copies/L only in the septic tank when 800 mg/L sodium hypochlorite was used. However, no detection of SAR-CoV-2 was found; the sodium hypochloride concentration range was 6700 mg/L. Several similar studies were reviewed by researchers to further confirm the treatment of SARS-CoV using various disinfection treatments and its infectivity on inanimate surfaces.[41,70—72]

Yet another disinfectant is hypochlorous acid (HOCl), which is a productive mediator against viruses for disrupting their genomic and proteomic mechanism.[73] Block and Rowan depicted the probable use of HOCl against SARS-CoV-2 virus disinfection.[74]

Another disinfectant, ethanol, has been used for disrupting the cell wall of the virus as reported by.[71] They reported that 62%—71% ethanol along with 0.5% hydrogen peroxide, or 0.1% sodium hypochlorite (NaOCl) as surface disinfection rapidly reduces the infectivity of SARS-CoV-2 on different surfaces within 1 min exposure time. According to WHO recommendation, higher concentration of NaOCl or 70% ethyl alcohol are suitable for disinfecting surfaces. Therefore, the ethanol and sodium hypochlorite are highly recommended for killing SARS-CoV-2 and protecting water workers from COVID-19 infection.[75]

The List N of the EPA has registered 431 commercial disinfectants which ascertain COVID-19 inactivation as of June 2020. Most of the products that follow the criteria chosen by EPA for its effectiveness against the removal of the virus possess some important component.[76]

Despite the promising results from treatment using disinfectant as reported above, various disadvantages accompanied them. Hence, there is a need for further research for deactivating SARS-CoV-2 viral infection.

The COVID-19 also demonstrated a high sensitivity toward ozone treatment in indoor spaces. Ozone has the ability for the disruption of protein and the lipid of the spikes of the enveloped viruses, specifically fatty acids, cysteine, methionine, and tryptophan along with linoleic and oleic acid with N-glycopeptides present on the spikes of subunits 1 and 2 proteins.[77] With the gradual enhancement of ozone from 48.83 to 94.67 $\mu g/m^3$, temperature from 13.17 to 19°C and a reduction of RH from 23.33% to 82.67% there is a decrease in the transmission of viral infections.[78]

Ultraviolet C (UV−C) could be a promising alternative for SARS-CoV-2 strain inactivation as it damages the viral genome.[79,80] Recently Inagaki and his coworker in 2020 have illustrated burgeoning deactivation method of COVID-19 using DUV-LED.[80] Another report revealed the inactivation of SARS-CoV-2 strain achieved with monochromatic UV-C (254 nm) irradiation, which is associated with multiple doses of illumination (3.7, 16.9, and 84.4 mJ/cm^2) in contrast to a sequence of different viral titers (0.05, 5, and 1000).[79]

Using the technology, International Advanced Research Center for Powder Metallurgy and New Material (ARCI) India has put forward a positive effort to develop a device. The device contains three disinfection systems that execute various physical, thermal, and chemical processes for sterilization of a selected area and surfaces efficiently at different health care departments as well as public locales for combating the COVID-19 pandemic. The system has a UV-C-based trolley for disinfection, an air heater of honeycomb type, and a fogging system to provide effective inactivation against SARS-CoV-2.[81] Therefore, it could be predicted that in the near future, if used for wastewater treatment, it would be credible to provide benefit to the society in alleviating the viral outburst.

Recently, another group of researchers from US Department of Homeland Security provided the first data on the effect of simulated sunlight on the survival of SARS-CoV-2 suspended in simulated saliva or culture media and suggested that sunlight could directly influence the survival of the virus.[82] The study showed that under simulated sunlight, 90% of infectious virus gets inactivated in every 6.8 min in simulated saliva

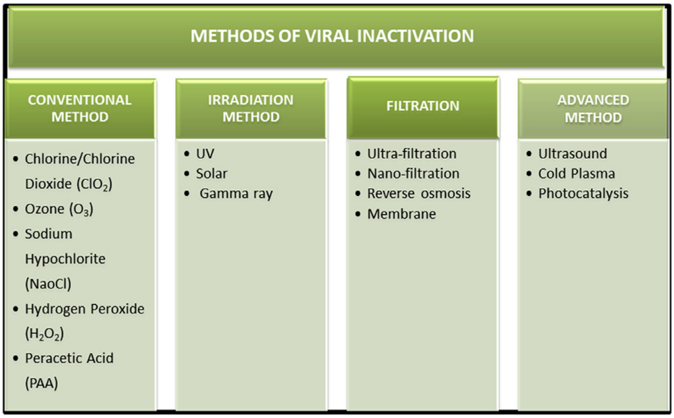

Figure 5.6 Different methods of inactivation of SARS-CoV-2 in wastewater.

and in every 14.3 min in culture media. Further, confirmation is yet to be explored.

Beside this, various advanced oxidation processes (AOPs) including photocatalysis, ultrasonic process, and Fenton processes have also been effectively utilized in treating wastewater.[7] Different deactivation methods used for COVID-19 were represented in Fig. 5.6. However, their use in wastewater treatment needs more momentum to gain robust solutions to meticulously eliminate the SARS-CoV-2 viral infection.

5.9 Remedial approach

In a recent report, the municipal and hospital wastewater estimated the SARS-CoV-2 load ranges from 56.6 million to 11.3 billion per infected individuals in a single day.[4] Such huge viral loads need to be addressed. Hence, apart from eradicating the disease, various prevention-based approaches has been undertaken for self-protection and also for the protection of personnel working in different sectors. WHO in 2020 had provided standard precautionary measures and guidelines to be followed by the workers who were working in the viral-suspected areas[75,83]:

(1) Maintenance of social distancing between two persons of six feet (about two arm's length).
(2) When outdoors, respirator masks (such as FFP2, FFP3, N95, and N99) should be properly used.
(3) Use of soaps for frequent hand washing along with alcohol sanitizer.
(4) Prohibition of touching mouth, eyes, and nose.

Short-term laboratory biosafety guidelines, released by Centers for Disease Control and Prevention (CDC) put forward various instructions regarding management along with specimen dispensation associated with COVID-19.[84] The instructions summarize the facts that

- ➢ Standard precautions should be followed while handling COVID-related clinical specimens.
- ➢ Use of EPA-registered disinfectants, which claims SARS-CoV-2 removal, should be practiced.
- ➢ Proper procedures should be maintained for management of laboratory waste as instructed by the administrator.

Although various effective measures were taken, the stigma in response to COVID-19 has put the life of health care workers and patients along with frontline workers in danger.[35,85] Therefore, the following propaganda was suggested, which might instigate us for future research in this area:

1. The wastewater treatment of the community should be decentralized with the purpose that the release of the waste from the isolation centers, having infected patients residues, will not contaminate the nearby area. Hence, decentralization of a community might reduce the contamination of a neighboring region.
2. The immense populace needs large-scale testing, which is quite strenuous. Consequently timely viral detection of the feces needs to be attained, while scrutinizing the virus genome in the aquatic waste of a community wastewater for early detection of the virus in an individual. This communal testing found to be a better alternative for mitigating the viral explosion.
3. The competent authorities should adequately address the sanitation and improve the water quality required. In a way the aquatic contamination could be surpassed with the avoidance of unpredicted SARS-CoV-2 viral RNA transmission to an infected person. Also, other human enteric viruses and associated viruses which emanate from feces could be diagnosed and prevented.
4. For deactivating and eliminating SARS-CoV-2 viral genome and its related species from the feces present in aquatic waste, an economically cheap point-of-use device should be developed, which eventually will be available to the public.
5. An appropriate legal policy, laws, guidelines, and so forth need to be recommended to certify sufficient agreement for the release of wastewater into the atmosphere with proper treatment. Also, there is a

need for various public awareness campaigns to tackle the stigma derived from the disease properly.

6. The government should implement a strategic plan for wastewater treatment plants at pilot scale, which could be beneficial in the long run. Also, there is a need for reforming the inconsistent health care system and setting proper guidelines for destroying such types of infectious disease outburst in the near future.

The current snowballing spread of COVID-19 can be curtailed with a rising level of community empowerment, with healthy literacy to respond appropriately in the situation.

5.10 Conclusion and future perspectives

The occurrence of the COVID-19 pandemic reminds us that the life in the world is impulsive and challenging. At present, trivial knowledge existed on the potentiality of aquatic waste in the spread of the viral dissemination and its magnitude of infectivity. Studies are under progress on existence and deactivation of COVID-19 in the waste aquatic environment. The background information indicated the firmness of the genome and its analogy with SARS-CoV. Based on the contextual knowledge, the following research directions should be explored:

1. Governments should take responsibility to develop economically cheap wastewater-based epidemiologies for controlling the spread of the pandemic in developed and underdeveloped countries from the global to local level.
2. Though various researchers have shown interesting and positive outcomes addressing the water quality, viral propagation, inactivation mechanisms of SARS-CoV-2 under solar, and other disinfection, evidence regarding their removal still need to be deciphered. Studies on disinfection kinetics with reference to the dosage concentration, activity, etc. should be addressed to gain a comprehensive knowledge regarding the inactivation strategy. Also, environmental impacts must be studied to be considered for disinfectants and other deactivating techniques.
3. Concern associated with the health of wastewater treatment workers and COVID-19 frontline workers need to be attended with priority at regular intervals. Proper guidelines related to hygiene and other preventive measures should be augmented by policymakers and health administration. Also, clear strategies should be guaranteed for the

support of infected health care workers to adequately cope with the disease.

4. Awareness programs for the public related to COVID-19 and its preventive practices need to be adopted to meet goals related to its elimination. For this, educational interventions might be considered.

Ideally, the COVID-19 pandemic has taught us to work together. The integration and synchronization of various actions can contribute to this work on a global scale so that a happy future for all can be achieved.

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