CHAPTER 4

Presence, detection, and persistence of SARS-CoV-2 in wastewater and the sustainable remedial measures

Bashir Adelodun[1,2], AbdulGafar Olatunji Tiamiyu[3], Fidelis Odedishemi Ajibade[4,5,6], Golden Odey[1], Rahmat Gbemisola Ibrahim[7], Madhumita Goala[8], Hashim Olalekan Bakare[3], Temitope F. Ajibade[4,6,9], Jamiu Adetayo Adeniran[10,11], Kamoru Akanni Adeniran[2], Kyung Sook Choi[1,12]

[1]Department of Agricultural Civil Engineering, Kyungpook National University, Daegu, Korea; [2]Department of Agricultural and Biosystems Engineering, University of Ilorin, Ilorin, Kwara State, Nigeria; [3]Department of Chemical Engineering, University of Ilorin, Ilorin, Kwara State, Nigeria; [4]Department of Civil and Environmental Engineering, Federal University of Technology, Akure, Ondo State, Nigeria; [5]Key Laboratory of Environmental Biotechnology, Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, PR China; [6]University of Chinese Academy of Sciences, Beijing, PR China; [7]Kwara State Ministry of Health, Ilorin, Kwara State, Nigeria; [8]Nehru College, Pailapool, Affiliated Assam University, Silchar, Cachar, Assam, India; [9]Key Laboratory of Urban Pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, PR China; [10]Environmental Engineering Research Laboratory, Department of Chemical Engineering, University of Ilorin, Ilorin, Kwara State, Nigeria; [11]Atmospheric Chemistry and Modeling Group, Department of Atmospheric and Oceanic Sciences, Peking University, Beijing, China; [12]Institute of Agricultural Science & Technology, Kyungpook National University, Daegu, Korea

4.1 Introduction

The world has experienced another global pandemic, the coronavirus disease 2019 (COVID-19), caused by novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was declared a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO) on January 30, 2020.[1] The generally known means of transmission was from direct contact or droplets from respiratory actions. However, the reports on the detection of SARS-CoV-2 in wastewater samples and water bodies may have been present via the gastrointestinal tract of an infected patient and contaminated sewage discharged (Fig. 4.1).[2–4] The possible presence of SARS-CoV-2 in wastewater is a serious issue that needs to be properly attended to and curbed before the widespread of the virus among vulnerable populations;[5] this, in addition to

Environmental Management of COVID-19
ISBN 978-0-323-85780-2
https://doi.org/10.1016/B978-0-323-85780-2.00014-7

a report that the resemblance of SARS-CoV-2 to SARS-coronavirus (SARS-CoV) is about 82%,[6] gives researchers and policymakers an area of focus to expedite actions toward finding lasting solutions.

In 2003, during the outbreak of SARS-CoV, the virus spread rapidly and was detected in feces (surviving for up to four days), water, and sewage systems for days to weeks, and also in the faulty sewage system of an apartment in Hong Kong.[8,9] The complications of SARS-CoV-2 transmission in water and wastewater environment may have become worrisome due to the high possibility of asymptomatic patients and high viral-shedders[10] who may contaminate the environment easily and spill over the transmission to other people, health care personnel, and front-line wastewater treatment plant workers.[11] Other areas of concern are treatment facilities with inefficient treatment processes,[12–14] which may also threaten the public for possible exposure to the virus. For instance, Radazzo et al.[12] confirmed the presence of SARS-CoV-2 RNA in 11% of the secondary-treated water samples investigated. Similarly, Zhang et al.[13] investigated the presence of SARS-CoV-2 in medical wastewater in septic tanks of a hospital in China, where it was revealed that the high load of the virus might be another secondary source of sporadic spread of COVID-19.

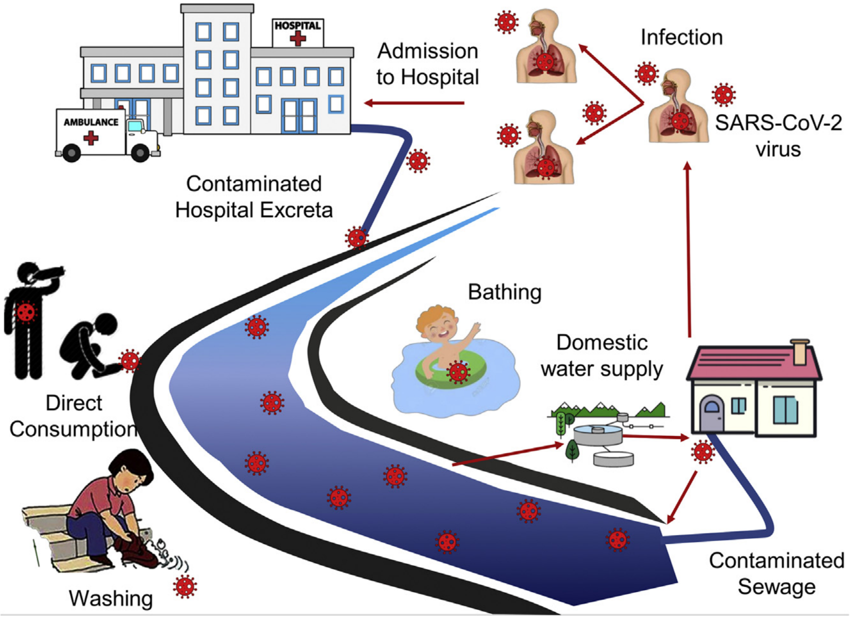

Figure 4.1 The pathways of SARS-CoV-2 in wastewater and water systems. (Adapted from Adelodun et al.[7]).

While the presence of SARS-CoV-2 in wastewater have been studied and confirmed,[15–18] only a few studies have so far investigated its persistence in the wastewater environment,[19] thereby creating an important information gap on the provision of sustainable approaches to mitigate the spread of the virus through this medium. Moreover, the previous knowledge on the virus removal methods, deactivation/inactivation, and treatment[20–22] can be further explored targeting the novel SARS-CoV-2. Therefore, it is important to review recent advances in wastewater treatment and the protection of wastewater plant personnel in the face of the pandemic and proffer effective techniques.

4.2 Occurrence, detection, and persistence of SARS-CoV-2 in wastewater, feces, slurry, or biosolids

4.2.1 Occurrence and detection of coronaviruses in wastewater

Viruses in wastewater have been studied extensively for nonenveloped enteric viruses, such as adenoviruses, polioviruses, enteroviruses, rotaviruses, and noroviruses, primarily due to their fecal-oral transmission routes. With the recent development on the detection of coronaviruses (both SARS-CoV and SARS-CoV-2) in wastewater, there is a need for a replica of the previous efforts on the studies of nonenveloped viruses for the enveloped ones.[23] Due to the report on the isolation of viable SARS-CoV-2 in the feces of infected patients and possible transmission through secondary routes,[10,24,25] the concern and awareness in the persistence of coronaviruses in wastewater has increased, leading to the adoption of the wastewater-based epidemiology (WBE) as a concept for wastewater analysis to serve as a caution and projection for a possible disease epidemic.[17,23,24,26] Fig. 4.2 shows the schematic flow of detection and quantification of SARS-CoV-2 from wastewater sources.

WBE is a new tool for the environmental engineers and researchers to harness the possibility of contaminants (including viruses) surviving in a relatively stable environment and subsequently released in the sewage/wastewater system by sampling and quantifying to get real-time information in early detection of viral outbreaks and preventing such occurrence via a well-defined inactivation and removal techniques.[2,27] The methods often used for the detection after sampling are the variants of polymerase chain reaction (PCR); reverse transcription-polymerase chain reaction (RT-PCR), and reverse transcription real-time polymerase chain

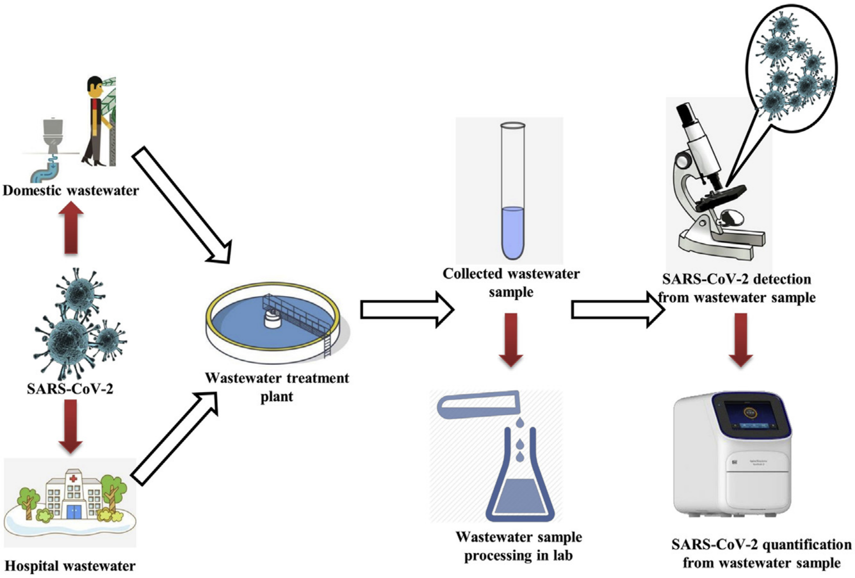

Figure 4.2 Schematic flow of detection and quantification of SARS-CoV-2 from wastewater sources.

reaction (RT-qPCR). Medema et al.[19] suggested surveillance for the sewage system as a sensitive tool to closely monitor for the circulation of the virus in the community. The authors studied the presence of SARS-CoV-2 in sewage samplings in six separate cities and an airport in the Netherlands. The confirmation of the genetic material of SARS-CoV-2 in the sampled sewage six days before the officially reported first COVID-19 cases, and the subsequent detection in the collected sewage samples after the announcement, even when the incidence is still low, signifies the importance of the WBE tool, especially for the monitoring of the municipal wastewater/sewage system including the ones at the airports, ports, and land-border facilities.

Municipal wastewater/sewage systems can be a hotspot for diseases outbreak due to the high dependence and usage by the community and, as such, can be monitored for viral outbreak containment. Therefore, wastewater-based epidemiological studies are important in this regard while improving the initial treatment techniques such as nanofiber filters as wastewater pretreatment techniques and upgrading the existing ones to have an efficient surveillance tool.[28] The limitation of clinical diagnostics in terms of the population coverage makes WBE an important tool for identifying disease hotspots and monitoring a campaign on the extent and duration of the spread for the population in a specific location.[28] Wastewater-based epidemiology can be properly used in areas where patients are asymptomatic, which may not immediately be known via clinical surveillance and possible underdiagnoses. This limitation is of growing concern because of retesting a large number of infected persons to ascertain valid results.[29]

Further, there are instances where the clinical diagnostic methods cannot detect viruses at low levels of prevalence due to a low level of viral loads/concentrations below 100 copies/μL (>40 Ct) among the infected individuals.[12,30,31] Hence, wastewater surveillance could adequately be employed to fill this important gap. The WBE is considered effective during the introduction of a new virus in the community or when there are seasonal variations and the survivability of such viruses is to be determined.[29] WBE is a tool that needs extensive research so that different regions can have an idea of the persistence, occurrence, and survival of different pathogenic viruses in hospital wastewater and rural waterways, as a means of signaling early detection to help in combating future outbreaks.[3]

Sharif et al.[6] investigated the existence of SARS-CoV-2 in 78 wastewater samples from 38 districts across Pakistan using the existing polio environment surveillance sites. The samples of SARS-CoV-2 infected areas and a drainage system at a COVID-19 quarantine center were analyzed using RT-qPCR, with 27% of the samples from 13 districts reporting positive. It was suggested that since the studies were able to replicate the surveillance network of polio sites, the potential for WBE is promising and may be necessary for situations where early detection is vital. At the same time, door-to-door contact tracing may prove difficult and strenuous, especially in densely populated areas.[6]

4.2.2 Methods for SARS-CoV-2 detection in wastewater

The urgency and dynamics of actions in responding to the challenges of containment, assessment, and risk mitigation of SARS-CoV-2 have revealed the shortcomings, which may need to be addressed in earnest, especially when there are emerging indications of the presence of the virus in water and wastewater environments, posing a serious threat to wastewater/sewage treatment workers and the general public.[32] In the preprint by Singer and Wray,[33] a detailed review of recent works showed that there are reports of SARS-CoV and SARS-CoV-2 presence in stool and urine samples with both virus detections carried out using either or both of

RT-PCR and real-time reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR). Other methods of SARS-CoV-2 detection available are categorized based on diagnostic tests such as nucleic acid detection and amplification (examples are PCR, RT-PCR, and qPCR); isothermal amplification technologies (examples are NASBA, LAMP, HAD, RCA, NEAR, SDA, and TMA); immunoassays (examples fluorescent antibody staining, EIA/ELISA); deoxyribonucleic acid (DNA) sequencing (such as sanger sequencers, next-generation sequencers, and DNA microarrays); mass spectrometric methods (MALDI-TOF); direct visualization of viruses (electron microscopy), and microelectronics and microfluidics-based techniques (such as lab-on-a-chip [LOC], point of care [POC] testing, and surface plasmon resonance [SPR] technique).[34] In this section, the RT-PCR and its variants will be discussed considering their widely used quick method for virus detection in water and wastewater, compared to other methods listed due to their disadvantages in the pilot setup stage, scalability for mass production, and cost implications of laboratory setup, reagents, highly trained and qualified personnel, and time.

4.2.2.1 Virus detection in wastewater using RT-PCR/RT-qPCR

The WHO technical brief statement on March 3, 2020 read,

> *[T]he presence of the virus (SARS-CoV-2) in drinking water is a possibility; however, there is no evidence as at the time of the brief, that surrogate human SARS-CoV-2 are present in surface or underground water routes or transmitted via contaminated drinking water; the statement went further to state that the virus has not been detected in the mentioned water supplies and the risk is thereby low.*[35]

The statement has many pointers that need further critical analysis to not misguide the nonscientist reader because "there is no current evidence" may imply that research is still ongoing in this area (water and wastewater). The low-risk claim is as a result of little or no evidence at the moment and may tend to become a high-risk source depending on the action taken over time. Lastly, the similarity between SARS-CoV and SARS-CoV-2 mean guards should not be let down because a secondary source of SARS-CoV was via an inadequate sewage system.[9] Therefore, researchers are vigorously working to understand the possible persistence of SARS-CoV-2 in water and wastewater, and devising some robust mitigation approaches to avoid its widespread.[7]

Recently, there have been different methods of SARS-CoV-2 detection, such as two-phase (PEG-dextran method) separation and aluminum hydroxide adsorption-precipitation method; unfortunately, these methods do not report the percentage recovery.[18,36] The investigation on the presence of SARS-CoV-2 in wastewater was carried out by Sherchan et al.[18] using two concentration methods. The SARS-CoV-2 detection in wastewater was by RT-qPCR with mean recovery efficiency of about 73% using a *Pseudomonas* bacteriophage as a control. Subsequently, the treated wastewater tested negative, thereby indicating the effectiveness of both the detection and treatment techniques. Westhaus et al.[15] carried out the detection of SARS-CoV-2 in wastewater treatment plants (WWTP) using RT-qPCR methods. The specificity and sensitivity were verified by comparing the different viral genes (N-gene, E-gene, M-gene, and RdRP) with three different sewage samples. The performance of RT-qPCR was notably good with the RdRP gene, and other false-positive results were not considered with suggestions for proper RT-qPCR sequencing or design of other suitable methods.[15] The efficiency and sensitivity of RT-qPCR were tested using three different assays, tagged as N1, N2, and N3 for several water samples collected before (October 2019) and after the emergence of 2019-nCoV (within two months).[12] The different inconsistencies were observed in the results, with few samples showing positive results, while others indicated false-positive results. It was concluded that while considering WBE for the emergence of SARS-CoV-2 in water and wastewater, digital RT-qPCR should be considered despite its cost implications.[12]

4.2.3 Persistence of coronaviruses in wastewater

There are several factors that may affect the survival of SARS-CoV-2 in wastewater, and these factors may be intrinsic or extrinsic depending on the environmental conditions. These factors include viral structure, the composition of the wastewater, pH, and temperature.

4.2.3.1 Composition of wastewater

The composition of wastewater is vital to the survival of the viruses in the medium and the level of their reduction.[37] The log reduction level of the viruses in the wastewater depends on the composition of such wastewater. For instance, in a reagent grade water, the 4 $\log_{10}$ (99.99%) reduction of transmissible gastroenteritis virus (TGEV) was achieved in 44 days, while it took 35 days at 25°C for murine hepatitis virus (MHV) to get inactivated under the same inactivation/reduction conditions.[23] Polo et al.[38] opined that the composition of the water, including other microorganisms, can influence the persistence of enveloped viruses in wastewater compared to nonenveloped viruses, thus affecting the virus recovery, inactivity assay results, underestimated infectivity rate, and concentration techniques.

4.2.3.2 pH

Extensive research is needed to investigate varying pH levels to determine the survivability of viruses in wastewater. A recent study that made a comparison between enveloped and nonenveloped viruses found that the stability conditions for MHV was within the pH ranges of 5—7.4 and 3—10 at temperatures of 37 and 4°C, respectively, which are regarded as the most stable conditions that depend on the removal, inactivation, and survival.[23] The stability and persistence of SARS-CoV-2 raised more concerns with the different levels of pH. For example, Tran et al.[39] observed that SARS-CoV-2 was stable at a pH range of 3.0—10 under room temperature, while that of SARS-CoV survived for one day at pH of 8.0, 5 days at pH of 9.0, and only for 3 h at pH of 6.0. Similarly, Chin et al.[40] confirmed that SAR-CoV-2 was stable at pH values of 3—10 at room temperature, indicating the importance of pH as an environmental factor in the survivability of the virus in different environmental conditions, including wastewater.

4.2.3.3 Temperature

Temperature is another important factor that largely determines the survivability of viruses in wastewater. Coronaviruses often get inactivated at a certain temperature (for instance, 20°C) and not at low temperatures (i.e., 4°C and below).[39] It was also reported that SARS-CoV survived in different wastewater samples, such as hospital wastewater, dechlorinated tap water, and domestic sewage for 14 days at 4°C, while the survival was just for two days at 20°C.[41,42] It is safe to say that virus survival will be longer in the colder regions than the tropical regions, which can be ascribed to the protein and nucleic acid denaturation and the upsurge in extracellular enzyme activities.[23]

4.3 Removal of viruses from water and wastewater environment

The consumption of virus-contaminated water can lead to various forms of acute illnesses.[43] According to WHO and UNICEF,[44] about 1.8 billion of the global population consumed contaminated water with pathogens in 2012, leading to gastroenteritis-related diseases.[45,46] The reclamation of water from wastewater for reuse has increased recently due to incessant water scarcity and population surge, especially in developing countries.[47] This has led to an increase in waterborne-related diseases due to poor sanitation and hygiene coupled with a lack of persuasive techniques in treating water and wastewater before usage.[48–50] Therefore, it is important to fashion out some effective treatment methods for the removal of viruses for advanced treatment, which will be made available for reuse for potable water and other agricultural use such as irrigation and food processing.[51]

Having ascertained the presence of SARS-CoV-2 in water and wastewater via several detection methods,[12,16,18] it is imperative to research wastewater treatment methods that are cost-effective and efficient to dispel the fear of the virus spread through secondary transmissions.[45] Technically, virus removal is different from virus inactivation since virus removal may involve some specific steps to concentrate/coagulate the viruses before the disinfection mechanism. However, virus disinfection or inactivation involves applying advanced methods to inactivate the virus before and/or after virus removal. Traditional wastewater treatment methods are primarily aimed at the removal of biodegradable organics and suspended solids,[49] which comprise of physical, biological, and chemical processes such as activated sludge process, filtration (including the use of sand filters), and membranes (nanomaterials, ultrafiltration, microfiltration, membrane bioreactor, ceramic membranes sedimentation, and reverse osmosis).[27,45,50]

Due to the lethargic nature of viruses to humans, there are guidelines sets for handling viruses (especially SARS-CoV-2); these give precautions needed to be adhered to for scientific testing to be carried out. The guidelines for Biosafety and COVID-19 by the Center for Disease Control and Prevention (CDC) for environment specimen testing include "[p]rocedures that concentrate viruses, such as precipitation or membrane filtration can be performed in a BSL-2 laboratory with unidirectional airflow and BSL-3 precautions."[27,52] Due to this, surrogates are used in place of dangerous pathogens and depend on the type of experiment to be carried out. The possible surrogates for SARS-CoV-2 are murine hepatitis virus (MHV), TGEV, feline infectious peritonitis virus (FIPV), bacteriophage φ6, and pepper mild mottle virus (PMMoV).[27]

The use of MHV was considered in four different environments to represent cold, tropical, subtropical, and temperate latitudes using the common RT-qPCR in untreated wastewater, autoclave wastewater, and dechlorinated tap water in order to investigate the survival of both

SARS-CoV-2 and MHV in these environments.[53] It was posited that the two viruses showed similar persistence in the selected environments. The findings indicated that at 37°C, the detection of SARS-CoV-2 RNA was not affected; however, the persistence of the virus was prolonged at 4 and 15°C. The difference in the decay rate between MHV and SARS-CoV-2 was observed and may be due to the gamma-irradiated SARS-CoV-2 used in the study. Conclusively, MHV indicated a viable surrogate for SARS-CoV-2 usage based on the CDC guidelines, with the inability to use viable SARS-CoV-2 considering the required high level of biological safety measures (BSL-3) that must be in place before handling infectious viruses.[53] Mohan et al.[24] also reported the use of bacteriophage φ6 as a surrogate for influenza and coronaviruses, where the inactivation varied based on factors such as temperature, aqueous media composition, and biological activity. It was further explained that both SARS-CoV and SARS-CoV-2 are affected by temperature compared to Poliovirus 1 LSc-2ab (PV-1), while having less survivability in the environment than nonenveloped viruses such as PV-1.

The question will always be on the readiness to test or determine the infection rate, humoral protection, herd immunity, and efficacy of vaccine (during and after clinical trials) using a readily available testing procedure that is faster, economical, and compact. During this pandemic, different challenges range from understanding the virus (infections, survival, replication, and persistence in different environments) to the search for economically viable testing methods and vaccine development. Therefore, researchers have investigated the use of a surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction.[54] In this study, it was revealed that the performance of the surrogate virus neutralization test (sVNT) is significantly high compared to the conventional virus neutralization test (cVNT) and pseudovirus neutralization test (pVNT). This implies that sVNT can be used rapidly in either research or clinical labs without using a live virus and biosafety environment, since it can easily detect total N-specific antibodies (NAbs) in an isotype-independent manner.[54] In addition to the previous position, the performance validation of sVNT was carried out using two cohorts of positive and negative sera from two countries, and the specificity and sensitivity were 100% and 98%, respectively.

Another cohort study was carried out to ascertain the performance level of sVNT with cVNT and pVNT. In this study, the specificity and selectivity was also very much superb, with the rapid screening ability of sVNT ascertained for larger samples. Further, it was posited that sVNT was precise, less complicated, economical, and faster, thereby making it more appropriate for use in a rapid and large number of samples.[55] It was concluded that sVNT at a standardized level will be vital for the selection of convalescent plasma donors for COVID-19 patients treatment, while the assay does not depend on anti-species antibodies. Therefore, it is suitable for use during preclinical testing of SARS-CoV-2 vaccines.[55]

Decision making such as lockdown, rapid testing, treatment, contact tracing, and vaccine developments depend on some conventional procedures, considering the spike in the infection rate of SARS-CoV-2 all around the world. Therefore, in a bid to provide timely information to assist in easy and fast decision making, the use of other surrogate testing procedures was investigated by Sharif et al.[6] The biochemical surrogate point-of-care tests (POCTs) were used to differentiate viral from a bacterial infection in patients with influenza-like illnesses. It was found that FebriDx (the surrogate POCTs) showed some positives for triage early detection, while large population testing and a UK-based studies (two precisely) indicated specificity and selectivity of 93% and 100%; and 86% and 100%, respectively.[6] However, the comprehensive assessment of the POCT field performance was not clearly defined by the authors.

4.4 Virus removal techniques from wastewater

This section is necessary for the SARS-CoV-2 studies and investigations since the knowledge of SARS-CoV-2 transmission routes, survival, and decay in wastewater will give an overview of the effective treatment (removal/inactivation) methods. Therefore, the following paragraph will explain the different removal techniques available and their effectiveness therein. Traditional wastewater treatment techniques are majorly used for organic matter and suspended solids removal.[49,50] However, during the process, it has been reported that some pathogens were also removed, making it considerable for use. Unfortunately, the reports are based on the effective removal of bacteria compared to viruses.[23]

4.4.1 Activated sludge treatment

In the wastewater treatment plant, the activated sludge is one of the most employed biological treatment technology,[56] which comprises multiple unit operations such as sedimentation (primary and secondary settlers), biological decomposition (including aerobic, anoxic, or anaerobic tanks or similar equipment) and followed by disinfection processes such as chlorination, UV irradiation, or ozonation. However, in the treatment plants where the wastewater is to be reused, either for agricultural or public consumptions, sand filters, membrane, and other tertiary treatment techniques may be used.[57] Having understood that the activated sludge treatment (AST) techniques are designed for other purposes such as removing suspended solids, nutrients, and organic substances, it was opined that since viruses are fine particles with colloidal features. It is possible to get absorbed on or within suspended particles in wastewater and further disinfected by other techniques or change of environments.[45,57] The activated sludge process has been widely adopted in most WWTPs. Randazzo et al.[12] employed six WWTPs with activated sludge in all treatment reclamation processes for wastewaters from public use, including irrigation. AST was also used in virus removal from wastewater in a subtropical environment. The removal rate recorded was 3 $\log_{10}$; however, the virus removed an enteric virus.[23]

Further, the use of MHV surrogates for human coronaviruses was investigated with a possible one-fourth of the MHV absorbed to the solids and about 99% at an increased retention time of 0.4−2.9 h at a primary settling stage until saturation is attained.[23] AST virus removal efficacy was put to the test in a study by Arraj et al.[58] where two types of bacteriophages and three types of enteric viruses were removed during sewage treatment. It was found very effective, especially with the enteric viruses, and could be tested for further studies on other types of viruses, especially SARS-CoV-2. This is a proven process that requires further intensive studies at different conditions while considering other factors that affect the persistence of the virus in water and wastewater.

4.4.2 Membrane bioreactor

A membrane bioreactor (MBR) is an example of a biological method of virus removal from wastewater that uses the cellular activity of the microorganisms for organic matter oxidation present in the wastewater. MBR is a combination of a filtration that solely membrane-based and a suspended growth biological reactor for virus removal from wastewater.[45] The major types of filtration used in MBR techniques are microfiltration and ultrafiltration with a size range of 0.1−0.2 μm and 0.005 ≈ 10 μm, respectively. The virus particles Log removal of more than 4 was earlier reported;[59] however, it was opined that the best MBR techniques for coronaviruses removal would be ultrafiltration since the average viral particle diameter and envelop diameter are 120 and 80 nm, respectively.[23]

Nanofiltration was also proposed as part of the processes of MBR techniques. The pore sizes of the membranes are usually less than 10 nm, which is smaller than any virus that may be present in wastewater. Generally, the use of MBR for surrogates may not be extensively applicable for human pathogenic viruses as the removal efficiency may differ, but it is a good pointer as a combination of different techniques will bring about the required efficiency.[27] In wastewater samples obtained for the SARS-CoV-2 detection from hospitals sewage, one of the samples obtained was pretreated using the following sequence of techniques: adjusting tanks → septic tank → adjusting tank → moving bed biofilm reactor (MBBR) → sedimentation → disinfection, while no virus was detected at the MBBR unit.[11]

4.5 Mechanism of inactivation of coronaviruses in water environment using disinfectants

Virus disinfection or inactivation is a general technique for preventing SARS-CoV-2 infection, transmission, and persistence in the environment. There are guidelines for the safe use of these disinfectants in wastewater treatment sources such as laboratories, hospitals, and homes.[60] Wastewater treatment is considered a means of reducing or complete expunging of dissolved and particulate organic matter, suspended solids, nutrients, and heavy metals; and the extent of the treatment is guided by the standards outlined by regulatory bodies such as WHO and local authorities. However, as for the current COVID-19 pandemic situation, there are no additional measures specially designed for the virus by WHO, US Centers for Disease Control and Prevention (CDC), or the Occupational Safety and Health Administration (OSHA).[61] However, it is vital to strictly adhere to the guiding techniques available for wastewater treatment, such as a well-designed and well-functional treatment plant, sufficient to curb the risk posed by fecal pathogens, such as SARS-CoV-2. Meanwhile, there are considerations for tertiary treatments, also known as disinfection procedures, to reduce the pathogen level further. The treatments include physical methods such as ionization by gamma-ray radiation, nonionizing radiation by ultraviolet light, photodynamic oxidation and heat, and chemical methods, which involve chlorine and chlorine dioxide, ozone,

Table 4.1 Comparison of inactivation methods for wastewater treatment.

Inactivation method	Advantage	Disadvantage
Liquid chlorine	Energy consumption is low.	High risk of storage.
UV light	Low costs of investment and operation.	High risks by operators and insufficient level of penetration.
Chlorine dioxide	Highly efficient and minimal operation costs.	Transport and storage risk are high.
Sodium hypochlorite	Less toxic, easy setup and operation and low cost.	Energy consumption is high and highly corrosive and high pollution.
Ozone	Excellent features of deodorizing, decoloring and decomposition of viruses.	Operation cost is high with high generation of harmful disinfection by-products (DBPs).

Source: Adapted from Ghernaout D, Elboughdiri N. Urgent proposals for disinfecting hospital wastewaters during COVID-19 pandemic. *OALib* 2020;**07**(05):1–18. https://doi.org/10.4236/oalib.1106373.

iodine, bromine, and bromine chloride.[61] The comparison of merits and demerits of selected inactivation methods for the virus in wastewater treatment is presented in Table 4.1.

4.5.1 Chlorination pretreatment

In chlorination pretreatment procedures, bleaching powder is mostly used in this regard in which the powder used should be emptied into the septic tanks alongside both the measured residual chlorine and water to ascertain the water quality.[60]

One of the most active disinfectants in the WWTPs is its high reactivity at low concentrations, comparatively low cost, and little residue formation at moderate doses. Chlorine can be used in the gaseous state (which is the most widely used) or hypochlorite. At the gaseous state application, the gas immediately reacts with water to form hypochlorous acid and hydrochloric acid; and the former, which is the primary form of active chlorine for the disinfecting features, subsequently dissociates to produce hypochlorite ion:

$$Cl_2 + H_2O \rightarrow HOCl + HCl$$

$HOCL \rightleftarrows H^+ + OC^-$ Source: Lahrich et al.[61]

The application of chlorine in virus disinfection was demonstrated by Wang et al.,[42] where SARS-CoV in low-level wastewater was inactivated using chlorine with 0.5 mg/L as free chlorine residual. However, a standard dosage was advised to reduce the rate of disinfection byproduct (DBP). The use of chlorine was advised in locations with high population density, especially if it is liquid chlorine, which possesses a high risk as regards storage and other concerns raised with its usages such as the use of chlorinator and corrosion-resistance materials (copper pipes and hard PVC pipes).[14]

4.5.2 Chlorine dioxide

Chlorine dioxide can efficiently be used as a disinfectant at the acidic level due to its high oxidization capacity. It has a solubility, which is 500% more than chlorine, and an oxidization capacity of 2.63 times compared to chlorine gas.[14] Chlorine dioxide tends to destroy protein's anabolic pathways and exterminate microorganisms including bacteria, fungi, spores, and viruses. It has the wonderful features of decolorization, oxidation, deodorization, and increasing oxygen content in wastewater; however, the storage risk often outweighs numerous advantages.[14]

4.5.3 Sodium hypochlorite

Sodium hypochlorite is more useful in disinfections of different wastewater categories, such as hospital wastewater and municipal wastewater. The ready availability of the material is due to its numerous advantages such as low level of toxicity, steady and less-tedious mode of operation, ease of control, less-tedious operation, and inadequate preparation and operation costs. However, it has some demerits such as higher pollution in terms of DBPs, high energy, and strong corrosiveness.[14] The use of sodium hypochlorite at the designed dosage may not wholly inactivate the viruses except when a high dosage is applied, bringing about more DBPs. It is unsuitable for disinfecting wastewater with amino acids because it can react to produce cyanogen chlorides and dissolved nitrogen substances in wastewater. This can also produce organochloramines, which are all toxic and more stable.[62]

SARS-CoV-2 inactivation from hospital wastewater was investigated using sodium hypochlorite at the prescribed dosage level by WHO and China CDC.[13] It was found that complete inactivation was not achieved at this set of guidelines. In fact, the viruses were embedded in the suspended solid; hence, a higher dosage was used for complete inactivation, causing more concerns for the DBPs. At 800 g/m^3, the free chlorine declines and paved the way for the SARS-CoV-2 viruses attached to the stools in the organic matter to be released slowly.[13] More research is needed by collecting more wastewater samples to have a range of dosage necessary for complete inactivation and reduce the DBPs to avert the release of more toxicity of solid substances into the environment.

4.5.4 Ozone

Disinfection using ozone has spanned many applications in water and wastewater treatment studies, particularly in developed countries. The ozonation procedures (15—20 mg/L for 10—15 min) come after double-staged sedimentation and purification procedures before discharge.[60] Ozone has a competing application with chlorine because of its highly effective inactivation properties. It has a disinfection efficiency, using the concept of log inactivation levels (or log reduction values) between 0.56 for an LRV of 1—1.32 for an LRV of 1.32, indicating a complete virus inactivation to a 4-log at a CT of 1.0 mg/min. However, the International Ozone Association (IOA) has stated its position on the use of ozone for SARS-CoV-2 inactivation.[27] Ozone has been reported to have an oxidation-potential of 2.07 V and often leads to the formation of secondary oxidants (also called hydroxyl radicals). This makes ozone have a high reactivity and can inactivate viruses in a shorter time of reaction. Despite the corrosive/active nature and costs, ozone has the vulnerability of virus inactivation selection.[24] Extensive research on the use of ozone for specific inactivation of SARS-CoV-2 should be considered, which will help in a future pandemic or emerging viruses. Table 4.2 shows the respective treatment for ozone for different viruses. From the table, only one inactivation was done for SARS-CoV-2 with doses of about 10—20 ppm, the contact time of 10—15 min, and the inactivation rate at 3.5 $\log_{10}$. However, the exposure limit of ozone to humans is 0.05 ppm for 8 h and should be closely monitored during application.

4.5.5 Ultraviolet irradiation

Ultraviolet light (UV) is an electromagnetic wave with a length between 200 and 400 nm. The first UV application dated back to 1910 in the disinfection of drinking water.[14] Ultraviolet light can be divided into four types: ultraviolet A, UV-A (320—400 nm; 315—400 nm), ultraviolet B, UV-B (280—320 nm; 280—315 nm), ultraviolet C, UV-C (200—280 nm), and vacuum ultraviolet (100—200 nm). However, the use of vacuum ultraviolet as a disinfectant is limited due to its absorption by the wastewater.[14,62]

Table 4.2 Viruses and their respective treatment with ozone.

Viruses	Ozone	Time	Medium	Log_{10} reduction
Murine coronavirus	10	10	Air (90%)	>3
SARS-CoV-2	15–20	10–15	Air	3
HSV	10	15	Stainless steel	4
Influenza virus	10	10	Glass	4
Rotavirus	10	10	Plastic	4
Hepatitis A virus	0.3–0.4	0.08	Water	3.9
Norovirus	7	15	Wastewater	5

Source: From Quevedo-León R, Bastías-Montes JM, Espinoza-Tellez T, Ronceros B, Balic I, Muñoz O. Inactivation of Coronaviruses in food industry: the use of inorganic and organic disinfectants, ozone, and UV radiation. *Sci Agropecu* 2020;**11**(2):257–266. https://doi.org/10.17268/SCI.AGROPECU.2020.02.14.

Ghernaout and Elboughdiri[60] posited that the wavelength range value of 200–300 nm is enough to disinfect or destroy the DNA and ribonucleic acid (RNA) structure of bacteria, viruses, and single-celled microorganisms, which can thereby block the synthesis of protein. The inactivation of coronaviruses was reviewed for the food industry using UV radiation by Quevedo-León et al.[62] It was reported that for effective inactivation of the viruses using UV-A and UV-B, the exposure time needs to be extended, while the types of microorganisms affecting the effectiveness of UV-C and the exposure limit to UV-C by a human should be maintained at 2.5 J/m^2 in 10–15 min; otherwise, there is a risk of erythema (the redness of the skin or mucous membranes). Exposure to UV-A is safe at a maximum level of 10 W m^2 for 8 h, at the eye level.

Simmons et al.[63] studied the deactivation of SARS-CoV-2 on surfaces (environmental surfaces and personal protective equipment) using a pulsed-xenon UV light. The authors obtained remarkable success in the disinfection rate for hard surfaces at 1, 2, and 5 min with 3.53 $\log_{10}$, >4.54 $\log_{10}$, and >4.12 $\log_{10}$ viral load reduction, respectively, and a viral load reduction of >4.79 $\log_{10}$ in 5 min for N95 respirators. Therefore, it was concluded that using a pulsed-xenon UV light, SARS-CoV-2 was significantly reduced, and this can be replicated on other mediums where the virus may persist.

4.6 Measures to ensure the protection of personnel and wastewater treatment workers from contacting COVID-19

4.6.1 Viral contamination risk assessment

In the aspect of viral risk assessment for viruses or majorly pathogens, the quantitative microbial risk assessment (QMRA), which has dated back to over 35 years for its usefulness in determining and developing measures for safeguarding public health in line with water, food, remediation, and so forth, is employed.[64] The data obtained from exposure assessment and dose-response assessment are initially used and are obtained from viability assays, including plate counts, plaque assays, or animal infectivity. Meanwhile, with increases in the use of molecular techniques for measuring microorganisms in the environment (food, water, soil, and air), it is now pertinent to investigate how to apply such data to estimate infectious disease risks. However, the limitations to the application of these data need redress.[64]

In a study aimed at estimating the viral risk assessment of SARS-CoV-2 (the current pandemic) using the QMRA, it was found that, while using data from the literature, SARS-CoV and SARS-CoV-2 have different exposure scenarios framework.[65] The dose-response model of a surrogate coronavirus was useful as an early health warning tool for the current and future pandemic.[65] While considering the extreme cases, a three-tiered method showed an estimated risk of infection on workers higher than the derived acceptable infection risk of SARS-CoV-2,[66] indicating the urgent need for hands-on emergency response. Since the pandemic could affect the activities in WWTPs, the population that solely depends on the WWTP facility is at varying levels of risk, depending on the level of their exposure to the contaminated wastewater. It is crucial to use essential tools like QMRA to establish risk management strategies, particularly to WWTP personnel's exposure as the risk level may be higher than anticipated.

A perfect example was the laxity in the 2003 outbreak of SARS-CoV, in which the role of wastewater or sewage systems aided the sporadic spread of the virus as a secondary source, which was never anticipated.[9] However, it occurred due to a faulty sewage system but resulted in full community transmission.[23] Exposure to untreated wastewater from the source poses a higher risk to WWTP workers and inhabitants living close to the sewage system line and is of great danger to the general public. The worst could have been imagined in a situation where there are inadequate WWTPs facilities, especially in developing countries where the majority depends on unclean water to drink, cook, and other household usages.[5,23]

4.6.2 Measures to protect wastewater treatment workers

Using the QMRA, it is now possible to safeguard public health and reduce contamination via proper surveillance, detection methods, analysis, and decision making, while also concentrating on the effective molecular methods for discovery, analysis, and policymaking.[66] Using QMRA involves five major steps that help predict the burden associated with waterborne diseases, risk reduction to the community, and measures to protect water safety (users, climate, aquatic animals, etc.). The steps include hazard identification: identification and quality assessment of the microbial hazard; exposure assessment: the estimation of the time of human exposures to the diseases by laid down routes; dose-response assessment: the classification of the connection between dose and incidence of consequential effect in the population unprotected to the pathogens; risk characterization: the incorporation of information from the identification of hazard (here it is the combination of dose-response and exposure assessment that gives the extent of health effects); and risk management and communication: the decision-making section based on all recent steps in the assessment of risks.[66]

Part of the measures to ensure that health and WWTP workers are properly protected against contracting the virus is to improve on the WBE and QMRA tools so that the viruses in wastewater may have been anticipated. The provision of easy to use testing kit that will be available at the WWTPs is essential. Advanced training of personnel on how to treat sewage/wastewater, either contaminated or not, while adequate provisions of PPEs that are disposable and out of reach, i.e., with proper disposal means, should be ensured. Lastly, the sorting of the waste type via proper tagging of the waste disposal points, which will help in proffering the best sewage treatment and disposal methods, should be implemented.

4.7 Conclusion

The presence of coronaviruses like SARS-CoV and the new SARS-CoV-2 (COVID-19) in wastewater has been ascertained via selected molecular testing methods and techniques, including PCR and its variant methods. However, the current knowledge on the persistence of the SARS-CoV-2 in the wastewater environment is not sufficient to make any concluding remarks. Meanwhile, since the research on the viability and virulence of the SARS-CoV-2 is still evolving, further development of important available tools/methods such as WBE and QMRA targeting the SARS-CoV-2 and related emerging disease outbreaks is inevitable to complement the existing molecular methods. These tools would ensure adequate surveillance and monitoring of the prevalence of the SARS-CoV-2 within the population, while also assessing the possible associated risk with the persistence of the virus in wastewater.

While the development of vaccines to combat the COVID-19 is ongoing, sustainable remedial approaches that can be employed to inactivate the virus in wastewater and water environment is essential to forestall probable spread through this medium. For the WWTPs workers that maybe the line of contact with the wastewater, their safety is paramount, and it is important to train them on waste handling management that involves the waste collection, waste sorting, waste treatment, and proper disposal of any resulting DBPs while they ensure strict compliance on the rules of engagement in order to prevent against viral infections of water-borne diseases.

References

1. *WHO. COVID-19 public health emergency of international concern (PHEIC) global research and innovation forum*. World Health Organization; 2020. Published, www.who.int/publications/m/item/covid-19-public-health-emergency-of-international-concern-(pheic)-global-research-and-innovation-forum. [Accessed 10 October 2020].
2. Foladori P, Cutrupi F, Segata N, et al. SARS-CoV-2 from faeces to wastewater treatment: what do we know? A review. *Sci Total Environ* 2020;**743**:140444. https://doi.org/10.1016/j.scitotenv.2020.140444.
3. Mandal P, Gupta AK, Dubey BK. A review on presence, survival, disinfection/removal methods of coronavirus in wastewater and progress of wastewater-based epidemiology. *J Environ Chem Eng* 2020;**8**(5):104317. https://doi.org/10.1016/j.jece.2020.104317.
4. Guerrero-Latorre L, Ballesteros I, Villacrés-Granda I, Granda MG, Freire-Paspuel B, Ríos-Touma B. SARS-CoV-2 in river water: implications in low sanitation countries. *Sci Total Environ* February 2020;**743**:140832. https://doi.org/10.1016/j.scitotenv.2020.140832.
5. Adelodun B, Ajibade FO, Ighalo JO, et al. Assessment of socioeconomic inequality based on virus-contaminated water usage in developing countries: a review. *Environ Res* 2021;**192**:110309. https://doi.org/10.1016/j.envres.2020.110309 (September 2020).
6. Sharif S, Ikram A, Khurshid A, et al. Detection of SARS-Coronavirus-2 in wastewater, using the existing environmental surveillance network: an epidemiological gateway to an early warning for COVID-19 in communities. *medRxiv* 2020. https://doi.org/10.1101/2020.06.03.20121426.
7. Adelodun B, Ajibade FO, Ibrahim RG, Bakare HO, Choi K-S. Snowballing transmission of COVID-19 (SARS-CoV-2) through wastewater: any sustainable preventive measures to curtail the scourge in low-income countries? *Sci Total Environ* 2020;**742**:140680. https://doi.org/10.1016/j.scitotenv.2020.140680.
8. Hui DSC. Severe acute respiratory syndrome (SARS): epidemiology and clinical features. *Postgrad Med* 2004;**80**(945):373–81. https://doi.org/10.1136/pgmj.2004.020263.
9. McKinney KR, Gong YY, Thomas G. Lewis. Environmental transmission of SARS at Amoy Gardens. *J Environ Health* 2006;**68**(9):26–30. quiz 51.
10. Wu Y, Guo C, Tang L, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol* 2020;**5**(5):434–5. https://doi.org/10.1016/S2468-1253(20)30083-2.
11. Zhang D, Yang Y, Huang X, et al. SARS-CoV-2 spillover into hospital outdoor environments. *medRxiv* 2020;**86**(0). 2020.05.12.20097105.

12. Randazzo W, Truchado P, Cuevas-Ferrando E, Simón P, Allende A, Sánchez G. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. *Water Res* 2020;**181**:115942. https://doi.org/10.1016/j.watres.2020.115942.
13. Zhang D, Ling H, Huang X, et al. Potential spreading risks and disinfection challenges of medical wastewater by the presence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viral RNA in septic tanks of Fangcang Hospital. *Sci Total Environ* 2020;**741**:140445. https://doi.org/10.1016/j.scitotenv.2020.140445.
14. Wang J, Shen J, Ye D, et al. Disinfection technology of hospital wastes and wastewater: suggestions for disinfection strategy during coronavirus Disease 2019 (COVID-19) pandemic in China. *Environ Pollut* 2020;**262**(114665). https://doi.org/10.1016/j.envpol.2020.114665. In press.
15. Westhaus S, Weber FA, Schiwy S, et al. Detection of SARS-CoV-2 in raw and treated wastewater in Germany – suitability for COVID-19 surveillance and potential transmission risks. *Sci Total Environ* 2021;**751**:141750. https://doi.org/10.1016/j.scitotenv.2020.141750.
16. Medema G, Heijnen L, Elsinga G, Italiaander R, Brouwer A. Presence of SARS-coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in The Netherlands. *Environ Sci Technol Lett* 2020. https://doi.org/10.1021/acs.estlett.0c00357.
17. Ahmed W, Angel N, Edson J, et al. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. *Sci Total Environ* 2020;**728**:138764. https://doi.org/10.1016/j.scitotenv.2020.138764.
18. Sherchan SP, Shahin S, Ward LM, et al. First detection of SARS-CoV-2 RNA in wastewater in North America: a study in Louisiana, USA. *Sci Total Environ* 2020;**743**:140621. https://doi.org/10.1016/j.scitotenv.2020.140621.
19. Bivins A, Greaves J, Fischer R, et al. Persistence of SARS-CoV-2 in water and wastewater. *Environ Sci Technol Lett* 2020. https://doi.org/10.1021/acs.estlett.0c00730.
20. Girones R, Carratalà A, Calgua B, Calvo M, Rodriguez-Manzano J, Emerson S. Chlorine inactivation of hepatitis e virus and human adenovirus 2 in water. *J Water Health* 2014;**12**(3):436–42. https://doi.org/10.2166/wh.2014.027.
21. Shimabuku QL, Arakawa FS, Fernandes Silva M, et al. Water treatment with exceptional virus inactivation using activated carbon modified with silver (Ag) and copper oxide (CuO) nanoparticles. *Environ Technol* 2017;**38**(16):2058–69. https://doi.org/10.1080/09593330.2016.1245361.
22. Van der Laan H, van Halem D, Smeets PWMH, et al. Bacteria and virus removal effectiveness of ceramic pot filters with different silver applications in a long term experiment. *Water Res* 2014;**51**:47–54. https://doi.org/10.1016/j.watres.2013.11.010.
23. Amoah ID, Kumari S, Bux F. Coronaviruses in wastewater processes: source, fate and potential risks. *Environ Int* July 2020;**143**:105962. https://doi.org/10.1016/j.envint.2020.105962.
24. Mohan SV, Hemalatha M, Kopperi H, Ranjith I, Kumar AK. SARS-CoV-2 in environmental perspective: occurrence, persistence, surveillance, inactivation and challenges. *Chem Eng J* 2021;**405**:126893. https://doi.org/10.1016/j.cej.2020.126893 (September 2020).
25. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *J Am Med Assoc* March 2020:3–4. https://doi.org/10.1001/jama.2020.3786.
26. Sims N, Kasprzyk-Hordern B. Future perspectives of wastewater-based epidemiology: monitoring infectious disease spread and resistance to the community level. *Environ Int* February 2020;**139**:105689. https://doi.org/10.1016/j.envint.2020.105689.

27. Lesimple A, Jasim SY, Johnson DJ, Hilal N. The role of wastewater treatment plants as tools for SARS-CoV-2 early detection and removal. *J Water Process Eng* July 2020;**38**:101544. https://doi.org/10.1016/j.jwpe.2020.101544.
28. Venugopal A, Ganesan H, Sudalaimuthu Raja SS, et al. Novel wastewater surveillance strategy for early detection of coronavirus disease 2019 hotspots. *Curr Opin Environ Sci Heal* 2020;**17**:8—13. https://doi.org/10.1016/j.coesh.2020.05.003.
29. Kitajima M, Ahmed W, Bibby K, et al. SARS-CoV-2 in wastewater: state of the knowledge and research needs. *Sci Total Environ* 2020;**739**:139076. https://doi.org/10.1016/j.scitotenv.2020.139076.
30. Jung Y, Park GS, Moon JH, et al. Comparative analysis of primer-probe sets for RT-qPCR of COVID-19 causative virus (SARS-CoV-2). *ACS Infect Dis* 2020;**6**(9):2513—23. https://doi.org/10.1021/acsinfecdis.0c00464.
31. Vogels CBF, Brito AF, Wyllie AL, et al. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT—qPCR primer—probe sets. *Nat Microbiol* 2020;**5**(10):1299—305. https://doi.org/10.1038/s41564-020-0761-6.
32. Barcelo D. An environmental and health perspective for COVID-19 outbreak: meteorology and air quality influence, sewage epidemiology indicator, hospitals disinfection, drug therapies and recommendations. *J Environ Chem Eng* 2020;**8**(4):104006. https://doi.org/10.1016/j.jece.2020.104006.
33. Singer AC, Wray R. Detection and survival of SARS-coronavirus in human stool, urine, wastewater and sludge. *Preprints* 2020:1—29. https://doi.org/10.20944/preprints202006.0216.v2.
34. Samson R, Navale GR, Dharne MS. Biosensors: frontiers in rapid detection of COVID-19. *3 Biotech* 2020;**10**(9):1—9. https://doi.org/10.1007/s13205-020-02369-0.
35. WHO. *Water, sanitation, hygiene and waste management for the COVID-19 virus: technical brief.* World Heal Organ; March 2020. p. 1—9.
36. Rusiñol M, Martínez-Puchol S, Forés E, Itarte M, Girones R, Bofill-Mas S. Concentration methods for the quantification of coronavirus and other potentially pandemic enveloped virus from wastewater. *Curr Opin Environ Sci Health* 2020;**17**:21—8. https://doi.org/10.1016/j.coesh.2020.08.002.
37. Gundy PM, Gerba CP, Pepper IL. Survival of coronaviruses in water and wastewater. *Food Environ Virol* 2009;**1**(1):10—4. https://doi.org/10.1007/s12560-008-9001-6.
38. Polo D, Quintela-Baluja M, Corbishley A, et al. Making waves: wastewater-based epidemiology for COVID-19 — approaches and challenges for surveillance and prediction. *Water Res* 2020;**186**:1—7. https://doi.org/10.1016/j.watres.2020.116404.
39. Tran HN, Le GT, Nguyen DT, et al. SARS-CoV-2 coronavirus in water and wastewater: a critical review about presence and concern. *Environ Res* 2020. https://doi.org/10.1016/j.envres.2020.110265.
40. Chin AWH, Chu JTS, Perera MRA, et al. Stability of SARS-CoV-2 in different environmental conditions. *The Lancet Microbe* 2020;**1**(1):e10. https://doi.org/10.1016/s2666-5247(20)30003-3.
41. Yeo C, Kaushal S, Yeo D. Enteric involvement of coronaviruses: is faecal—oral transmission of SARS-CoV-2 possible? *Lancet Gastroenterol Hepatol* 2020;**5**(4):335—7. https://doi.org/10.1016/S2468-1253(20)30048-0.
42. Wang XW, Li J, Guo T, et al. Concentration and detection of SARS coronavirus in sewage from Xiao Tang Shan Hospital and the 309th hospital of the Chinese People's Liberation Army. *Water Sci Technol* 2005. https://doi.org/10.2166/wst.2005.0266.
43. World Health Organisation (WHO). *Potable reuse: guidance for producing safe drinking-water. Geneva.* 2017. https://www.who.int/water_sanitation_health/publications/potable-reuse-guidelines/en/.
44. WHO and UNICEF. *Progress on sanitation and drinking water: 2015 update and MDG assessment.* 2015.

45. Bhatt A, Arora P, Prajapati SK. Occurrence, fates and potential treatment approaches for removal of viruses from wastewater: a review with emphasis on SARS-CoV-2. *J Environ Chem Eng* 2020;**8**(5):104429. https://doi.org/10.1016/j.jece.2020.104429.
46. Bosch A, Guix S, Sano D, Pintó RM. New tools for the study and direct surveillance of viral pathogens in water. *Curr Opin Biotechnol* 2008;**19**(3):295—301. https://doi.org/10.1016/j.copbio.2008.04.006.
47. Santos SD, Adams EA, Neville G, et al. Urban growth and water access in sub-Saharan Africa: progress, challenges, and emerging research directions. *Sci Total Environ* 2017:607—8. https://doi.org/10.1016/j.scitotenv.2017.06.157. 497—508.
48. Pooi CK, Ng HY. Review of low-cost point-of-use water treatment systems for developing communities. *npj Clean Water* 2018;**1**(1). https://doi.org/10.1038/s41545-018-0011-0.
49. Adelodun B, Ajibade FO, Ogunshina MS, Choi K-S. Dosage and settling time course optimization of *Moringa oleifera* in municipal wastewater treatment using response surface methodology. *Desalin Water Treat* 2019;**167**:45—56. https://doi.org/10.5004/dwt.2019.24616.
50. Adelodun B, Ogunshina MS, Ajibade FO, Abdulkadir TS, Bakare HO, Choi KS. Kinetic and prediction modeling studies of organic pollutants removal from municipal wastewater using *Moringa oleifera* biomass as a coagulant. *Water* 2020;**12**(7):2052. https://doi.org/10.3390/w12072052.
51. Morrison CM, Betancourt WQ, Quintanar DR, Lopez GU, Pepper IL, Gerba CP. Potential indicators of virus transport and removal during soil aquifer treatment of treated wastewater effluent. *Water Res* 2020;**177**:115812. https://doi.org/10.1016/j.watres.2020.115812.
52. CDC. Interim Laboratory. *Biosafety guidelines for handling and processing specimens associated with coronavirus disease 2019 (COVID-19).* Centers for Disease Control and Prevention; 2020. Published, https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html. [Accessed 9 October 2020].
53. Ahmed W, Bertsch PM, Bibby K, et al. Decay of SARS-CoV-2 and surrogate murine hepatitis virus RNA in untreated wastewater to inform application in wastewater-based epidemiology. *Environ Res* August 2020;**191**:110092. https://doi.org/10.1016/j.envres.2020.110092.
54. Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2—spike protein—protein interaction. *Nat Biotechnol* 2020;**38**(9):1073—8. https://doi.org/10.1038/s41587-020-0631-z.
55. Bošnjak B, Stein SC, Willenzon S, et al. Novel surrogate virus neutralization test reveals low serum neutralizing anti-SARS-CoV-2-S antibodies levels in mildly affected COVID-19 convalescents. *medRxiv* 2020. https://doi.org/10.1101/2020.07.12.20151407. 2020.07.12.20151407.
56. Gernaey KV, Sin G. *Wastewater treatment models*. Elsevier Inc.; 2013. https://doi.org/10.1016/b978-0-12-409548-9.00676-x.
57. Zhang CM, Xu LM, Xu PC, Wang XC. Elimination of viruses from domestic wastewater: requirements and technologies. *World J Microbiol Biotechnol* 2016;**32**(4):1—9. https://doi.org/10.1007/s11274-016-2018-3.
58. Arraj A, Bohatier J, Laveran H, Traore O. Comparison of bacteriophage and enteric virus removal in pilot scale activated sludge plants. *J Appl Microbiol* 2005;**98**(2):516—24. https://doi.org/10.1111/j.1365-2672.2004.02485.x.
59. Chaudhry RM, Nelson KL, Drewes JE. Mechanisms of pathogenic virus removal in a full-scale membrane bioreactor. *Environ Sci Technol* 2015;**49**(5):2815—22. https://doi.org/10.1021/es505332n.

60. Ghernaout D, Elboughdiri N. Urgent proposals for disinfecting hospital wastewaters during COVID-19 pandemic. *OALib* 2020;**07**(05):1−18. https://doi.org/10.4236/oalib.1106373.
61. Lahrich S, Laghrib F, Farahi A, Bakasse M, Saqrane S, El Mhammedi MA. Review on the contamination of wastewater by COVID-19 virus: impact and treatment. *Sci Total Environ* 2021;**751**:142325. https://doi.org/10.1016/j.scitotenv.2020.142325.
62. Quevedo-León R, Bastías-Montes JM, Espinoza-Tellez T, Ronceros B, Balic I, Muñoz O. Inactivation of Coronaviruses in food industry: the use of inorganic and organic disinfectants, ozone, and UV radiation. *Sci Agropecu* 2020;**11**(2):257−66. https://doi.org/10.17268/SCI.AGROPECU.2020.02.14.
63. Simmons S, Carrion R, Alfson K, et al. Deactivation of SARS-CoV-2 with pulsed xenon ultraviolet: implications for environmental COVID-19 control. *Infect Control Hosp Epidemiol* 2020:1−4. https://doi.org/10.1017/ice.2020.399.
64. Haas CN. Quantitative microbial risk assessment and molecular biology: paths to integration. *Environ Sci Technol* 2020;**54**(14):8539−46. https://doi.org/10.1021/acs.est.0c00664.
65. Zaneti RN, Girardi V, Spilki FR, et al. Quantitative microbial risk assessment of SARS-CoV-2 for workers in wastewater treatment plants. *Sci Total Environ* 2021;**754**:142163. https://doi.org/10.1016/j.scitotenv.2020.142163.
66. Ramírez-Castillo FY, Loera-Muro A, Jacques M, et al. Waterborne pathogens: detection methods and challenges. *Pathogens* 2015;**4**(2):307−34. https://doi.org/10.3390/pathogens4020307.