

# Prevalence, environmental fate, treatment strategies, and future challenges for wastewater contaminated with SARS-CoV-2

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## Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been detected in untreated and treated wastewater and studies have shown that the concentration of SARS-CoV-2 is proportional to the prevalence of the coronavirus disease 2019 (COVID-19) in communities. This article presents a literature review of the prevalence of SARS-CoV-2 in wastewater, its environmental fate, recommended treatment strategies for contaminated wastewater, and treatment challenges to be faced in the future. The environmental fate of SARS-CoV-2 in wastewater is not straightforward because it can be a source of infection when present in the treated wastewater depending on the permeability of the wastewater treatment plant containment area, and can also leach into aquifers, which may serve as drinking water supplies. Secondly, there are different practices that can mitigate the SARS-CoV-2 infection rate from infected feces and urine. The World Health Organization has recommended the use of ultraviolet radiation (UV), disinfection, and filtration for wastewater contaminated with SARS-CoV-2, processes also common in wastewater treatment facilities. This article discusses these strategies referencing studies performed with surrogate viruses and shows that SARS-CoV-2 treatment can be complicated due to the interference from other aqueous chemical and physical factors. Considering that COVID-19 is not the first and certainly not the last pandemic, it is imperative to develop an effective multitreatment strategy for wastewater contaminated with contagious viruses and, preferably, those that are compatible with current wastewater treatment methods.

## 1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of COVID-19 (coronavirus disease 2019), is genetically related to the other two coronaviruses, SARS-CoV and MERS-CoV (Middle Eastern Respiratory Syndrome coronavirus), the cause of the outbreaks in 2002 and 2012, respectively (Chang et al., 2020; Petersen et al., 2020; Wu et al., 2020). After entering the respiratory system via inhalation, SARS-CoV-2 binds to the angiotensin converting enzyme 2 (ACE2) receptor on epithelial cells in the nasal cavity. It then

propagates via the typical virus cycle and migrates to the respiratory tract where the innate immune system is triggered. The virus attaches to the alveolar type II cells in the lungs and these cells eventually undergo apoptosis, releasing self-replicating pulmonary toxins and viral particles that affect more type II cells and the infection cycle continues. The damage done to the alveolar cells in this viral infection cycle leads to severe scarring and fibrosis (Mason, 2020). The initial binding of the SARS-CoV-2 to the ACE2 receptor on the ciliated epithelial cells in the nasal cavity is the most widely reported method of entry for the SARS-CoV-2 and SARS-CoV.

However, Mason (2020) mentioned that this concept might need to be revised for SARS-CoV-2 because research has shown that there is no obvious cell type preference for this virus. SARS-CoV-2 has a similar transmissibility value to SARS-CoV, 2.5 versus 2.4, and as of the year 2020, lower percentage requiring hospitalization, 20% versus more than 70% for SARS-CoV, and overall lower case fatality, 2.3% versus 9.6% for SARS-CoV (Ceccarelli et al., 2020; Petersen et al., 2020). Even though the transmissibility values are similar, there is zero interval between symptom onset and maximum infectivity for SARS-CoV-2 compared to 5.7 days interval for SARS-CoV and so SARS-CoV-2 viral shedding begins a few days before symptom onset, a very different scenario than for SARS-CoV. The fatality rate can change as the virus mutates and, as the study by Davies et al. (2021) showed, there was 61% higher risk of death associated with one of the SARS-CoV-2 mutations, the B.1.1.7 variant.

The vast global spread of SARS-CoV-2 infections and the detection of new variants give rise to the concerns about the possibility of this coronavirus contaminating the environment and leading to health risks. SARS-CoV-2 viral RNA (the genetic material to replicate the virus) has been detected in the stool of infected patients. The detection of SARS-CoV-2 RNA depends on which nucleocapsid gene, N1, N2, or N3 gene, is targeted and the targeted gene affects the correlation with the frequency of hospitalization. When the N1 gene was targeted for the detection of SARS-CoV-2 RNA, the frequency of hospitalization due to COVID-19 was proportional to the concentration of this gene detected in untreated wastewater samples. However, this correlation was weaker for the N3 gene (Hong et al., 2021).

Foladori et al. (2020) presented a summary of several studies where stool samples tested positive for SARS-CoV-2 viral RNA for up to 84% of the infected patients. This includes the study by Xiao et al. (2020) for which 23% of the stool samples tested positive for SARS-CoV-2 viral RNA for infected patients in China after their respiratory tests were negative for the virus. In other studies, the stool of all the infected patients with diarrhea in Macau (Lo et al., 2020) and 16% of infected patients in Hong Kong (Cheung et al., 2020) tested positive for the SARS-CoV-2 viral RNA. Positive results in stools for the SARS-CoV-2 viral RNA was also found in 50% of patients in Singapore (Young et al., 2020) and also in the first case in the United States although this patient's serum and urine tested negative for the virus (Holshue et al., 2020). A positive result for SARS-CoV-2 viral RNA in the above mentioned studies indicates the presence of the virus in the stool can be grown. The sputum can have a higher content of the SARS-CoV-2 RNA than in the stool and a value of  $7 \times 10^6$  SARS-CoV-2 RNA per mL of sample for sputum samples was reported by Wölfel et al. (2020) for infected patients in Germany showing negative results for viral RNA in the stool. This is not surprising because COVID-19 infections usually start with inhalation of the SARS-CoV-2, as described earlier in this article. The respiratory secretions containing the virus in the upper respiratory tract can enter the digestive system where, due to gastric acidity in the stomach, the virus could be killed. However, the virus could also be

protected from this acidity when mixed with food or if the virus has resistance to low pH, in which case it could pass into the intestine, replicate there, and could be detected in the stool samples (Foladori et al., 2020).

Similar to SARS-CoV-2, MERS-CoV was found in the stool samples of infected patients (Corman et al., 2016) and can grow in the small intestine (Zhou et al., 2017). Like the MERS-CoV, the SARS-CoV and SARS-CoV-2 were not only found in stool samples but also in the liver (Chau et al., 2004; Ding et al., 2004; Gu et al., 2020; Mackay & Arden, 2015). SARS-CoV-2 also infects the colon cells due to the presence of the ACE2 receptors there (Ng & Tilg, 2020). Since these receptors are also found in the gastric, duodenal, and rectal epithelial cells, SARS-CoV-2 could infect these cells, replicate, and pass into the stool, implying that oral-fecal transmission is likely for SARS-CoV-2 (Xiao et al., 2020).

In addition to the presence of SARS-CoV-2 genetic material and live virus in stool, live SARS-CoV-2 was isolated from the urine of an infected patient and found to be infectious, meaning it could infect new cells and then spread to others (Sun et al., 2020). According to a World Health Organization (WHO) (2020a) report, SARS-CoV-2 could also spread via respiratory droplets from infected people; this infection mode is known as the aerosolized transmission mode.

Fomite transmission of SARS-CoV-2, that is, infection transmitted from contaminated surfaces to people, can also occur as the median half-life of SARS-CoV-2 on surfaces is 1–7 h depending on the surface, and the virus has been detected for up to 7 days and could be detected even longer depending on the analytical method and its concentration. The ability of SARS-CoV-2 to stay viable on hard surfaces is due to its charged surface, which is in part due to the presence of glycan in its composition (Yao et al., 2020). Other viruses have also shown the ability to remain on hard surfaces. For example, the virus *Escherichia coli* phage has a high degree of retention on silica (Qin et al., 2020). World Health Organization (WHO) (2020a) recommends the use of disinfectants, such as 70% ethanol or 0.1% sodium hypochlorite, to decontaminate surfaces to reduce fomite transmission of SARS-CoV-2.

The objectives of this article, in addition to discussing the prevalence and fate of SARS-CoV-2 in wastewater, is to assess the treatment strategies recommended by WHO and other agencies for wastewater contaminated with SARS-CoV-2 and to discuss the challenges posed by these strategies. Relevant published studies, including case studies on coronaviruses, are also presented as supporting material and whenever possible, relevant data from these studies are reported in a consistent manner.

## 2 | PREVALENCE OF SARS-COV-2 IN WASTEWATER

SARS-CoV-2 genetic material, that is, the RNA, has been found in wastewater in a number of studies. Examples include raw municipal wastewater and, in one study in Italy, rivers near wastewater treatment plants (Rimoldi et al., 2020) and in sewage at different

locations in The Netherlands where the SARS-CoV-2 RNA concentration ranged from  $1.2 \times 10^1$  to  $1.8 \times 10^3$  copies of RNA per mL of sample (Medema et al., 2020). The latter study also reported that the detected quantity of SARS-CoV-2 RNA was correlated with the prevalence of infection. SARS-CoV-2 RNA was also found in sewage sludge in New Haven, Connecticut, USA, ranging in quantity from  $1.7 \times 10^3$  copies of RNA per mL of sample to  $4.6 \times 10^5$  copies of RNA per mL of sample (Peccia et al., 2020).

## 2.1 | Data dashboards for monitoring of SARS-COV-2 in wastewater

The concentration of SARS-CoV-2 RNA is proportional to the prevalence of infection, that is, the disease it causes, COVID-19, and this relationship has been shown in a number of studies, some of which were discussed earlier in this article. This relationship is the key point for the creation of surveillance dashboards to monitor COVID-19 outbreaks. Surveillance of SARS-CoV-2 in sewage wastewater is an unbiased and efficient way to monitor the outbreak of COVID-19. Not everyone who has COVID-19 gets tested because the disease may be asymptomatic and people might not be aware they are infected. However, the SARS-CoV-2 virus can be detected in the stool discharge in wastewater and so wastewater surveillance can provide information about the prevalence of the disease for the population in an area or region utilizing sectors of a wastewater treatment conveyance system (Gajewski, 2021).

A number of states have online dashboards for monitoring the presence of SARS-CoV-2 RNA in wastewater and some of the states have data in the form of graphs showing the daily trend in the concentration of the SARS-COV-2 RNA. Examples of these dashboards include the states of Michigan (Michigan COVID Wastewater Testing Dashboard, 2021) and Ohio (Ohio Department of Health COVID-19 Dashboard, 2021), both accessible from the state governments' websites. The purpose of these dashboards is to monitor COVID-19 outbreaks and to provide the data publicly so that decision-makers can manage the outbreaks.

In addition to these individual dashboards, the US Centers for Disease Control (CDC) within the US Department of Health and Human Services (HHS) in collaboration with several other federal agencies including the US Environmental Protection Agency (EPA), the US Geological Survey (USGS), and the National Institutes of Health (NIH) initiated the National Wastewater Surveillance System (NWSS) to generate data on the presence of SARS-CoV-2 in wastewater (NWSS, 2021). The NWSS' website includes guidelines for performing the surveillance including sampling strategies and data reporting. SARS-COV-2 is quantified in terms of the RNA, its genetic material, specifically SARS-CoV-2 RNA using polymerase chain reaction (PCR) technologies. According to NWSS, the submitted data by the states' health departments are analyzed and the results are reported back to the health departments (US Centers for Disease Control CDC, 2021a).

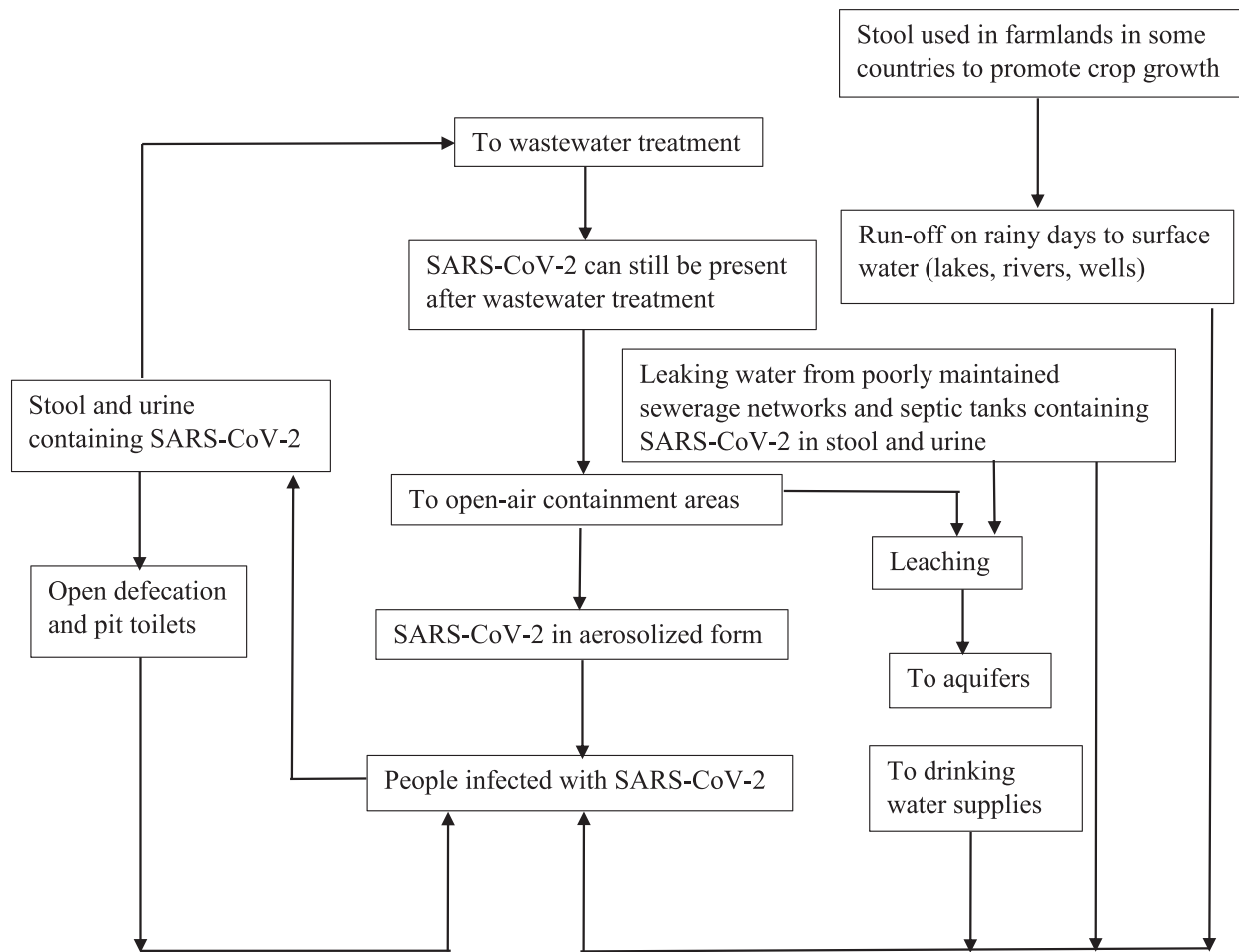
Besides these surveillance websites, there is also a publicly available map showing countries participating in the monitoring of SARS-CoV-2 in wastewater, operated by the University of California Merced (University of California Merced maps, 2021). Currently, 53 countries participate in this surveillance including The Netherlands, Turkey, India, Japan, Australia, in addition to the United States, Canada, and a number of European countries. The map can be enlarged to see individual sites in different countries monitored for SARS-CoV-2 in wastewater.

While COVID-19 surveillance is a larger scale surveillance compared to past surveillance for tracking noroviruses and other epidemiological pathogens in wastewater, it has its technical challenges, such as using standardized methods and data normalization, and also a global challenge: wastewater monitoring of SARS-CoV-2 is not performed by all states in the United States and not by all countries, as shown by the UCMERGED map for SARS-CoV-2 surveillance (Naughton et al., 2021; University of California Merced maps, 2021). For example, currently the UCMERGED map shows no record of wastewater monitoring for SARS-CoV-2 in South Dakota even though it had the second highest number of COVID-19 cases in the United States per capita and the seventh highest per capita death rate from this virus (Naughton et al., 2021). The UCMERGED map also shows that wastewater monitoring for SARS-CoV-2 is not widespread in the Middle East or Africa. Therefore, the dashboards have a limited number of entries in some areas of the world.

## 3 | THE FATE OF SARS-COV-2 IN WASTEWATER

The fate of SARS-CoV-2 in wastewater is explained by Foladori et al. (2020) who reported the stool of infected people typically contains  $5 \times 10^3$  to  $5 \times 10^{7.6}$  copies of SARS-CoV-2 RNA per mL of sample and this virus load is less after stool discharge because the stool is diluted after it enters the sewage. After treatment in the wastewater treatment plant, Foladori et al. (2020) reports there are  $2 \times 10^{-2}$  to  $3 \times 10^3$  copies of SARS-CoV-2 RNA per mL of sample, and this concentration depends not only on how widespread the outbreak is, but also on factors that affect its viability in the wastewater, namely temperature, pH, presence of solids, and the disinfection processes.

As discussed earlier in this article, SARS-CoV-2 is genetically related to and also similar in its infectivity to SARS-CoV, which spreads through the air. This is exemplified by a particular outbreak event in a large, private apartment complex in Hong Kong where 321 SARS-CoV cases were found at this location in 2003, accounting for 18% of all reported SARS-CoV cases in Hong Kong (McKinney et al., 2006). According to the World Health Organization (WHO) (2003), many residents had installed high-powered fans that created negative pressure and drew air from any available sources including from sanitary risers through the floor drain. Aerosolized droplets of SARS-CoV, in sufficiently large quantities, were drawn into the bathrooms from plumbing systems resulting in the high number of people infected. Other microorganisms including *Pseudomonas putida* and



**FIGURE 1** Fate of SARS-CoV-2 in wastewater and the infection cycle

*Legionella pneumophila*, which as the name implies causes Legionnaires' disease and other viruses, such as human adenoviruses, can also spread in the aerosolized forms (Gormley et al., 2020; Naddeo & Liu, 2020; Verani et al., 2014). Recently, the CDC (US Centers for Disease Control CDC, 2021b) mentioned in a scientific brief that SARS-CoV-2 infection can occur from air farther than six feet from the infectious source, implying that SARS-CoV-2 infection can occur via the aerosolization mode.

The transport and infection cycle of SARS-CoV-2 in wastewater is illustrated in Figure 1. SARS-CoV-2 can transport into wastewater from infected stool and urine and, depending on the treatment method, can remain in the water after the effluent has been treated, as shown in studies discussed earlier in this article. The treated wastewater could be discharged into a containment area (Thakur et al., 2021) where it could spread to people via aerosolization, an infection process discussed in the above paragraph and, if leaching occurs, the virus could contaminate the underlying aquifers and eventually infect people. According to WHO (2017), open defecation areas and pit toilets are used by 900 million people worldwide and this is a problem, especially if the feces is not treated or runs off into other water bodies, and if people drink from these water bodies they could be exposed to SARS-CoV-2. In theory, SARS-CoV-2 infection

via inhalation of aerosols is possible, similar to SARS-CoV infection via aerosolization from the plumbing system in Hong Kong as described earlier in this article. A study found SARS-CoV-2 RNA in soil (0.21 to 0.056 copies of RNA per mg of sample) and aerosols (0.29 to 1.1 copies of RNA per and omit /mm<sup>3</sup>) in addition to its presence in wastewater (0.26–18.7 copies per mL of sample) and postulated that the virus genetic material was in soil due to deposition and in wastewater due to aerosolization from infected patients at a nearby hospital (Zhang et al., 2021). While Lednicky et al. (2020) found that a SARS-CoV-2 strain in air matched the strain from infected patients within a hospital room, Correia et al. (2020) pointed out that airborne transmission of SARS-CoV-2 is possible from building ventilation systems. In some countries, feces is used in farmlands to promote crop growth (Naddeo & Liu, 2020) and, with the SARS-CoV-2 in it, the feces in stormwater run-off from agricultural lands could transport to water bodies such as lakes, rivers and also into wells, thereby posing sources of exposure to the virus. Furthermore, the applicators and farmers could potentially be exposed to contaminated feces. Water leaking from septic tanks (Qin et al., 2020) and sewage networks is also a common occurrence in municipalities with older infrastructure (Paleologos et al., 2020), resulting in the exposure to wastewater contaminated with SARS-CoV-2.

## 4 | RECOMMENDED TREATMENT STRATEGIES FOR SARS-COV-2 CONTAMINATED WASTEWATER

### 4.1 | World Health Organization (WHO)

Factors facilitating virus reduction include high temperature, high/low pH, and sunlight (Abraham et al., 2020). WHO published an extensive and comprehensive report describing not only the mode of transmission of SARS-CoV-2, but also recommended treatment strategies for wastewater contaminated with SARS-CoV-2 WHO (2020b). The report recommends that wastewater, and sludge from wastewater treatment, should be contained and treated onsite; however, if it is treated off-site, it should be in a well-designed and managed treatment facility. Each stage of wastewater treatment should combine physical, chemical, and biological processes and a final disinfection process should be considered if the treatment processes are not capable of removing the virus. The WHO report also recommended filtration and disinfection for drinking water treatment at the point of distribution to improve water safety. Where centralized water treatment and safe piped drinking water is not available, the WHO report recommends boiling, solar irradiation, and the use of chlorine products such as sodium hypochlorite.

Like the WHO recommendation, some agencies, such as the International Water Association (IWA), also recommend multiple disinfection steps for wastewater treatment such as the recommended use of ozonation, UV irradiation, and sodium hypochlorite. In a study mentioned by the IWA, the concentration of the different disinfection steps were optimized and, after treatment, no SARS-CoV-2 was detected in the effluent (International Water Association IWA, 2020). The US Occupational Safety and Health Administration (OSHA) also recommends multi-steps for disinfection in wastewater treatment. Oxidation with hypochlorous acid or peracetic acid and UV as is normally performed are expected to be sufficient for treating SARS-CoV-2 in wastewater (Phillips P. J U.S. Occupational Health and Safety Administration OSHA, 2020).

### 4.2 | United States Environmental Protection Agency and other regulatory agencies

The US EPA states on their website that, based on evidence, the risk of SARS-CoV-2 infection is low from water supplies and so people can continue to drink and use tap water (US Environmental Protection Agency EPA, 2020). The website also has a template that water treatment utilities can provide to workers and it mentions that water and wastewater workers are essential critical infrastructure workers needed to maintain and operate drinking water and wastewater infrastructure. Additionally, it also has a "Frequent Questions" link and the questions include (under Wastewater and Septic Tanks): "Will my septic system treat COVID-19?". The answer mentioned that, while decentralized wastewater treatment does not disinfect, the US EPA expects septic systems to treat COVID-19 the

same way they manage other viruses found in wastewater which is via disinfection.

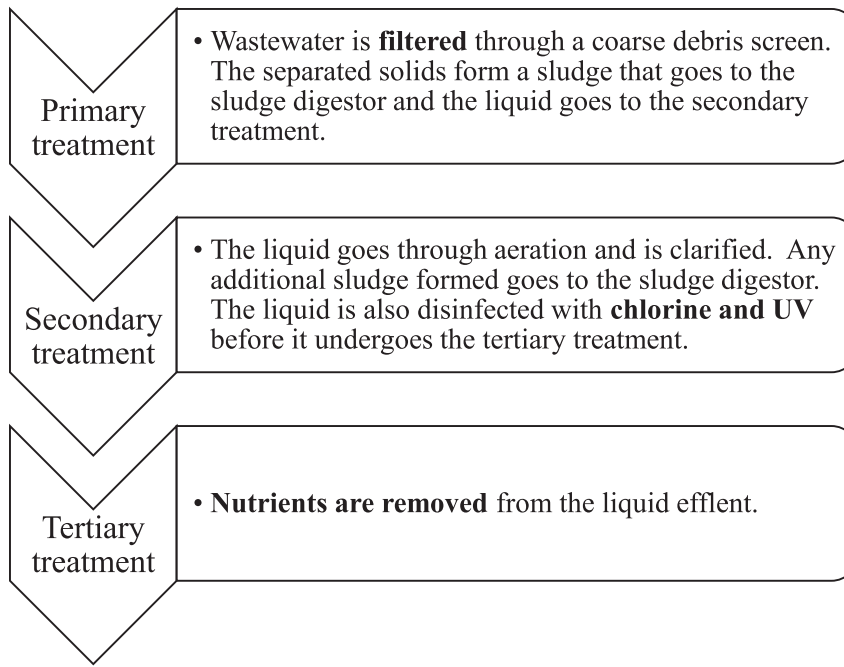
Some agencies have specified recommended disinfection methods. For example, the UK Health and Safety Executive (HSE) mentions that a chlorine dose of 15 mg per liter of water per minute inactivates nonenveloped viruses and, therefore, a lower chlorine dose could inactivate SARS-CoV-2, which is an enveloped virus, because according to the HSE, enveloped viruses require a lower dose of chlorine for inactivation compared to non-enveloped viruses (U.K. Health and Safety Executive HSE, 2020). However, as discussed later in this paper, this correlation has not always been reliable in research studies.

## 5 | INSUFFICIENCY OF PUBLISHED STUDIES ON WASTEWATER TREATMENT TECHNOLOGIES USING SARS-COV-2 AND THE USE OF SURROGATE VIRUSES

There are very few published studies testing different wastewater treatment strategies to address the presence of SARS-CoV-2 in wastewater and there are several reasons for this insufficiency. Working with SARS-CoV-2 requires a biosafety level 3 or higher in the U.S., not to mention federal and state regulations, and not all laboratories have the required biosafety level facility and the research expertise to handle this highly contagious coronavirus. The majority of published research studies on SARS-CoV-2 has been performed using "surrogate viruses," i.e., viruses that are good indicator microorganisms for SARS-CoV-2, an enveloped virus with single stranded RNA, and the selection and logic for a good indicator microorganism as a surrogate virus for SARS-CoV-2 differs among researchers. For example, some research studies used the non-enveloped virus bacteriophage MS2, the enveloped human coronavirus 229E (HCoV 229E), or the enveloped murine hepatitis virus (MHV), all possessing single stranded RNA, while some of the other studies used the enveloped bacteriophage phi6 possessing a double stranded RNA as the surrogate viruses for comparing the theoretical outcome of the results for treating SARS-CoV-2.

## 6 | SARS-COV-2 CONTAMINATED WASTEWATER TREATMENT STRATEGIES

There are a number of published review articles on SARS-CoV-2 in wastewater and, while some of these articles touch on important topics such as the need for research to look into the migration of SARS-CoV-2 in wastewater due to the COVID-19 outbreak and poor water sanitation in some countries (Paleologos et al., 2020), and the use of biosensor technologies to detect SARS-CoV-2 in wastewater (Tetteh et al., 2020), these and a number of review articles also discuss treatment strategies for wastewater contaminated with SARS-CoV-2. Some of the suggested treatment strategies discussed in such review articles include promoting decentralized wastewater



**FIGURE 2** General steps in the wastewater treatment process

treatment (Kataki et al., 2021). Promoting decentralized wastewater treatment in this context means that certain facilities, including virus hotspots such as hospitals and quarantine centers, that are not connected to a centralized sewer, as is the situation in some countries, could contain the wastes and disinfect it to reduce the virus concentration before releasing the waste into the environment so to decrease secondary transmission from the virus. The use of ozonation, UV, chlorine, sodium hypochlorite, membrane bioreactors, and algae (an emerging technology) are considered viable treatment technologies for wastewater contaminated with SARS-CoV-2 (Lahrich et al., 2021; Tetteh et al., 2020; Thakur et al., 2021; Wang et al., 2020b).

The use of UV, chlorination, and filtration, the methods recommended by the WHO, are widely used in wastewater treatment systems (Figure 2). Membrane bioreactors are also common while algal based methods for wastewater treatment, being relatively new, could be effective and should not be ignored as a potential technology. The following sections of this article summarize the capability of these technologies to treat SARS-CoV-2 contaminated wastewater based on published research studies performed with SARS-CoV-2 and surrogate viruses.

## 6.1 | Ultraviolet Light

UV light rays cause viruses to lose the ability to replicate and thus halts the spread of the virus in the environment. When the UV rays hit the virus particle, the phosphodiester bonds and the crosslinks in the viral DNA are disrupted and the thymine bases react with the UV forming thymine-thymine double bonds, and these bonds inhibit the replication of the viral genomic material and, therefore, prevent the virus from further replication.

UV light has proven to be effective against SARS-CoV. In a study by Darnell et al. (2004) the virus was inactivated when treated with UV light at 254 nm and, in another study by Duan et al. (2003) a strain of this virus, SARS CoV-2 strain Pa, was in undetectable amount after exposure to UV light for one hour.

The wavelength intensity of the UV light affects the virus. UV light at 282 nm was less effective than at 222 nm against the human coronavirus 229E, the murine hepatitis virus (MHV), and the bacteriophage phi 6 as it reduced the virus inactivation rates by 32%, 42%, and 6.9%, respectively (Ma et al., 2021). These values indicate that bacteriophage phi 6 is more resistant to UV and MHV is the least resistant. The difference in these three enveloped viruses is the presence of double stranded genetic material in bacteriophage phi 6 compared to the single stranded genomic material in the other two viruses; however, whether this difference contributed to the resistance is not understood.

UV-C light is also effective against other viruses. For examples, the exposure of Surficide™ UV-C to MERS-CoV on glass coverslips for 10 min in a laboratory study resulted in an undetectable concentration of MERS-CoV (Bedell et al., 2016) and, in another study, increasing the duration of UV-C exposure at unspecified wavelength from 10 s to 30 s resulted in approximately two and four times the reduction in concentration of the bacteriophage MS2 and phi 6, respectively (Cadnum et al., 2020). In addition to Surficide™ UV-C, a microplasma UV lamp is another UV technology that is germicidally (germicidal equals inhibition) efficient on bacteriophage MS2 (Raeiszadeh & Taghipour, 2021).

UV and UV-C treatments in combination with other factors have proven more effective than UV treatment alone against viruses. For example, a UV intensity of 200 mJ per cm<sup>2</sup> was required to achieve 99% reduction of adenovirus, and less was required to achieve this reduction (120 mJ per cm<sup>2</sup>) when hydrogen peroxide was used in

addition to UV (Bounty et al., 2012). The use of hydrogen peroxide created hydroxyl radicals which in turn likely damaged the attachment proteins in addition to the DNA damage in the adenovirus. The damage to the attachment proteins was unlikely with the use of UV as the sole treatment. Hydroxyl radicals have been successful in reducing the concentrations of coronaviruses including SARs-CoV-2 in wastewater (Randazzo et al., 2020). While discussing the use of other factors along with UV-C, it should be noted that air flow, specifically rapid air along with UV-C, has been shown to be effective in reducing the ambient concentration of SARS-CoV-2. The use of a wind tunnel at an air flow rate of 2,439 liters per minute and UV-C at  $253 \pm 1$  nm in a laboratory study resulted in a 99.98% virus removal efficiency (Qiao et al., 2020).

There are several variables in aquatic systems that could affect the outcome of treatment strategies to inactivate or eliminate viruses in wastewater. These factors include extracellular algal organic matter, that uses organic matter excreted by algae, which affects the UV treatment on viruses. For example, when bacteriophage MS2 was in the presence of extracellular organic matter formed by the alga *Microcystic aeruginosa*, it was not inactivated by the use of UV at 254 nm and was inactivated when UV at 220 nm was used. In addition, the inactivation for bacteriophage MS2 was higher when UV of 220 nm was used if there was organic-free phosphate buffer solution in lieu of the extracellular organic matter (Wang et al., 2019). Why UV treatment of lower wavelength (220 nm) was effective for the inactivation of the virus (bacteriophage MS2) when a UV treatment of higher wavelength (254 nm) was not might be due to the effect of the lower wavelength on the extracellular algal extract, considering that the study found detectable concentration of hydroxyl and oxygen radicals only in the treatment irradiated at 220 nm. The UV of lower wavelength (220 nm) was probably absorbed by the extracellular algal extract, causing the production of hydroxyl and oxygen radicals, and these radicals in turn caused genomic damage in the virus that resulted in the inactivation of the virus.

## 6.2 | Ozonation and chlorination

Ozonation destroys viruses by attacking their proteins. The ozone first breaks the lipid molecules and when it comes in contact with the proteins, hydroxides and peroxides are produced, and the oxidative stress from these destroys the virus. In theory and using molecular modeling, it is believed that ozone could be effective for the elimination of SARs-CoV2 (Schwartz & Martínez-Sánchez, 2020; Tizaoui, 2020). However, there are practical challenges in using ozonation as a treatment technology for wastewater. It increases the acidity of the water, and it is toxic, reactive, expensive, has a short half-life, and viruses, more than bacteria, could develop resistance to ozone. After ozone treatment, chlorination is usually applied as a secondary treatment.

Chlorination for treating water containing SARS-CoV-2, as mentioned above and recommended by the WHO, is the most widely used disinfectant because it is effective at low concentrations and it is relatively inexpensive compared to other disinfectants. Among the

different chemical forms of chlorine, hypochlorous acid is the one that gives chlorine its disinfecting property, and it is formed when chlorine is mixed with water, with more formed at neutral or low pH because it dissociates in water.

Chlorine when combined with other treatment methods has proven to be virucidally effective (rendering the virus noninfectious or destroyed) for treating viruses in wastewater, especially the use of chlorine in acidic electrolyzed water containing SARS-CoV-2. A study showed that while this was virucidally effective, it was in the contrary in the absence of the chlorine treatment and the virucidal activity for the acidic electrolyzed water against SARS-CoV-2 was proportional to the chlorine dose used (Takeda et al., 2020).

However, the opposite effect of using chlorination has also been observed. In one such study SARS-CoV-2 RNA was detected in untreated wastewater and also in secondary treated wastewater after chlorination and UV were used as the treatments (Randazzo et al., 2020). Also, there seems to be no correlation between the chlorine dose required to eliminate the virus and the viral structural features, namely the presence or absence of the envelope and a single or double-stranded RNA. For example, in a study to determine the effects of chlorination on different viruses, the results showed that bacteriophages MS2 and phi 6, both nonenveloped viruses and possessing single stranded RNA, required 3,800 mg per L and 400 mg per L of free chlorine respectively while bacteriophage X-174, an enveloped protein with double stranded RNA, required 960 mg per L of free chlorine to achieve 99% reduction in the viral concentration (Strasser, 2017). While indicating the need for substantial chlorine doses, the results of this study imply that there is no correlation between the chlorine dose required to eliminate the virus and the above mentioned viral structural features.

Successful results for the virucidal effects of chlorine alone for wastewater treatment have been reported for human coronaviruses such as for SARS-CoV for which 100% virucidal effect was observed when 20 mg per L of free chlorine was applied to the wastewater samples containing the virus before chemically neutralizing the samples. However, a lower virucidal effect of 94% was achieved for the same treatment with the use of 40 mg per L of chloride dioxide, indicating that free chlorine worked better than chlorine dioxide for inactivating SARS-CoV (Wang et al., 2005). This does not imply that chlorine dioxide is a weak virucidal agent. It damages different structural component of the enveloped and nonenveloped viruses. Chlorine dioxide, formed from the reaction of sodium chlorite with chlorine, damages the genome of non-enveloped viruses such as the polio virus, enterovirus, and hepatitis A virus and the protein coat in enveloped viruses such as the rotavirus and influenza A virus (Ge et al., 2021). Also, the time for the virucidal effect of chlorine dioxide is longer compared to for chlorine, as shown in the above study with SARs CoV-2 (an enveloped virus) and also with other viruses such as the murine coronavirus A59 (also an enveloped virus) in wastewater, where a chlorine dioxide dose of 0.16 ppmv per minute required 12 h to achieve no viable A59 virus in the wastewater sample (Kim et al., 2016).

In addition to the use of chlorine in the form of chlorine dioxide, chlorine can also be used as sodium hypochlorite (bleach) formed by

the reaction of chlorine with caustic soda (sodium hydroxide), and it has been effective for eliminating SARS-CoV-2 in wastewater. For example, SARS-CoV-2 RNA was found in all samples collected from the influent of a hospital's wastewater disinfection system that used sodium hypochlorite, while the effluent samples were all negative (Wang et al., 2020a). Some bleach products have color additives used as an application indicator that also promote the disinfection properties of the product. For example, the additive colorizes the bleach blue and turns colorless after a certain elapsed contact time. In one such study, a blue color additive in combination with 0.5% sodium hypochlorite reduced the concentration of human coronavirus 229E by more than 4.50 log<sub>10</sub>, the U.S. EPA's acceptable limit in water, and this effect probably occurred because, while sodium hypochlorite beads up when used alone, it spreads in the presence of the dye allowing it to be more effective against the virus (Tyan et al., 2018).

### 6.3 | Carbon materials as adsorbents

Due to the presence of glycan and other biochemicals, viruses can have ionic charges on their surfaces and, therefore, have the ability to adsorb to solid surfaces, as mentioned earlier in this article, as shown in a number of studies discussed in this article, and can also adsorb to carbon-based nanomaterial such as graphene, that besides being corrosion-resistant, also has excellent electrical and thermal conductivity (Yu et al., 2021) and can be used as a biosensor for the detection of different viruses. Examples include the detection of influenza A strain H9N2 by graphene and influenza virus strains H1N1 and H5N1 by graphene oxide. The concentration range detected by either graphene or graphene oxide can be quite high, 25–500 picomolar (Anik et al., 2018; Ono et al., 2017; Veerapandian et al., 2016).

Graphene electrodes including the use of laser-induced graphene, i.e., carbon material exposed to a laser that converts it into graphene, when used with a power supply can also be used for the inactivation of the pox virus. Due to its electrochemical properties and porous texture, laser-induced graphene has antimicrobial surfaces and can reduce 99.9% of the concentration of the pox virus, *Vaccinia lister*, at 20 V. However, no virus concentration was reduced at 2.5 V. It is theorized that viral inactivation in this case involves the formation of hydrogen peroxide and associated reactive oxygen species at the anode, both potentially involved in the reduction of the viral concentration (Barbhuiya et al., 2020). When 20V was used, possibly more of these two chemicals were produced than when 2.5V was used and therefore, 2.5V was not effective in reducing the virus concentration.

In addition to graphene electrodes, graphene tubes could be stacked one inside of another in a concentric arrangement or graphene sheets could be rolled, and these two structures are known as multi-walled carbon nanotubes (MWCNT), which can also be made of graphite rather than graphene. MWCNT, when coated with copper(I) oxide, have been effective in the adsorption and, thus, removal of bacteriophage MS2 (a virus) from water, and the permeation

property of copper is postulated to contribute to the adsorption of this virus (Domagała et al., 2020). However, the presence of dissolved organic carbon can make the MWCNT ineffective in removing viruses from aquatic systems. For example, a study showed that removal of the bacteriophage MS2 by MWCNT only works in the presence of dissolved organic carbon at concentrations close to zero, and any higher concentration was not effective for the virus removal (Jacquin et al., 2020).

While the use of carbon-based material in an electrochemical system can inactivate viruses, as shown in several studies previously discussed in this article, it can also produce toxic chemicals. For example, an electrochemical disinfection system that included iridium-antimony-tin coated titanium anode and high salt concentration inactivated bacteriophage MS2 when a current was applied. However this electrical system, in addition to producing significantly high concentrations of chlorate ions, also produced trihalomethanes and haloacetic acids, although in lower concentrations compared to the amounts commonly produced in the chlorination of surface water supplies (Fang et al., 2006).

A charged surface, in addition to being present on carbon electrodes as discussed above, can also be created from biological materials such as lignin from plants, which has been shown to remove viruses from the surrounding aquatic system. A study showed that the positively charged lignin particles were able to remove cowpea chlorotic mottle viruses. Transmission electron microscopy showed that this virus forms chemical complexes with the charged lignin particles. These complexes can be removed from water by filtration or centrifugation (Riviere et al., 2020).

### 6.4 | Algal-based strategies

High rate algal ponds are effective in decreasing the concentration of viruses. These ponds are shallow, mixed lagoon water treatment systems and require smaller space than waste stabilization ponds. By creating optimal conditions for algal growth and oxygen production, not only nitrogen and organic waste is removed from the wastewater in high rate algal ponds (Young et al., 2017), but the concentrations of viruses such as the F-RNA bacteriophage can be reduced. A study showed the median virus concentration of F-RNA bacteriophage in effluent samples taken from a high retention algal pond was 2-fold less compared to in the wastewater influent, showing that the high retention algal pond reduced the concentration of F-RNA bacteriophage in the wastewater (Young et al., 2016).

The alga *Microcystic aeruginosa* can decrease the concentration of the bacteriophage MS2 in wastewater when sodium hypochlorite is used as a disinfectant; however, without the alga, sodium hypochlorite was less effective in reducing the concentration of MS2 in the wastewater. When the cells of *Microcystic aeruginosa* bind to the sodium hypochlorite molecule, hypochlorous acid is formed, and while the chlorine formed from this acid is consumed by the algae, the acid kills the bacteriophage MS2. However, this process to reduce the virus concentration is influenced by



the presence of calcium ions and natural organic matter (Tang et al., 2021).

However, not all algae remove viruses from wastewater. An example of this scenario is the alga *Nannochloropsis salina*. When bacteriophage MS2 in secondary treated wastewater was co-incubated for up to three hours with this alga, the concentration of MS2 was higher than without the alga (Unnithan et al., 2014).

As discussed previously in this article, algal organic matter is a factor that influences the effects of UV on the concentration of viruses in wastewater. It can also directly affect the inactivation of viruses in wastewater. For example, a study showed that a high intracellular algal organic matter concentration of 13 mg carbon per L had a negative effect on the inactivation of bacteriophage MS2 (Wu et al., 2019).

## 6.5 | Other treatment strategies

While the treatment technologies commonly used in wastewater treatment discussed above can also be used to treat viruses, there are also other treatment strategies such as the use of photocatalytic membrane reactors, electrochemical membrane bioreactors, and ferric chloride coagulation, which are reviewed in the following discussion.

A membrane bioreactor uses a membrane for micro- or ultra-filtration to separate activated sludge from the water. After passing through a fine screen to remove solids, the wastewater enters an anoxic zone to treat nitrogen and phosphorus before entering the aerobic zone where microorganisms, with the help of oxygen, metabolize organic matter and, in the process, clump together to produce an activated sludge. The activated sludge enters the membrane bioreactor where the membrane separates the sludge from the water and the water can then be fed back into the anoxic zone for further treatment. Despite the high cost and complex maintenance along with the problems of frequent fouling and formation of foam, membrane bioreactors can remove SARS-CoV-2 using membrane sizes ranging from 60 to 140 nm (Lesimple et al., 2020).

When membrane filtration is combined with photocatalysis, i.e., when a semiconductor is activated by sunlight or other light source and used as a catalyst, the system is called a photocatalytic membrane reactor and, like the membrane bioreactor discussed above, it can also remove viruses from the water. For example, the use of a photocatalytic aluminum oxide membrane reactor coated with titanium dioxide was able to remove bacteriophage MS2 from water at a higher efficiency when supplemented with 500 mg per L chlorine and operated at a neutral pH (Horovitz et al., 2018).

The use of a membrane as an electrode in an electrochemical system is known as an electrochemical membrane bioreactor and it has been shown to be highly efficient in the removal of viruses. For example, the use of a membrane as the cathode and iridium (IV)-tantalum oxide anode, which forms an electrochemical membrane bioreactor, led to 100% removal of bacteriophage MS2 from wastewater. The same wastewater treated without this electrode removed a relatively low amount of MS2 (average of 20% removal).

The production of a reactive oxygen species on the cathodic membrane and reactive hydroxide in the form of iridium (IV) oxide attacked and destroyed bacteriophage MS2 while the electricity produced in this bioreactor mitigated fouling issues, a problem common with membrane bioreactors (Chen et al., 2021).

In addition to the use of membrane bioreactors, coagulation is also a strategy for virus removal from wastewater and, among inorganics, ferric chloride is a common coagulant. Ferric chloride in the presence of calcium bicarbonate in addition to calcium chloride and oxygen produces ferric hydroxide and via further reactions, this hydroxide can form ferrous oxide. Both ferric chloride and ferrous oxide as coagulants have the ability to remove viruses from water. The use of both of these led to a 5-log removal (99% removal) of phi6 bacteriophage in 20 min. The adhesion of this bacteriophage to the precipitated ferric hydroxide led to damage of its vital envelope structure, causing its inactivation, as seen by Fourier transform infrared spectroscopy (Kim et al., 2021).

## 7 | IMPLICATIONS FOR SARS-COV-2 CONTAMINATED WASTEWATER AND THE ROAD AHEAD

SARS-CoV-2 has been detected in treated wastewater in numerous studies as discussed in this article. This virus can remain infectious for days in sewage and, depending on the fate of the treated wastewater, there is a potential for SARs-CoV-2 to spread as shown in Figure 1, even though its concentration in treated wastewater is less than in untreated water. This presents a concern because the amount detected in treated wastewater is proportional to the prevalence of the disease, as pointed out in several studies discussed in this article, and also because of its long half-life, its ability to spread in aerosolized forms, and its ability to survive on hard surfaces. SARS-CoV-2 is viable in the aerosolized form and its half-life in the viable form on hard surfaces is one to three hours on copper, two to five hours on cardboard, and six to seven hours on plastic, due to the ability of this virus to possess a charged surface. All of these viability time frames are similar to SARs-CoV (van Doremalen et al., 2020).

The different wastewater treatment strategies discussed in this article are technologies based on the WHO recommendations and several are also common in typical municipal wastewater treatment plants. They all point to one issue: variability in their effectiveness because of the influence of the chemical factors present in the wastewater, as shown in Table 1, which summarizes the various treatment strategies. Also, as is the case for any treatment strategy, the strategies discussed in this article also have drawbacks. For example, ozonation can increase the water acidity, it is expensive, and has a short half-life. UV is energy intensive and also expensive. In chlorination, when chlorine reacts with ammonia present in the water, chloramine is formed and it behaves differently than free chlorine during disinfection. Considering all this, particularly keeping in mind that the concentration of SARs-CoV-2 in treated wastewater is proportional to the prevalence of COVID-19 in the community,

**TABLE 1** An overview of some of the wastewater treatment strategies discussed in this article and factors influencing the treatments

Wastewater treatment	Virus tested	Factor(s) influencing the treatment for the elimination/concentration decrease of the virus	References
UV	SARS-CoV	UV wavelength intensity	Darnell et al. (2004)
	SARS-CoV-2 strain PA	Exposure duration	Duan et al. (2003)
	Adenovirus	Use of hydrogen peroxide	Bounty et al. (2021)
	Bacteriophage MS2	Algal extracellular organic extract	Wang et al. (2019)
UV-C	MERS CoV	UV wavelength intensity and exposure duration	Bedell et al. (2016)
	Bacteriophages MS2 and phi6	Exposure duration	Cadnum et al. (2020)
	SARS-CoV-2	Air flow	Qiao et al. (2020)
Chlorination	SARS-CoV-2	Acidic electrolyzed water	Takeda et al. (2020)
	Bacteriophages MS2, phi6, X174	Concentration of chlorine	Strasser (2017)
Chlorine dioxide	Murin coronavirus A59	Dose	
Sodium hypochlorite	Human coronavirus 229E	Color additive	Tyan et al. (2018)
Multi-walled carbon nanotubes	Bacteriophage MS2	Dissolved organic carbon	Jacquin et al. (2020)
Titanium anode	Bacteriophage MS2	Salt concentration	Fang et al. (2006)
High rate algal pond	F-RNA bacteriophage	Conditions for algal growth and oxygen production	Young et al. (2017)
Alga - <i>Microcystis aeruginosa</i>	Bacteriophage MS2	Sodium hypochlorite, calcium ions, natural organic matter	Tang et al. (2021)
Algal organic matter	Bacteriophage MS2	Intracellular and extracellular algal organic matter concentrations	Wu et al. (2019)
Membrane bioreactor	SARS-CoV-2	Membrane size range	Lesimple et al. (2020)
Photocatalytic membrane reactor	Bacteriophage MS2	Chlorine and pH	Horovitz et al. (2018)
Electrochemical membrane bioreactor	Bacteriophage MS2	Electrochemical membrane	Chen et al. (2021)

what can be done to treat wastewater if the concentration of SARS-CoV-2 rises to a concerning level? Increasing the treatment doses might not be feasible because of the concern that their drawbacks, as just pointed out, will be amplified and lead to more problems in terms of operations and water quality and offset the effect of eliminating SARS-CoV-2.

Some of the published articles on SARS-CoV-2 in wastewater have emphasized the importance of wastewater-based epidemiology to monitor the virus and identify hotspots for COVID-19 (Lahrich et al., 2021; Tetteh et al., 2020). There are data dashboards for such monitoring including states data dashboards, the NWSS and UC-MERCED, as discussed in this article, and these could be used to develop wastewater treatment strategies if the SARS-CoV-2 concentrations rise to a concerning level keeping in mind that studies have shown that the concentration of SARS-CoV-2 in wastewater, including in treated wastewater, is proportional to the prevalence of COVID-19 in the community. We need to be prepared to implement wastewater treatment strategies that use a combination of different treatment technologies, preferably technologies that could be

compatible with the current methods and infrastructure of our wastewater treatment plants because, as history has shown, COVID-19 is not the first pandemic and will certainly not be the last.

## 8 | CONCLUSION

SARS-CoV-2 has been detected in wastewater and in relatively lower concentrations in treated wastewater. This virus can be transmitted in the aerosolized form and can remain viable for hours on hard surfaces due to its charged surface arising from its biochemical composition. The fate of SARS-CoV-2 in wastewater is complicated as shown in Figure 1. The virus can leach with the treated wastewater into underlying aquifers and eventually get in drinking water supplies. In addition, the agricultural practices in some countries that use feces as a fertilizer along with the poor conditions of sewage treatment can increase the prevalence of SARS-CoV-2 in wastewater. The use of UV, ozonation, disinfection, and carbon materials as adsorbents as recommended by the WHO, in addition to novel

methods such as the use of algae, are generally effective against viruses, including against SARS-CoV-2. However, the effectiveness of these treatment technologies can be compromised due to the interference from physical and chemical factors present in the aqueous environment making the treatment of wastewater contaminated with SARS-CoV-2 a not-so-straightforward process. While the current trend in effluent research is on wastewater-based epidemiology to monitor and identify COVID-19 hotspots, it is imperative to develop multi-trained treatment strategies to eliminate SARS-CoV-2 from wastewater, preferably strategies that are effective and compatible with current treatment methods so that we are prepared in times of future pandemics.

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