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Detecting and disinfecting SARS-CoV-2 in wastewater: techniques, challenges, and strategies

Pedro Henrique Mainardi [1,2,*], Ederio Dino Bidoia [2]

[1] Municipal Department of Education of São Paulo (SME-SP), São Paulo, Brazil

[2] São Paulo State University Júlio de Mesquita Filho (UNESP), Institute of Biosciences, Department of General and Applied Biology, Rio Claro, SP, Brazil

* Corresponding author e-mail: pedro.h.mainardi@unesp.br

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ABSTRACT: Numerous studies have reported the detection of SARS-CoV-2 genetic material in wastewater networks and treatment plants. The presence of SARS-CoV-2 in wastewater has raised concerns about the potential indirect transmission of COVID-19 via fecal-oral route, and the possibility of virus spread in domesticated and wild animals, which could lead the dissemination of the pathogen in future outbreaks through cross-infection. Knowing that inhibiting the spread of SARS-CoV-2 through aquatic matrices has proven to be of great importance as a control of COVID-19, in this review, the main techniques for detecting the SARS-CoV-2 in aquatic matrices and the main reports on the occurrence and viability of the virus in these environments were described. It was presented the mechanisms of inactivation or removal SARS-CoV-2 through primary sedimentation, secondary biological processes, tertiary chlorination, ozonation, ultraviolet irradiation, membrane filtration, and some of the current emerging technological perspectives, such as retention ponds, disinfection by sunlight, treatment with algae, photocatalysis, thermal treatment and ceramic membranes. The article also highlighted strategies concerning the integration of disinfection methods, decentralization of treatment plants, sewage monitoring, and major challenges faced by researchers and professionals engaged in the disinfection of the new coronavirus in this complex matrix.

Keywords: Collective health; Inactivation; Prophylaxis; Sanitization; Sewage; Viral pathogens.

1. INTRODUCTION

SARS-CoV-2 is a new type of coronavirus capable to infect humans and cause the Coronavirus Disease 2019 (COVID-19), a notorious disease that has been responsible for causing enormous social and economic impacts around the globe [1]. The virus has an approximate diameter of 100 nm, a genome of $30x10^3$ single-stranded positive-sense RNA nucleotides and, according to electronic microphotographs, an outer lipid envelope with spike-shaped glycoproteins, structures that derived its name ("corona" is the Latin word for crown) [2]. The spike protein was seen to have a strong binding affinity to human host cells that contain angiotensin-converting enzyme 2 (ACE2) receptors, regarded as a key component that allows the virus to attach, enter and infect human cells [3,4]. The SARS-CoV-2 belongs to the *Coronaviridae* family, *Betacoronavirus* genus, and was firstly detected in late December 2019 in the city of Wuhan, China [2,5]. Due to its rapid spread and high mortality rate, the World Health Organization declared the SARS-CoV-2 virus and COVID-19 disease a global health emergency in January 2020 and it reached pandemic proportions by March of the same year [6].

COVID-19 has been observed to produce a wide range of effects across various human systems, including the cardiovascular, renal, musculoskeletal, neurological, immunological, visual, gastrointestinal, dermatological, reproductive, endocrine and most notably, the respiratory system [7-16]. Clinical manifestations reported in symptomatic patients have included fever, cough, fatigue, dyspnea, diarrhea, vomiting and, in drastic cases, severe pneumonia with respiratory failure and septic shock, similar to other coronavirus diseases [17,18]. By the end of January 2023, nearly three years after the beginning of the pandemic that has caused over 660 million cases and 6.7 million deaths worldwide, [19] COVID-19 was still maintained at the highest alert level under International Health Regulations [20].

SARS-CoV-2 spread has mainly occurred through the direct contact with contaminated secretions, including respiratory droplets, saliva, nasal mucus and aerosolized particles dispersed through the air [3,5,21]. Additionally, there is growing evidence that the virus has also been transmitted through the indirect contact with contaminated surfaces and objects [22]. The persistence of the virus has been confirmed through studies, which have shown that it can remain infectious in aerosolized particles for up to 16 hours and on surfaces such as plastic, stainless steel, and surgical masks for up to 7 days [23-25]. To control the spread of SARS-CoV-2, various protective measures have been implemented, including personal hygiene practices, the use of face masks, eye protection, physical distancing, proper ventilation of enclosed spaces, disinfection of surfaces, and vaccination [2,5,18,21,22].

Detection of SARS-CoV-2 viral particles in various bodily fluids, such as nasopharyngeal secretions, saliva, urine and stool specimens has been commonly observed in patients with COVID-19, including presymptomatic, asymptomatic and mildly symptomatic cases [26-28]. The presence of viral particles of the new coronavirus in those samples, infectious in some cases, not only highlights its transmission potential through direct contact or through aerosols generated by the excreted materials, [3,22,29,30] but also suggests the possibility of the contamination of sewage and natural waters by the SARS-CoV-2 [31,32].

The potential spread of SARS-CoV-2 through aquatic environments could, therefore, result in an indirect transmission of COVID-19 via the fecal-oral route [4,5,33,34]. Transmission by this route, through sewage contaminated with SARS-CoV-2 infectious viral particles, was indeed evidenced in a survey conducted in a low-income community in China [35]. This type of viral spread was also proven during the 2003 SARS-CoV-1 outbreak, in which studies indicated that airborne droplets of water contaminated with feces had facilitated the spread of this virus in a residential building in Hong Kong [36,37].

The presence of viable SARS-CoV-2 viral particles in natural waters, as a result of inadequate sanitation or the release of untreated sewage into surface waters like lakes, rivers, and streams, has also raised concerns about the potential spread of virus to humans through recreation and fishing activities, as seen by other diseases transmitted by enteric viral pathogens [31,38]. The dissemination of the new coronavirus via untreated wastewater, besides representing a threat to humans, also poses a considerable risk of causing devastating impacts on populations of susceptible species, particularly terrestrial and marine mammals [39,40]. Additionally, the emergence of the new coronavirus in those environments has also raised concerns about the transmission of the SARS-CoV-2 to both domesticated and wild animals, novel hosts that would tend to favor the spread, resurgence and evolutionary adaptation of the pathogen in future outbreaks through cross-infection [41-45].

Considering that limiting the spread of SARS-CoV-2 in the environment has proven to be of great importance in controlling the COVID-19, and that the safe disposal or reuse of wastewater has directly depended on the effectiveness of the processes for treating them, [46,47]. The aim of this review was to describe the main techniques for detecting the SARS-CoV-2 in aquatic matrices, and to report its occurrence and viability when present in those environments. The review provided a comprehensive overview of the conventional and emerging technological methods that are capable of inactivating or removing the SARS-CoV-2 from wastewater, including their mechanisms of action. The article also highlighted important strategies that could be used to mitigate the dissemination of the virus, such as the integration of treatment methods, the decentralization of treatment plants, the epidemiological surveillance of wastewater, and major challenges encountered by researchers and professionals engaged in the disinfection of SARS-CoV-2 in this complex matrix.

2. METHODOLOGY

The review article was based on scientific works published in English or Portuguese and indexed in virtual databases such as Google Scholar, Science Direct, Web of Science, and Scopus. The electronic searches were conducted until March 08, 2023, and included documents available through academic institutions and open access sources. The searches used a combination of terms including "SARS-CoV-2", "COVID-19", "Characteristics", "Manifestations", "Symptoms", "Prophylaxis", "Sewage", "Wastewater", "Detection ", "Viability", "Risk", "Hazard", "Treatment", "Disinfection", "Primary", "Decantation", "Coagulation", "Secondary", "Biological", "Activated Sludge", "Biological Digestion ", "Tertiary", "Chlorination", "Ozonation", "Ultraviolet", "Membranes", "Filtration", "Emerging", "Technology", "Trends", "Retention Ponds", Sunlight", Algae", "Photocatalysis", Thermal", "Heat", "Ceramic", "Integration", "Decentralization", "Monitoring", "Surveillance", "Early warning", and "Challenges". To verify the presence of duplicates and ensure the relevance of the works to the article's theme, the documents were refined based on their titles and contents. After refining the documents, they were then classified into the following topics: (a) Characteristics, manifestations and prophylaxis of SARS-CoV-2/COVID-19, (b) Detection and risks regarding the occurrence of SARS-CoV-2 in sewage, (c) Conventional treatments related to SARS-CoV-2, (d) Emerging technologies related to SARS-CoV-2 disinfection, (e) Wastewater surveillance and epidemiology, and (f) Major challenges and issues.

3. REVIEW

After combining the keywords mentioned in the previous topic, the databases were searched and 608 scientific articles were retrieved. After screening the articles based on their titles and contents, 601 were found to fit the topic proposed in this review, while 7 were either duplicates or did not fit. Out of the 601 relevant articles, 172 were classified as belonging to topic (a), 201 as belonging to topic (b), 43 to topic (c), 60 to topic (d), 76 to topic (e) and 49 to topic (f). After fully reading all 601 articles, 253 were selected as a theoretical basis for this review and included in the references. Of the 253 articles selected, 236 were novel investigations or literature reviews, and 17 were preprints. The methodological path used to prepare this review is illustrated in Figure 1.

3.1. Occurrence of the SARS-CoV-2 in aquatic matrices

SARS-CoV-2, commonly found in bodily fluids excreted by individuals with COVID-19, such as nasopharyngeal mucus, urine, feces and vomit, has commonly entered in sewer systems through wastewater discharges from hospitals, isolation centers, and residences populated or frequented by infected people [4,37,48]. The virus's presence in that environment has been previously reported in samples collected from various countries, including Spain, Brazil, Italy, United Kingdom, Netherlands, United States of America, Japan, Australia, China, Finland, India, Pakistan, Mexico, and Turkey (Table 1). Nontreated or inadequately treated wastewater discharges into natural bodies of water, possibly through leaks, illegal discharges or infrastructure failures, has also leaded in the detection of the SARS-CoV-2 in receiving natural water bodies, such as streams, rivers and groundwater. The following table summarized detection reports of fragments of the SARS-CoV-2 viral genome in different samples from aquatic environments.

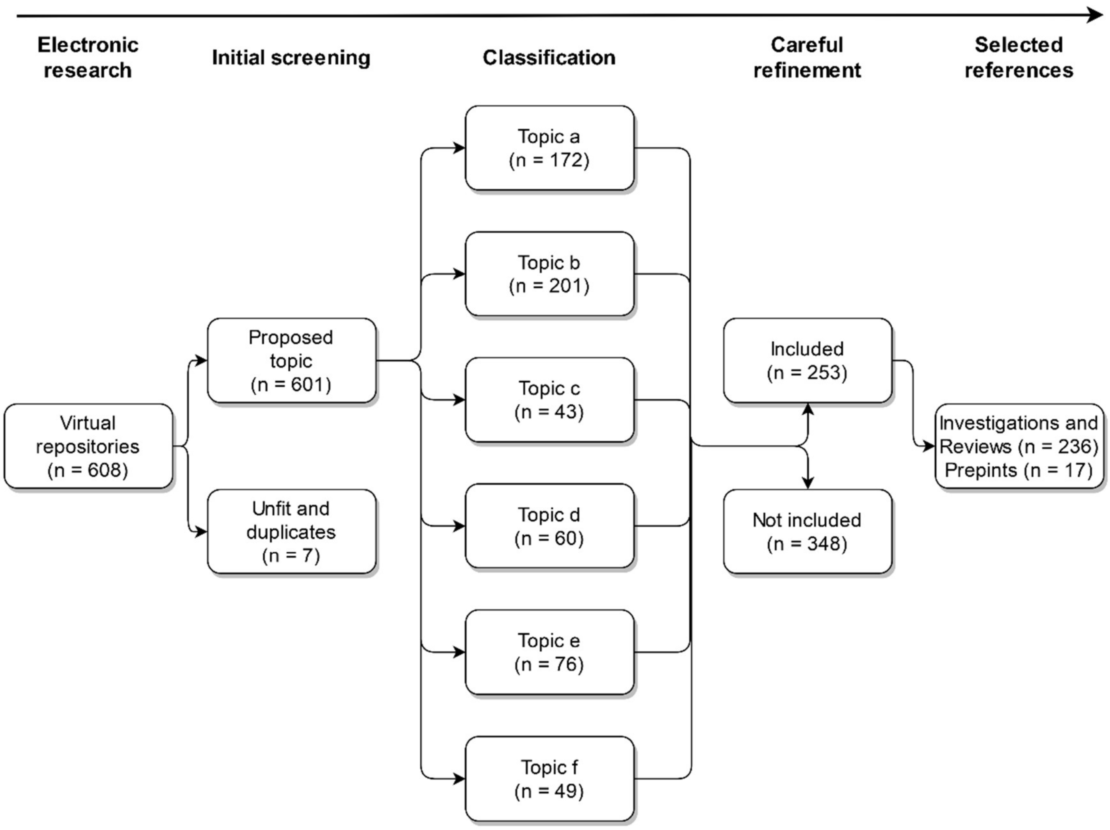

Figure 1. Methodological workflow used in the preparation of this review.

Table 1. Detection of fragments of the SARS-CoV-2 genome in samples obtained in untreated wastewater, waste sludge from treatment plants, treated wastewater, natural waters and environments.

Country	Location	Sample source	Detection date	Genome concentration	Detection assay	Reference
Spain	Barcelona	Untreated sewage	2019/03/12	$6.4x10^2$ and $8.3x10^2$ per L	RT-qPCR	[49]
Brazil	Florianópolis, Santa Catarina	Untreated sewage	2019/11/27	5.49±0.02 log_{10} per L	RT-qPCR	[50]
Italy	Bologna	Untreated sewage	2019/12/18	4.1×10^3 per L	RT-qPCR and Nested RT-PCR	[51]
United Kingdom	Southeast Region	Untreated sewage	2020/02/11	Qualitative	Nested RT-PCR	[52]
Spain	Valencia	Untreated sewage	2020/02/24	5.22 and 5.99 log_{10} per L	RT-qPCR	[53]

Country	Location	Sample source	Detection date	Genome concentration	Detection assay	Reference
Netherlands	Den Haag	Untreated sewage	2020/03/04	1.2×10^{1} and 2.2×10^{1} per mL	RT-qPCR	[54]
United States of America	Southeastern Virginia	Untreated sewage	2020/03/11	Less than 300 per 100mL	RT-ddPCR	[55]
United States of America	Massachusetts	Untreated sewage	2020/03/18	~50 per mL	RT-qPCR	[56]
Japan	Ishikawa and Toyama municipal administrations	Untreated sewage	2020/03/19	2.8×10^{4} per L	RT-qPCR and Nested RT-PCR	[57]
Australia	Southeast Queensland	Untreated sewage	2020/03/27	12 per 100 mL	RT-qPCR	[58]
United States of America	Bozeman, Montana	Untreated sewage	2020/03/30	528.9±249 and 665.6±261.5 per L	RT-qPCR	[59]
United States of America	Southern Louisiana	Untreated sewage	2020/04/08	3.2±0.4 and 3.0±0.3 per L	RT-qPCR	[60]
United States of America	Detroit, Michigan	Untreated sewage	2020/04/08	$9.87x10^{4}\pm3.43x10^{4}$ per L	RT-qPCR	[61]
China	Dongxihu district	Untreated sewage	2020/04/11	$7.4x10^{3}$ per L	RT-qPCR	[62]
Japan	Yamanashi municipal administration	Untreated sewage	2020/04/14	$<4.0x10^{3}$ and $<6.8x10^{4}$ per L	RT-qPCR	[63]
Brazil	Niterói, Rio de Janeiro	Untreated sewage	2020/04/15	-	RT-qPCR	[64]
Finland	Helsinki	Untreated sewage	2020/04/19	2.6±0.2 $\log_{10}$ per 100 mL	RT-qPCR	[65]
Czech Republic	Various Regions	Untreated sewage	2020/04/26*	-	RT-qPCR	[66]
India	Amedabad, Gujarat	Untreated sewage	2020/05/08	-	RT-qPCR	[67]
Pakistan	Lahore, Punjab	Untreated sewage	2020/07/13	0.60 and 4.55 $\log_{10}$ per mL	RT-qPCR	[68]
United States of America	New Haven, Connecticut	Primary sludge	2020/03/19	$1.7x10^{3}$ to $4.6x10^{5}$ per mL	RT-qPCR	[69]
Spain	Orense	Primary sludge	2020/04/07	1.3 per mL	RT-qPCR	[70]
Mexico	Santiago de Queretaro	Activated sludge	2020/04/23	3.5 $\log_{10}$ per mL	RT-qPCR	[71]
Turkey	Istanbul	Activated sludge	2020/05/07	$1.17x10^{4}$ to $4.02x10^{4}$ per L	RT-qPCR	[72]
Sweden	Gothenburg	Treated sewage	2020/02/18*	0.14 to 1.87 $\log_{10}$ per L	RT-qPCR	[73]
France	Paris	Treated sewage	2020/03/05	-	RT-qPCR	[74]
Iran	South of Tehran	Treated sewage	2020/04/20	-	RT-qPCR	[75]
Germany	North Rhine-Westphalia	Treated sewage	2020/04/08	$\sim10^{1}$ per mL	RT-qPCR	[76]
Israel	Jerusalem	Treated sewage	2020/04/21	-	RT-qPCR	[77]

Country	Location	Sample source	Detection date	Genome concentration	Detection assay	Reference
Italy	Padua, Veneto region	Treated sewage	2020/04/23	-	RT-qPCR	[78]
Chile	Santiago	Treated sewage	2020/05/25	10 and 20 per mL	RT-qPCR	[79]
Iran	Tehran	Treated sewage	2020/06/30	-	RT-qPCR	[80]
Tunisia	Monastir region	Treated sewage	2020/09/22	-	RT-qPCR	[81]
India	Ahmedabad	Treated sewage	2021/02/08	-	RT-qPCR	[82]
China	Wuhan	Effluent from a hospital septic tank	2020/02/26	$14.7 \pm 2.2 x 10^3$ per L	RT-qPCR	[83]
Italy	Milan metropolitan area	River water	2020/04/14	-	RT-qPCR	[84]
Brazil	São Paulo City	Stream water	2020/05/04	$2.26x10^6$ and 1.44×10^6 per L	RT-qPCR	[85]
Ecuador	Quito	River water	2020/06/05	$2.07x10^5$ to $3.19x10^6$ per L	RT-qPCR	[86]
Brazil	State of Minas Gerais	River water	2020/08/early days*	$1.1x10^2$ per mL	RT-qPCR	[87]
Mexico	Mexico City	River water	2020/09/22	18 to 79 per mL	RT-qPCR	[88]
Serbia	Belgrade	River water	2020/12/10	$5.97x10^3$ to $1.32x10^4$ per L	RT-qPCR	[89]
Nepal	Kathmandu Valley	River water	2020/07 to 2021/02	4.0 ± 1.2 to 5.1 ± 0.6 $\log_{10}$ per L	RT-qPCR	[90]
Mexico	Monterrey metropolitan area	Groundwater	2020/10/29	-	RT-qPCR	[91]
Tehran	South region	Irrigation water	2020/09 to 2021/01	-	RT-qPCR	[92]
Spain	Galicia	Estuarine sediment	2020/05/06	3.34 and 3.60 log per g	RT-qPCR	[93]

* approximate date

3.2. Detection methods of the SARS-CoV-2 in aquatic matrices

The detection of SARS-CoV-2 in aquatic matrices, including in wastewater, have been commonly performed using a molecular biology technique named RT-PCR (Reverse Transcription followed by Polymerase Chain Reaction), which amplifies and identifies fragments of the virus' genetic material from *in-vitro* samples [94]. Most of the detections, however, have been conducted through a similar technique, called real-time PCR or quantitative PCR (RT-qPCR), which simultaneously amplifies fragments of the viral RNA and quantifies the presence of specific target sequences [95]. Both RT-PCR and RT-qPCR techniques have been considered as highly sensitive, specific, and the gold standard for detecting low amounts of genetic material in different samples [94]. The methods, according to previous studies, were successfully able to determine the diversity and abundance of several viral pathogens in wastewater, including Enterovirus, Hepatitis A and E, Rotavirus, Adenovirus, Norovirus, Sapovirus, Human Herpesvirus 6 and 8, Polyomavirus, Papillomavirus and Zika virus [96-99].

The SARS-CoV-2 unique sequences used in the molecular methods, called primers or initiator oligonucleotides, have targeted the viral RNA polymerase gene RdRP, the nucleocapsid proteins N, N1 and N2, the envelope protein gene E, and the spike protein gene S [95,100]. In the RT-qPCR reaction, the target sequences have been labeled with fluorescent dyes like 6-carboxyfluorescein (FAM), carboxyrhodamine (ROX) and tetrachlorofluorescein (TET), as well as quencher dyes like Blackberry quencher (BBQ) or Black Hole quencher (BHQ1), commonly referred as hybridization probes or reporters. Those dyes have allowed the quantification of the target RNA through the measurement of the fluorescence emitted from the labeled probes during the RT-qPCR amplification [95,101].

3.3. SARS-CoV-2 viability in aquatic matrices

Methods based on RT-PCR and RT-qPCR molecular techniques for detecting the SARS-CoV-2 genomic material, although reliable, have not been able to determine the viability of the viral particles, in other words, the ability of the virus to attach to the host cell, inject its nucleic acids, and replicate enough to cause infection in susceptible organisms [102]. Thus, in order to assess the potential threat to human and animal health, virological methods based on *in vitro* cell culture techniques have been used to provide estimates of the infectious potential or the viability that the SARS-CoV-2 has when present in aquatic matrices [103].

Research indicated that the SARS-CoV-2, despite highly dependent on the characteristics of the environment, such as the physical-chemical composition, temperature, pH, and the presence of antagonist microorganisms [100,104,105], was able to remain infective for up to 4 days in sewage at a temperature of 24°C and 17.5 days at a temperature of 4°C [106]. When it comes to natural waters, it was found that the SARS-CoV-2 was able to remain infective for up to 6.4 days in river water at 24°C and 18.7 days at 4°C [106].

Due to the fact that the survival time of the SARS-CoV-2 in wastewater could be potentially long enough for the virus to reach wastewater treatment plants (WWTPs) and be disseminated through aquatic matrices, effective wastewater treatment processes have been considered as crucial in minimizing the spread of the virus and reducing the risk of its transmission [40,83,107-110]. The viability that infectious particles of SARS-CoV-2 were seen to have when artificially inoculated in different aqueous media was summarized in Table 2.

Table 2. Persistence of the viability of SARS-CoV-2 in different samples of sewage and natural waters when artificially inoculated.

Country	Sample Location	Matrices	Temperature	T_{90} (days)*	T_{99} (days)**	Virion survival time (days)	Author
Brazil	Nova Lima, Minas Gerais State	River water	24°C	1.9	6.4	-	[106]
			4°C	7.7	18.7	-	
		Wastewater	24°C	1.2	4.0	-	
			4°C	5.5	17.5	-	
Ireland	Dublin	River water	4°C	3.8	-	-	[111]
			20°C	2.3	-	-	
		Seawater	4°C	2.2	-	-	
			20°C	1.1	-	-	
United States	Northern Indiana	Wastewater	20°C	1.6–2.1	3.2–4.3	-	[112]
		Tap water	20°C	2.0	3.9	-	
France	Paris area	Wastewater	4°C	-	-	up to 1	[113]
		Wastewater	20°C	-	-	up to 1	
United Kingdom	River Thames	River water	Room	-	-	<3	[114]
		Sediment	Room	-	-	<3	

Country	Sample Location	Matrices	Temperature	T_{90} (days)*	T_{99} (days)**	Virion survival time (days)	Author
Japan	Nagasaki	Mineral water	4°C	-	-	35	[115]
		Tap water	4°C	-	-	56	
		Distilled water	4°C	-	-	77	
Korea	Inje-gun and Sokcho	Tap water	23°C	-	-	6	[116]
		Fresh water	23°C	-	-	2	
		Seawater	23°C	-	-	1	

*Reduction time of 90% of viable virions. **Reduction time of 99% of viable virions.

3.4. Inactivation and removal of SARS-CoV-2 from wastewater

Wastewater treatment methods have been conventionally designed to reduce or eliminate suspended and dissolved solids, organic matter, contaminants, heavy metals and pathogens from aqueous matrices [117,118]. In most cases, the process has been carried out in specific treatment units, known as preliminary, primary, secondary, and tertiary units. Preliminary and primary treatment units have aimed to equalize the pH and the temperature, as well to remove coarse particles, such as sand, fibers, oils, fats, and colloidal matter from the wastewater using grids, sieves, sedimenters, floaters or primary clarifiers [108,119]. The secondary treatment unit, situated subsequent to the primary one, have employed biological processes in aerobic and anaerobic bioreactors to remove the biodegradable organic matter and residual solids from the wastewater [118,120]. The treatment process has often included a third unit to remove the remaining compounds from the wastewater, such as dissolved salts, recalcitrant molecules and pathogens [119].

Studies have indicated that all three operational units have played key roles in inactivating and removing viral particles from wastewater [121]. The researchers have been crucial in understanding the mechanisms involved in the decay of pathogenic viruses during the removal or disinfection steps by treatment technologies, and in developing better strategies to decrease the spread of pathogenic viral strains, including the SARS-CoV-2 [122]. It is noteworthy that, given the potential lethality of SARS-CoV-2 for humans, appropriate measures have been implemented in regards to scientific investigations. In general, researchers have used non-pathogenic viral strains that structurally and morphologically resemble the SARS-CoV-2, such as the Bovine coronavirus (BCoV), Pepper Mild Mottle virus (PMMoV), *Pseudomonas* phage φ6, Murine Hepatitis virus (MHV), and non-infectious nucleotide sequences like Hep G Armored RNA [55,123-125]. The main primary, secondary and tertiary methods that have been used to inactivate or remove SARS-CoV-2 and its surrogates in WWTPs were described in the sections below.

3.4.1. Primary sedimentation

The first treatment process in conventional WWTPs, following sieving and preliminary equalization, has been typically the removal of solids and colloidal particles through coagulation, flocculation, or adsorption methods using specific equipment such as sedimenters, floaters, or clarifiers [118-120]. The methods are based on the effects of attraction and repulsion that the electric charges of the particles exert on each other, in other words, the affinity between the constituents. Due to their negative liquid electrostatic charge, viral particles can be adsorbed and agglomerated on solid surfaces with a positive charge, enabling subsequent removal using phase separation methods [126-128]. The mechanism is influenced by the hydrophobicity of the viral particle surfaces and mainly involves destabilization and coordination reactions between the ionized species of the sedimentation reagents and the carboxyl groups of the viral capsids [108]. Another process reported in this step involves viricidal effects exhibited by some reagents used as sedimentators. These effects included damage to the viral capsid and blockage of the viral particles' binding sites, thus preventing their connections with host cells [108,129].

According to research, based on the selected chemical agents, the primary sedimentation process could achieve considerable removal of human enteric viruses, including Poliovirus, Norovirus, Coxsackievirus and the bacteriophages models Q*β*, MS2, P1 and T4 [130-132]. Studies carried out in real WWTPs, however, have demonstrated that the removal of fragments of the SARS-CoV-2 viral genome has been insufficient in the primary sedimentation stage [70,77,133,134]. Thus, while the primary sedimentation process has been deemed crucial in removing colloidal particles and also highly important in removing a substantial portion of SARS-CoV-2 viral particles, [135] complete elimination of the new coronavirus in the treatment of wastewater necessitates the implementation of additional treatment processes.

3.4.2. Secondary biological processes

The secondary treatment stage, which has been commonly placed after the primary treatment unit, generally has employed biological treatment steps conducted under aerobic, anaerobic, or combined conditions [119,120,136]. The aerobic treatment process involves the use of aerated tanks to promote the activity of bacterial consortia that degrade organic matter in the presence of free oxygen. The anaerobic one, employed in bioreactors in the absence of free oxygen, has been used to catabolize the organic matter in the effluent and stabilize residual sludge from aerobic treatments, reducing the amount of biomass produced in biological units [121,135]. Biological treatment stages were also seen to provide secondary sedimentation processes that had the capability to adsorb, agglomerate, and retain organic compounds and suspended solids present in the waste sludge [46,137].

In the biological treatment unit, viral particles have been inactivated by the activity of microorganisms, including heterotrophic bacteria, actinomycetes, algae, protozoa, metazoan, and other higher organisms [121,138,139]. Those microorganisms, which play a crucial role in the treatment process, have effectively removed viral particles through predation or by synthesizing antagonistic substances, such as antiviral compounds and extracellular enzymes such as hydrolases, proteases, and nucleases [43,138,140,141]. Furthermore, the secondary sedimentation process that occurs in the biological treatment unit was seen to provide a sorption effect of viral particles in organic compounds, allowing the decantation of these particles through agglomeration into larger biomass flakes [46,142,143].

Another aspect refers to the abiotic factors provided by the environmental conditions in the treatment bioreactors, such as pH, exposure to UV light and temperature [104,105]. Among those factors, temperature was seen as a central factor in the inactivation of viral pathogens in the biological treatment units, specially enveloped viruses such as SARS-CoV-2 [121,140]. Anaerobic processes that reached temperatures of 56°C for 90 minutes, for example, were responsible for significantly reducing the infectivity of Coronavirus strains [144]. The effects were believed to be related to an increase in the activity of extracellular enzymes excreted by antagonistic microorganisms, in addition to the denaturation of proteins, viral nucleic acids, and destruction of the viral capsid [139,140].

It is noteworthy that despite the fact that studies have shown that secondary treatment units in WWTPs have been effective in removing and inactivating a considerable proportion of enteric viruses, [145,146] recent research has demonstrated that those units have not yet been successful in completely removing the SARS-CoV-2. The studies, which were conducted in difference locations in the globe and considered significantly operational differences among the WWTPs, have shown that fragments of the SARS-CoV-2 genome could still be detected in secondary effluent samples [63,77,81,134,147-149]. Hence, the implementation of tertiary treatment units has been strongly recommended in order to minimize the risk of SARS-CoV-2 transmission through aquatic matrices.

3.4.3. Tertiary disinfection processes

As previously mentioned, the complete elimination of SARS-CoV-2 in WWTPs has primarily been accomplished through the use of disinfection methods, which are typically applied after the secondary biological unit, commonly referred to as the tertiary treatment stage [105,110,150]. Those tertiary strategies, when dealing with contaminated wastewater with SARS-CoV-2, have been developed by separating or applying environmental stress on essential structures of the virus, that is, its three functional constituents: the genomic material, the protein capsid or the lipid envelope [102,118]. The available technologies have mainly included chemical methods, such as chlorination and ozonation, and physical methods like ultraviolet radiation and membrane filtration. The characteristics of each of those treatment technologies were discussed in more detail in the following topics.

3.4.3.1. Chlorination

The chlorination method has been based in the use of chemical oxidants that have chlorine in their composition and the capacity to inactivate pathogenic viruses suspended in aquatic matrices, such as sodium hypochlorite (NaClO), chlorine gas (Cl_2), and chlorine dioxide (ClO_2) [121]. The addition of those compounds in aqueous solvents has led to the formation of oxidizing chemical species, such as hypochlorous acid HClO or hypochlorite ion OCl^-, that attack the viral genome and capsid, prevent the replication of infectious viral particles, and reduce the ability of viruses to infect hosts [102,117,127]. According to research, Coronavirus strains have been shown to be extremely sensitive to chemical oxidants, such as sodium hypochlorite and chlorine dioxide [151]. Dosages above 10 mg/L of chlorine from sodium hypochlorite at a temperature of 20°C, with a contact time of 30 minutes and a free residual chlorine value above 0.4 mg/L, according to Wang *et al.* [152], were sufficient for completely inactivating the SARS-CoV-1 virus present in wastewater. Chlorine dioxide, in turn, was responsible to complete inactivate the virus after 30 minutes of disinfection at concentrations of 40 mg/L and 2.19 mg/L of free residual chlorine under the same conditions as in the previous study [152].

Due to the similarity between SARS-CoV-2 and SARS-CoV-1, it has been believed that the new coronavirus is also sensitive to environmental factors and disinfectants [153]. To date, evidence includes research by Chin *et al*. [23], which showed that SARS-CoV-2 was inactivated after 5 minutes of contact time with bleach diluted 1:49, and a study by Chan *et al.* [154], which proved that sodium hypochlorite at a concentration of 10% could inactivate the new coronavirus in 1 minute. Other studies include a research promoted by Takeda *et al.* [155], who indicated that the virucidal effect of acid electrolyzed water on SARS-CoV-2 depended on the amount of available free chlorine, and an investigation by Xiling *et al.* [156], who demonstrated that available chlorine concentrations of 250, 500 and 1000 mg/L required respectively 20 min, 5 min and 0.5 min to completely inactivate the new coronavirus in aqueous suspension. In the case of samples obtained from real WWTPs, Hemalatha *et al.* [157] showed that concentrations above or equal to 2% of sodium hypochlorite were responsible for completely inhibiting the detection of the SARS-CoV-2 genome, indicating a likely inactivation of the viral genetic material.

Chlorination, due to its operational simplicity, technical viability and cost-effectiveness, has been widely recommended and used as a disinfection unit in WWTP [117,127,158]. In those places, the chlorination process has been carried out in a tertiary stage, generally in tanks that allow the application of chlorine in the form of gas or sodium hypochlorite (NaClO). The disinfection guidelines recommended by the World Health

Organization have been a residual concentration of free chlorine of more than 0.5 mg/L for at least 30 min of contact at pH less than 8.0 [159]. It should be noted, however, that the efficiency of chlorine in inactivating viral particles, including enveloped viruses such as SARS-CoV-2, has been influenced by several critical factors, such as contact time, dose, pH, temperature and wastewater composition, in addition to the operational conditions of the treatment plants [75,121].

Studies performed at real WWTPs in different locations, for example, indicated that, in some cases, the chlorination process in the tertiary stage was not 100% effective in removing SARS-CoV-2 genetic material from wastewater [75,77]. The situation tended to get worse in places whose wastewater was more likely to receive the new coronavirus, such as sewage from hospitals that received patients with COVID-19. In those environments, it was seen that the dosage of 800 g/m^3 of sodium hypochlorite and concentrations greater than 0.5 mg/L of free chlorine with a contact time above 1.5 h were not able to guarantee the total disinfection of SARS-CoV-2 [83]. According to the study, the complete deactivation of SARS-CoV-2 was only guaranteed when doses of 6700 g/m^3 of NaClO were added to hospital wastewater [83]. The use of high doses of chlorine, however, has the possibility of leading to the formation of disinfection by-products (DBPs), an undesirable problem that can cause damage to the environment and human health, including mutagenic, carcinogenic, and teratogenic effects [127]. Thus, modeling and optimizing the amount of residual chlorine required to inactivate the SARS-CoV-2, as well as developing new disinfection methods for the virus and other pathogens in WWTPs have been endorsed [160-162].

3.4.3.2. Ozonation

The wastewater treatment method of ozonation has involved injecting ozone gas (O_3) during the tertiary disinfection process. The addition of ozone gas, which exhibits a high oxidation potential of 2.07 V, was seen to form secondary oxidants, known as hydroxyl radicals, that cause the inactivation of numerous pathogenic microorganisms, such as bacteria, protozoa, parasites and also viruses [118,121,163]. Ozone is believed to attack viral particles through either a direct reaction mechanism or indirectly through free radicals and reactive oxygen species (ROS) produced in the decomposition of the ozone, such as $\cdot OH$, $O_2{\cdot}^-$ and H_2O_2 [164]. The reaction has acted by destroying the essential structures of viruses, both externally and internally by diffusion, such as lipids, proteins, and viral nucleic acids [136,165,166]. Additionally, the reactions between the ozone-generated free radicals and the viral constituents have tended to form other reactive radicals, such as $RCOO^-$, which propagate the oxidation through a chain reaction [164].

According to Wang *et al.* [163], the disinfection of domestic sewage with 6 mg/L of ozone, after conventional activated sludge and chemical coagulation, reduced the concentration of adenovirus by 55% to 91%, norovirus GII by 85% to 100%, astrovirus 4 and parvovirus by 99% to 100%, and pecovirus, picobirnavirus, and gokushovirus to undetectable levels. The use of ozone gas with doses of 50 mg/m^3 and a contact time of 10 minutes was also able to reduce by more than 99.9% the presence of several infectious viruses on contaminated surfaces, including the Herpes simplex, Rhinovirus, Poliovirus, Influenza, Adenovirus III and XI and Murine coronavirus [167,168]. Regarding SARS-CoV-2, ozone gas exposure for 30 seconds at 10,000 ppm (20 g/m^3), 5 min at 4,000 ppm, and 10 min at 2,000 ppm was responsible to completely eliminate the presence of the virus's genetic material on personal protective clothing (PPE) and face masks [169]. Exposure to ozone gas at a concentration of 6 ppm for a contact time of 55 min was also found to significantly inactivate the SARS-CoV-2 virus when deposited on stainless steel plates [170]. Furthermore, a research has established that an aqueous solution containing 0.6 ppm of ozone was capable of significantly reducing the infectivity of SARS-CoV-2 after just 1 min of exposure [171].

It should be noted, however, studies performed in real WWTPs have indicated that, occasionally, the ozonation process in the tertiary treatment stage, although it appeared to reduce the presence of SARS-CoV-2 fragments, was not always effective in completely removing the new coronavirus genetic material from wastewater [76]. Moreover, substantial practical challenges have been considered regarding the application of ozonation in wastewater treatment. According to research, further studies have been necessary to confirm the effectiveness of ozonation in large-scale wastewater treatment and its cost-effectiveness. Additionally, optimized protocols have been needed to define the best methods for disinfecting pathogens, including the SARS-CoV-2 virus [166,172]. Researchers, moreover, have recommended the development of safety protocols in order to promote a safe and effective application of ozonation in wastewater treatment, especially regarding the formation of by-products associated with the use of ozone gas [172].

3.4.3.3. Ultraviolet irradiation

The disinfection process by ultraviolet (UV) irradiation has been based on a system that involves passing water through tubes that emit electromagnetic waves with wavelengths ranging from 200 nm to 400 nm [127,173]. Light within those wavelengths, traditionally generated by mercury lamps, has been shown to compromise the stability of the C=C bonds of some molecules, such as pyrimidines, purines and flavins, disrupt phosphodiester bonds and cross-links in viral DNA/RNA, and lead to the formation of pyrimidine dimers: uracil and cytosine in RNA, thymine and cytosine in DNA [158,166]. The use of this type of method, therefore, tends to cause direct damage to viral nucleic acids, either DNA or RNA, and disrupt several biochemical processes, including the DNA replication, RNA transcription and translation of proteins [127,158]. The effects include the inhibition of viral replication due to the destruction of their genomes and proteins, and consequently the interruption of their propagation in the environment [166,173].

UV radiation has been shown to effectively control the growth of microorganisms in various matrices, including air, water, and surfaces [118]. When applied in real wastewater treatment plants, after the stage of the activated sludge process, the UV disinfection method was able to significantly reduce the concentration of Rotavirus and Human Adenovirus, and completely inhibit the detection of Norovirus II and Human Astrovirus [174]. In samples obtained from combined sewage, the method was also responsible for reducing the concentration of Human Adenoviruses by 1.0±3.3 $\log_{10}$, Human Polyomaviruses by 1.5±3.9 $\log_{10}$, Enteroviruses by 1.3±2.6 $\log_{10}$, Norovirus II by 0.8±2.4 $\log_{10}$, and reducing Rotavirus levels below detection limits [175]. When it comes to coronavirus species, studies have indicated that exposure to UV radiation for 6 minutes was able to completely inactivate the SARS-CoV-1, 5 minutes the MERS-CoV, and 9 minutes the SARS-CoV-2 [176-178].

It is noteworthy that, according to research, viruses, in particular, were seen to be more persistent towards the UV irradiation than other pathogenic organisms, such as non-bacterial spores and protozoa, like cysts of *Cryptosporidium* and *Giardia* [179]. Furthermore, the efficiency of disinfection by UV irradiation was seen to be drastically reduced through the biofouling of the lamp due to the growth of microorganisms and the existence of colored substances that provide a protective shield to pathogens [118]. Therefore, it has been recommended that disinfection by UV irradiation be carried out after the secondary treatment, when the wastewater being treated has lower turbidity rates and, consequently, lower microbial shielding effects caused by suspended colloidal particles [118]. Even thought, studies performed in a real WWTP have indicated that disinfection using terminal UV lamps as a tertiary treatment was occasionally not effective in completely removing the SARS-CoV-2 genetic material from wastewater [78]. In that matter, it has been widely recommended processes optimizations regarding adequate inactivation efficiencies for SARS-CoV-2 and other pathogenic viruses [128].

3.4.3.4. Membrane filtration

The membrane disinfection process has been based on the separation of pathogens from the aqueous matrix through a physical barrier, typically a porous structure where treated water passes and solid particles are intercepted and attracted [127,121]. This type of technology, also called membrane filtration or membrane disinfection, has been considered a robust and non-toxic technique that has gained wide attention in recent decades due to its compactness, modularity, and effectiveness [46,128,136]. Several types of membranes have been used in this type of treatment, classified according to the size of their pores [47]. Microfiltration has used membranes with pore sizes ranging from 50 to 500 nm, ultrafiltration membranes with pore sizes 2-50 nm, and nanofiltration membranes with pores smaller than 2 nm [180]. Viral particles, which have sizes from 5 to 300 nm, have been removed through methods that use ultrafiltration and nanofiltration membranes [126]. As SARS-CoV-2 is approximately 125 nm, the use of ultrafiltration or nanofiltration filters has also been considered to be effective in removing this pathogen [126]. In addition, the filtration capacity was seen to be improved by using a filtration membrane with specific characteristics that attract viral envelopes due to their physicochemical interactions, for example, hydrophobic and electrostatically charged regions [143,136].

According to studies, the microfiltration method with a 0.22 μm hydrophobic membrane was capable of achieving a removal rate of over 91% of the Poliovirus, while ultrafiltration was able to remove concentrations above 4 $\log_{10}$ of Adenovirus, Coxsackievirus, Hepatitis A virus and Murine Norovirus strains [181,182]. The SARS-CoV-2, moreover, was removed from the air at rates above 90% by electrostatically charged nanofiber filters, which provided an interaction with its negatively charged surface [183]. It is significant to note that, however, the use of a filtration method as a tertiary treatment in real WWTP was not effective in completely removing the SARS-CoV-2 viral RNA from wastewater [76]. Although the filtration method mentioned in the study above was not fully detailed, the use of membrane technology as a virus disinfection strategy has required optimization of several parameters that have been shown to impact its effectiveness, including salt concentration, residual water flow, temperature, pH, and other factors [162,184].

3.5. Emerging technological perspectives

Although conventional primary sedimentation, secondary biological, and tertiary disinfection methods have been shown to remove significant amounts of SARS-CoV-2 from wastewater, occasionally, those processes were not always effective in completely removing the new coronavirus' RNA in real WWTPs [75-78]. In this context, novel disinfection technologies have been researched to improve the efficiency of removing SARS-CoV-2 and other pathogens from wastewater. The researches, in addition to increasing the removal and inactivation rate of pathogens, have aimed to decrease the amount of energy and chemicals required for the treatments, which reduces the environmental impacts and promotes more sustainable wastewater treatment process. Studies in this field have also been used to implement more cost-effective processes, making them accessible to low-income areas and remote locations. The following topics addressed the characteristics of some of the emerging treatment technologies and their disinfection mechanisms. These included retention ponds, sunlight disinfection, algae treatment, photocatalysis, thermal treatment, and ceramic membranes (Figure 2).

3.5.1. Retention ponds

Retention ponds, also known as detention ponds, have been defined as a technique that involves temporarily retaining and treating the wastewater in specifically designed structures. In the treatment process, the removal of viral particles has consisted of the combination of mechanisms, including sedimentation, predation by antagonistic organisms, and sunlight-mediated inactivation [185].

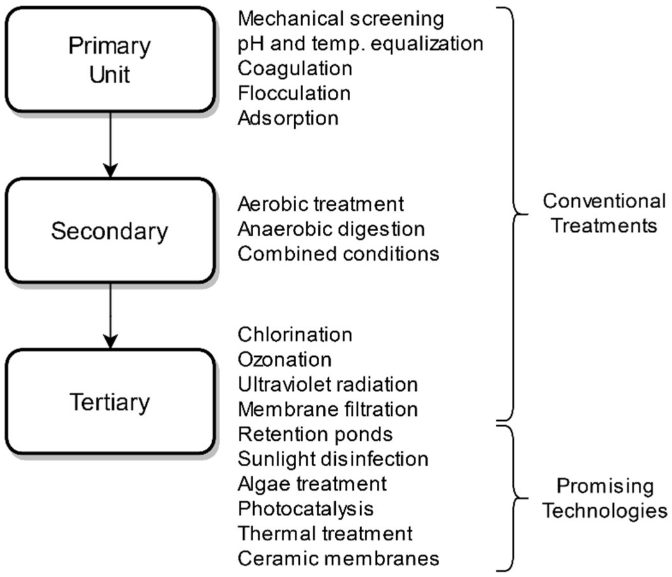

Figure 2. Conventional and promising sewage treatment units and methods.

The retention pond method, considered one of the simplest ways to handle wastewater, was capable of achieving an average reduction of 1 log_{10} of virus for every 15–21 days of retention [47,185]. The method could be applied as a precautionary measure in the management of residual sludge, particularly before its reuse in agriculture [150]. The principle of the method could be applied to provide longer hydraulic retention times (HRT) in conventional WWTPs, resulting in increased microbial inactivation, a particularly desirable effect in plants that operate under low-temperature conditions [104,118,186]. The effectiveness of systems based on retention ponds, moreover, could be improved through operational optimizations, such as the implementation of greater numbers of treatment stages and systems that promote the increase of wastewater temperature [47,118].

3.5.2. Sunlight disinfection

Sunlight disinfection has been based on systems developed to promote the inactivation of viruses through electromagnetic radiation emitted by the sun in specific locations, such as drains and stabilization ponds, as well as natural bodies of water like streams. This technology, known as solar disinfection (SODIS), has been shown to facilitate the inactivation of viruses through both endogenous and exogenous processes. Endogenous processes include the absorption of sunlight in the UVB range, while exogenous processes involve the adsorption of sunlight by chromophores or external sensitizers that generate reactive species [121]. Furthermore, the increase in water temperature resulting from sunlight irradiation has also been observed to contribute to the inactivation of viral particles [185]. SODIS technology has been considered a promising approach for disinfecting wastewater due to the high availability of solar radiation, low cost, and sustainable nature of the treatment methods [136,187].

According to reports, the SODIS technique, using simulated sunlight, was capable of completely inactivating the Coxsackievirus-B5, Poliovirus-2 and Hepatitis A virus on aqueous suspensions after 1-2 hours of exposure [188]. The method, using natural solar light, resulted a loss of infectivity for Hepatitis A virus ranging from 33.4% to 83.4% after exposure times of 4 to 8 hours, and a loss of infectivity ranging from 33.4% and 66.7% for Murine Norovirus after 6 to 8 hours of exposure [189]. Noteworthy, studies on the effectiveness of SODIS process against viruses commonly found in water, including the SARS-CoV-2, have been scarce [190]. To ensure safe and effective viral inactivation of wastewater, it has been crucial to have reliable data on the effectiveness of SODIS treatment. Researchers have recommended to consider factors that affect the treatment, including the intensity of solar radiation, optical, physical, and chemical properties of the wastewater, the type of virus present, and also the seasonal variations in solar UV radiation [185,190].

3.5.3. Algae treatment

Algae-based wastewater treatment has also been considered an effective and sustainable method for removing organic compounds, nutrients, and viral particles from wastewater [191]. The technology has relied on the symbiotic relationship between photoautotrophic algae and heterotrophic bacteria, which work together to degrade and remove pollutants without the need for artificial aeration [192]. The cultivation of microalgae has generally been done in oxidation ponds and specialized photo-bioreactors [139]. Oxidation ponds have been designed as shallow lakes, similar to stabilization ponds, that allow the natural development of algae and bacteria to biodegrade pollutants [166]. Photo-bioreactors, on the other hand, have been designed as devices that provide a controlled environment for algae and other microorganism to grow and degrade pollutants using an artificial light source [193]. Under ideal conditions, algae-based wastewater treatment has been shown to promote algae growth and oxygen production, while effectively removing organic waste and nutrients from wastewater, as well as viral particles, including pathogens [161,162,166].

Algal ponds, according to studies, were able to reduce the concentration of the F-RNA bacteriophage indicator by 1.59 ± 0.82 $\log_{10}$, and to remove the concentration of somatic coliphages by 3.13 ± 0.34 log, F-specific coliphages by 1.23 ± 0.34 log, Enterovirus by 1.05 ± 0.32 log, and Norovirus GI by 1.49 ± 0.16 log in domestic wastewater [192,194]. The efficiency of algal ponds in removing somatic coliphages, F-specific coliphages, Enterovirus, and Norovirus GI from wastewater without the use of chlorination has been shown to be comparable to conventional WWTPs that used chlorination [195]. According to the mentioned study, the use of algal ponds resulted in a lower viral diversity in the effluent compared to common treatment systems.

With regard to SARS-CoV-2, recent studies indicated that algae ponds, when conducted after an anaerobic UASB reactor, were able to reduce the RNA concentration of the new coronavirus by 0.74 $\log_{10}$ [196]. The research also indicated that the algae system was able to remove the Human Adenovirus DNA concentration by 0.92 $\log_{10}$, and the F-specific coliphages by 1.70 $\log_{10}$ [196]. Microalgae-based treatments, although further evaluation is needed to determine their effectiveness in removing viral particles from wastewater, have been considered a promising alternative to commonly used and costly disinfection techniques, especially in rural and remote communities [139,194,192].

3.5.4. Photocatalysis

Photocatalytic disinfection has been based in a process that utilizes a semiconductor (typically TiO_2) to produce reactive substances, such as singlet oxygen, superoxide radicals, hydrogen peroxide, hydroxyl radicals, and perhydroxyl radicals, through the activation of light irradiation, both solar and artificial [127]. The formation of those substances, besides promoting the oxidation of inorganic compounds, also decomposes the structure of living organisms that are suspended in liquids or adhered to surfaces in contact with the semiconductor [197]. The reactive substances generated in photocatalytic processes have been shown to cause the destruction of the capsid of viral particles, leading to the release of the genetic materials, minerals, and proteins from the interior of the viruses, and resulting in their inactivation [198]. The compounds produced in photocatalytic processes also promote the complete mineralization of the organic components within the structures of viral particles [199].

Due to its high oxidative capacity and the possibility of using sources of synthetic or natural irradiation of radiant energy, photocatalytic disinfection has attracted considerable attention in recent years. Disinfection systems using the UV-assisted TiO_2 photocatalyst, for example, were able to inactivate 3.5 log_{10} of the Bacteriophage Qß suspended in pure water, and decrease more than 90% of the relative concentration of Noroviruses in a sewage effluent [200,201]. Photocatalytic methods have also been used to develop antibacterial and antiviral surfaces, known as photocatalytic surfaces, for inactivating bacteria and viruses. Those surfaces can oxidize and permanently inactivate pathogens in environments that receive sunlight or ambient lighting conditions, including indoor settings illuminated by lamps that emit visible light [202]. TiO_2 nanoparticles immobilized on a glass plate, for example, were able to reduce the concentration of the influenza virus by more than 4 log_{10} [203]. TiO_2 photocatalyst, when externally applied on a solidified agar matrix and activated by UVC light radiation, was able to reduce the presence of Murine Norovirus to undetectable levels after 5 minutes of treatment [204].

The use of other photoactive oxides, such as ZnO, CuO, Fe_2O_3, MgO, as well as the development of new semiconductors, such as those based on g-C_3N_4, metal-organic frameworks, layered double hydroxides, MXenes and doped metals, has also been prominent areas in the development of antimicrobial and antiviral materials [198,205]. The metal-doped catalyst Mn/Co-TiO_2, when irradiated by natural sunlight for 45 minutes, for example, was found to cause the inactivation of nearly 99% of the MS2 Bacteriophage in sewage samples [206]. A TiO2 film activated at the temperature of 30°C under visible light for 24 h, in turn, was able to reduce concentration of the Human Rotavirus by 1.50 log_{10}, the Simian Rotavirus by 2.78 log_{10}, the Human Astrovirus by 2.42 log_{10}, and the Feline Calicivirus by 1.95 [207].

A study involving SARS-COV-2 found that copper oxide nanoclusters grafted with titanium dioxide (Cu_xO/TiO_2) were able to inactivate the virus to undetectable levels after 2 hours of visible light irradiation and 3 hours of dark incubation [208]. In another study, boron-doped bismuth oxybromide (B-BiOBr) photoparticles resulted in a 5.32 log inactivation of the SARS-CoV-2 in 5 min under LED light irradiation [209]. AgNPs@TiO_2 particles coated on industrial ceramic tiles, in turn, resulted in 1.775 and 2.620 log_{10} inactivation of the Novel coronavirus under UVA irradiation in 4 and 7 hours of contact time respectively, and 2.210 log_{10} inactivation under LED light irradiation in 7 hours of contact time [210]. While, coated photocatalysts that contained rutile TiO_2 and Cu_xO nanoparticles inactivated the SARS-CoV-2 under fluorescent light irradiation for 3 hours [211]. The researchers reported that the photocatalyst particles not only caused direct destruction of the SARS-CoV-2 membrane and its proteins, including spike, but also damaged its genetic material [208,210].

Surfaces coated with photocatalyst particles, therefore, have been considered highly promising, especially if used in environments with a high likelihood of contamination, such as hospitals, supermarkets, gyms, restaurants, airports, metro stations, schools [208,210]. Additionally, those materials have also been utilized as coatings in air filters, respiratory masks, and antiviral fabrics to prevent the transmission of COVID-19 and other diseases [208]. It should be noted, however, the use of photocatalysts in wastewater treatment still presents significant challenges, such as mass transfer limitations due to their size, surface, dispersibility, and optical transparency [197.211]. In addition, it has been crucial to evaluate the safety of using photocatalysts in order to prevent potential harm to both the environment and human health [197,208].

3.5.5. Thermal treatment

Temperature has been considered one of the most influential parameters for the inactivation of viral particles [212]. The increase of temperature has been shown to cause structural alterations in viral proteins due to the differential expansion of their constituent parts under the action of heat [213]. It could also induce the denaturation of their membrane, viral capsid proteins and nucleic acids, which are highly sensitive to heat [121,212,214]. Additionally, higher temperatures may also promote the growth and activity of extracellular enzymes produced by antagonistic microorganisms [215,216]. Thus, thermal treatments, which use high temperatures, have been considered as an effective inactivation method for disinfecting pathogenic viruses in WWTPs [105,139].

According to studies, a temperature of 47°C for 20 min was able to reduce the infectivity of the Transmissible Gastroenteritis virus by 4.2 log_{10}, and temperatures of 60°C for 15 minutes were able to reduce the infectivity of the Mouse Hepatitis virus by more than 3.9 log_{10} [217]. Concerning coronavirus strains, research has indicated that a temperature of 75°C for 30 minutes was able to completely inactivate the SARS-CoV-1, and a temperature of 65°C for 15 minutes was able to inactivate the MERS-CoV [218]. SARS-CoV-2, in turn, was completely inactivated when exposed to a temperature of 56°C for a period of 30 minutes, or when exposed to a temperature of 60°C for 15 minutes [105,156,218]. It should be noted, however, that aqueous matrices with high protein content were seen to make the virus more resistant to heat treatments, which could considerably reduce the efficiency of the method [156]. Thus, if heat treatment is implemented as a SARS-CoV-2 disinfection method, it has been suggested to add a safety factor [219]. The recommendations were to apply temperatures above 75°C for 3 minutes, temperatures above 65°C for 5 minutes, or temperatures above 60°C for 20 minutes [219].

3.5.6. Ceramic membranes

Ceramic membranes have recently been considered as an interesting method for removing viruses from wastewater. This type of membrane, usually made with hexyl or octyl triethoxysilanes, has increased the hydrophobic properties of their surfaces and facilitated the adhesion of viral particles [47]. Ceramic membranes have been developed to overcome the deficiencies observed in polymeric membranes, including low thermal, chemical, and mechanical stability [220]. They have also shown a great potential when coated with materials that improve their viral particle removal properties, such as hydrophobic and electrostatic attraction capabilities [220]. Studies in the literature, for example, have shown that high-performance ceramic membranes were able to remove over 3 to 4 log of the MS2 Bacteriophage virus in large-scale experiments using river water [221]. Iron oxide ceramic membranes were also able to remove 1.5 log reduction values of Bacteriophage P22 in a survey made from viral suspensions, while ceramic capillary membranes made of yttria-stabilized zirconia almost completely removed Bacteriophages MS2 and PhiX174, with respective removal indices of 8.8 and 8.9 log [222,223]. To date, however, no report has yet been made related to the removal of the new coronavirus using this type of membrane.

3.6. Integration of methods

In order to increase the treatment efficiency and performance of WWTPs, studies have experimented the integration of two or more technologies, referred to as hybrid technology, mixed systems, or combined systems [166,224]. Compounds such as H_2O_2, Cl_2, O_3, when used together with UV radiation, for example, were also seen to improve the process of disinfection of viral particles by increasing the photolytic production of highly reactive hydroxyls oxidants that tend to damage the replicative machinery of particles [47,128]. Ozone application, when integrated with H_2O_2 or persulfate/monopersulfate, has also been seen to promote the production of hydroxyl radicals, and thus possibly improve disinfection [121]. Photocatalic methods, when integrated with membrane filtration processes, have as well been considered as a promising technology for efficient removal of pathogens from contaminated water [139]. The hybrid system, named PMR, demonstrated high efficiency to disinfect viruses and other microorganisms under mild temperature and pressure conditions, in addition to providing the recycling of treated water and the aggregation of photocatalysts [225].

The use of membrane bioreactors, commonly called MBRs, have also shown great potential to be used in treatment of wastewater. Those methods have consisted in the use of equipment that, in a single unit, combines biological treatment, chemical sedimentation, and physical separation through membranes [128]. In the system, the biological process biodegrades organic matter and chemically sediment mineral compounds, while the membrane step separates residual solid components from the liquid phase [143,166]. MBRs, which can be carried out in aerobic and/or anaerobic conditions, have been capable of retaining the microbial community that metabolizes organic compounds, while also allowing for the recovery of the energy potential of organic matter [144]. MBR technology has been shown to be an effective system in the removal of pathogenic microorganisms, including viral particles [136,139]. Although not fully elucidated, the mechanisms by which MBRs remove and inactivate viruses have been mainly attributed to their attachment to biological solids, enzymatic action, microbial predation, size retention and exclusion by membrane and cake layer formation, and membrane backwashing [120].

According to studies, full-scale MBRs were able to remove 3 to 2 log concentration of Sapoviruses and Rotaviruses, approximately 5.1 log of Human Enterovirus, 5.5 log of Human Adenovirus, 5.7 log of Norovirus GII, and 7.1 log of F+ Coliphage from wastewater [143,226,227]. When it comes to SARS-CoV-2, researches conducted by Wang *et al.* [228] and Serra-Compte *et al.* [134] indicated that real-scale MBRs were able to reduce respective indices of 3.6±0.62 $\log_{10}$ and 1.97±0.93 log in domestic wastewaters. It is noteworthy that the removal of viruses in MBR systems depended on the operational aspects, such as the microbial community and membrane properties, including constituent materials and pore size [229]. The use of ultrafiltration membranes with a nominal pore size smaller than the size of the SARS-CoV-2 viral particles, hence, has been strongly advised [228]. Moreover, selecting microbial communities best suited for breaking down organics and removing viruses from wastewater can allow MBRs to produce effluent of higher quality with a smaller ecological footprint [136,143].

3.7. Treatment decentralization

The decentralization of wastewater treatment stations has also been considered as a cost-effective and sustainable strategy for mitigating the spread of SARS-CoV-2 through the wastewater system. The approach, particularly useful in locations where centralized treatment strategies are not feasible, has involved the use of smaller and decentralized treatment systems that can be installed at individual homes or small communities, such as septic tanks, constructed wetlands, and biofilters [162,230]. Decentralized wastewater treatment systems, in the COVID-19 pandemic context, could be used to inactivate the SARS-CoV-2 at critical points that have a higher probability of receiving the new coronavirus, such as wastewater from hospitals, community clinics, and nursing homes [37]. Being able to manage the local wastewater flows, without the need for long-distance transport, could help to reduce the risk of transmission and exposure to the virus, both for those working at the treatment plants and for the wider community [136,162,166]. The decentralization of wastewater treatment stations, however, still present considerable difficulties, mainly related to need to develop efficient installations that consider the peculiarities of each location and type of wastewater [119].

3.8. Wastewater monitoring

The detection of the SARS-CoV-2 in wastewater, which collects and concentrate human excreta, has been extremely useful as a tool for monitoring the spread of the virus within local communities. The noninvasive approach, named Wastewater-Based Epidemiology (WBE), has frequently been used as a fast and low-cost diagnostic tool to provide real-time information on the circulation dynamics of pollutants, chemicals, and pathogens in communities [231,232]. When focused on the detection of viral pathogens, such as SARS-CoV-2, surveillance in wastewater has the potential to vastly improve global public health efforts. SARS-CoV-2 surveillance in wastewater could be used as a quick alert about emerging and reemerging COVID-19 epidemics, thereby promoting better practices to coordinate resources and administer vaccines [46,233-235] (Table 3).

The WBE regarding the new coronavirus would enable the enumeration of pre-symptomatic and asymptomatic cases, as well as people who do not have access to health care, thus, provide better forecasts about the COVID-19 spread in the communities and be used to construct more accurately epidemiological models [101,236]. Along that, the approach could be used to detect the SARS-CoV-2 variants of interest that are circulating in communities and assess the dynamics of the spread of these variants in populations across temporal and geographic scales [237,238]. Wastewater-based epidemiological research could be conducted on previously frozen and archived wastewater samples and be used to promote future studies to trace the evolution and origins of SARS-CoV-2 [239]. Furthermore, studies could be conducted to assess the efficiency of disinfection systems and evaluate environmental risks to promote strategies for improving the suitability of water WWTPs [83,105]. Potential uses of the Wastewater-Based Epidemiology approach were illustrated in Figure 3.

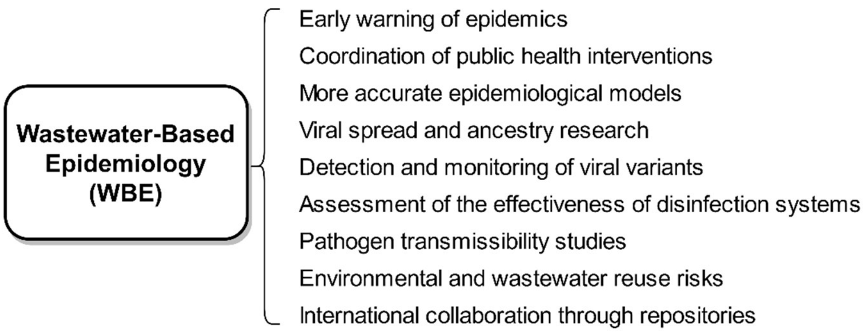

Figure 3. Possible applications of epidemiological monitoring of SARS-CoV-2 in sewage systems by wastewater-based epidemiology (adapted from Mainardi & Bidoia [34]).

Table 3. Early detections of SARS-CoV-2 in liquid and solid phases of wastewater samples.

Country	Location	Sample source	Signal detection	Reference
Netherlands	Amersfoort, Utrecht	Sewage	1 week after the first official COVID-19 case reported in the country and 6 days before the first reported case in Amersfoort city	[54]
United States	Bozeman, Montana	Wastewater	Increase of SARS-CoV-2 RNA in wastewater preceded by a day or two the detection of new COVID-19 cases in a community.	[59]
United States	New Haven, Connecticut	Primary sewage sludge	1–4 days before local hospital admissions and 6–8 days before SARS-CoV-2 positive test results	[69]
Canada	Ottawa, Ontario	Primary clarified sludge	48 hours before COVID-19 clinical tests and 96 hours before local hospitalizations	[240]
Denmark	Solrød, Zealand	Liquid and solid phases of wastewater	3 days before the first official COVID-19 case reported in the country	[241]
England	South East Region	Wastewater	3 days before the first reported case in the sewage plant catchment area	[52]
United States	New Haven, Connecticut	Sewage sludge	Average of 3 to 5 days before the admissions in a local hospital	[242]
Brazil	São José do Rio Preto-SP	Wastewater	Average lag of 5 days between the wastewater signal and new positive cases reported	[243]

Country	Location	Sample source	Signal detection	Reference
Mexico	Monterrey Metropolitan Area	Wastewater	Preceded 2–7 days the rise of reported clinical cases	[149]
Spain	Madrid region	Wastewater	Early SARS-CoV-2 detection in wastewater within 3 to 11 days	[244]
India	Gandhinagar, Gujarat	Wastewater	1-2 weeks before the official clinical cases in the studied location	[245]
Spain	Lorca, Cieza and Totana municipalities	Wastewater	12-16 days before official COVID-19 cases in the studied municipalities	[147]
United States	San Diego county	Wastewater	Emerging variants of concern detected up to 14 days earlier in wastewater samples	[246]
United States	Minnesota	Wastewater	2 weeks (15-17 days) before new clinical cases in rural and large metropolitan areas	[247]
Sweden	Gothenburg	Wastewater	19-21 days before new local hospitalized patients	[73]
United States	Houston, Texas	Wastewater	2 weeks before positive cases of nasal tests	[234]
Australia	Brisbane South	Wastewater	3 weeks before the first clinical case reported in sewerage catchment area	[248]
United States	San Diego, California	Wastewater	3 weeks before local newly reported cases	[249]
Spain	Barcelona	Wastewater	Nearly 1 year before the first official COVID-19 case reported in the country	[49]
Brazil	Florianópolis, Santa Catalina	Sewage	Nearly 3 months before the first official COVID-19 case reported in the country	[50]
Italy	Milan, Lombardy and Turin, Piedmont	Sewage	Nearly 1 month before the first official COVID-19 case reported in the country	[51]

3.9. Major challenges

Despite recent advancements in technologies for disinfecting pathogenic viruses, including SARS-CoV-2, significant challenges remain to be addressed. The removal of viral particles, primarily, was found to be significantly affected by the quality of the raw water, which can vary by geographical location and seasonal variations [108,129]. The presence of suspended particles such as colloidal matter, algae, bacteria, and chemical or biological flakes present in wastewater matrices can provide a physical barrier against disinfection and serve as reservoirs for several pathogens [46,140]. In addition, the presence of organic matter and nitrogenous substances, such as ammonia, can react with sodium hypochlorite used in chlorination treatments, forming less effective active principles, with a lower concentration of active chlorine, such as chloramines [46,107,118]. Excessive chlorine doses can also lead to corrosion of pipe networks and the formation of harmful chlorinated by-products (DBPs) [127,160,162]. The phenomenon may be enhanced by the application of UV light irradiation, which results in the formation of compounds like trichloronitromethane (TCNM), a class of DBP that poses a hazard to public health and the environment [250].

The ability of SARS-CoV-2 RNA to persist through primary and secondary wastewater treatment and accumulate in sewage sludge has also been cause for concern [105]. Sludge, for example, could serve as a reservoir for the virus, spreading it onto the soil and contaminating agricultural fields, as well as surface and groundwater during the process of treatment and disposal [145,150]. Thus, it has been recommended that WWTPs be specifically designed not only for water treatment but also for proper treatment of the sludge generated during the treatment processes [173]. Stabilization processes for sludge, such as longer retention times, thermal treatments, dehydration, aerobic stabilization, anaerobic digestion, and composting, have been suggested to reduce the spread of SARS-CoV-2 through sludge [110,121,150]. The treatments have relied on microbial antagonism and environmental conditions to inactivate viruses, such as elevated temperatures and extreme pH values [150,251].

Another factor to consider has been the potential transport of SARS-CoV-2 viral particles through aerosols generated during runoff, sewage treatment, and handling of sludge, similar to what has been observed with other pathogens [5,102,145,252]. Under certain environmental conditions, the aerosolized virus could theoretically be carried by winds and pose a significant risk to large numbers of people, as well as potential animal hosts [43,104,145]. In this regard, preventive measures, such as reducing reactor surface area, using smaller-diameter bubble-generating aerators, and covering sand removal chambers and aeration tanks, have been recommended [33]. Additionally, precautionary measures for occupational protection, such as using appropriate protective equipment (PPE), maintaining personal and installation hygiene, regular training, and good ventilation of treatment plants, have also been suggested [5,102,145,252].

Another concern has been the undesirable stimulation of antimicrobial resistance by disinfection treatments, as has been observed in both drinking water and wastewater treatments [161]. This could result in the increase of antibiotic-resistant pathogens, commonly known as superbugs, a crucial concern around the world [118]. Researchers, furthermore, have emphasized the need to investigate the impact of SARS-CoV-2 on the microbial community that degrades contaminants in wastewater. In this context, as previous research has shown that foreign viruses could affect bacterial populations in treatment systems by lysing their cells, studying changes in the microbial community that degrade contaminants in wastewater and water supply has been recommended [101]. The possibility of horizontal gene transfers to microbial hosts, which could increase the pathogenicity of microbial communities, should also be considered in the context of SARS-CoV-2 presence in wastewater [135,253]. Researches, therefore, are needed to determine the exact degree to which specific viruses impact host bacterial communities and to investigate potential implications of the dissemination of virulence genes through horizontal transfer on the environment [37,101,135].

4. CONCLUSIONS

In conclusion, the COVID-19 pandemic has highlighted the crucial role of sanitation, water resources, and environmental monitoring in safeguarding public health. It is necessary to invest in research and development of efficient and cost-effective disinfection technologies, surveillance systems, and strategies for risk management in wastewater treatment plants. Furthermore, it is essential to understand the stability and persistence of SARS-CoV-2 and other pathogens in wastewater and their potential impacts on human health and the environment. These efforts will be critical in mitigating the spread of diseases and preventing future outbreaks.

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