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A State-of-the-Art Review on SARS-CoV-2 Virus Removal Using Different Wastewater Treatment Strategies

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Abstract: In addition to the numerous health effects caused by the COVID-19 pandemic, the scientific community has considered other emerging effects such as water-related impacts worthy of deep investigation. In this regard, the transmission cycles of the SARS-CoV-2 virus from fecal, vomiting, and sputum routes to sewage have led health authorities to diagnose, prevent, and use novel wastewater treatment technologies. Once they enter the gastrointestinal canal of a healthy person, viral particles can infect via the nominal amount of Angiotensin-Converting Enzyme 2 (ACE2) present in alimentary canal epithelial cell surfaces and further infect lung, heart, kidney, and other organs. The current review highlights the detection, status, and fate of SARS-CoV-2 from sewage treatment facilities to water bodies. Besides, it addresses the potential wastewater treatment processes to cope with various viruses, especially SARS-CoV-2. Many processes can manage contaminated wastewater and solid wastes over the long term, including membrane technologies, disinfectants, UV-light and advanced oxidation methods like photocatalysis, ozonation, hydrogen peroxide, nanomaterials, and algae. Future work must focus on implementing the selected actions for the treatment of the wastewater released from the COVID-19 hospitals and self-quarantine centers to better regulate future waves of SARS-CoV-2.

Keywords: wastewater treatment plant; SARS-CoV-2 transmission; centralized treatment; decentralized treatment

1. Introduction

The infection arising from the new coronavirus SARS-CoV-2 has been formally known to cause Coronavirus Disease-19 (COVID-19), which is considered a global pandemic with considerable impacts on human beings. At the time this literature review was finished, on 1 April 2022, there were 507,267,013 confirmed cases of COVID-19, and 6,233,224 had succumbed to death around the globe. However, it is important to mention that these numbers are miscalculated given the considerable number of asymptomatic infected people. In addition, the SARS-CoV-2 virus can rapidly be transmitted from humans to humans via respiratory activities [1]. Nevertheless, other transmission pathways are still under discussion. Communities have experienced several issues such as over-saturation of hospitals because of limited early detection. Several research groups have developed clinical tests to detect SARS-CoV-2 RNA using analytical techniques such as droplet digital PCR (ddPCR), loop-mediated isothermal amplification (LAMP), quantitative reverse-transcription quantitative polymerase chain reaction (RT-qPCR), and digital PCR technique (RT-dPCR) assays. These techniques are considered of high sensitivity and specificity. However, the most used technique to diagnose an infected person with COVID-19 is RT-qPCR [2]. This technique has been used in various applications, including the genetic detection of SARS-CoV-2 (RNA-dependent RNA polymerase, nucleocapsid, and envelope protein genes) and infection research. Moreover, it has been performed in body fluids of human beings' such as saliva, sputum, fecal, and urine samples, and several types of water bodies such as river water, sewage, and wastewater [3]. In this venue, selecting the viral concentration method should be a priority.

SARS-CoV-2 RNA has been identified in various water bodies like sewage treatment facilities (STFs) and its derived sludges so it may penetrate to water bodies if proper treatment strategies are not provided [4–9]. In this sense, wastewater management is a crucial aspect that needs to be brought to light for a better understanding of public health risks. A typical STF includes preliminary, primary, secondary, and, in some cases, tertiary treatment units. Through chlorination, ozonation, or UV irradiation, the disinfection process causes viral elimination [9,10]. However, it is essential to analyze the physicochemical properties of wastewater and, consequently, the operating parameters to get rid of the virus.

Wastewater analysis could be equivalent to community-based urine and fecal analysis [11]. In near real-time, comprehensive information on the community health status should be provided to investigate the occurrence and quantity of chemical and biological markers such as pathogens, pharmaceuticals, and other human biomarkers in community wastewater. This methodology is termed as wastewater-based epidemiology (WBE) [12]. The method of WBE was first used to monitor some illicit drugs in 2005 under the term sewage epidemiology. However, several years before, in the 1980s, Israel and then the Netherlands since 2005 identified the poliovirus in wastewater [13]. This method could estimate the prevalence of some illnesses and become an early warning system for population-wide infectious diseases as investigated for hepatitis A and norovirus outbreaks where the pathogenic virus were identified days before the patient was diagnosed [14].

Fast and effective surveillance systems are the bedrock of preventing and controlling an infectious outbreak. With the increasing frequency of zoonotic outbreaks, the need for an efficient and effective manner such as wastewater surveillance and WBE to program future action plans against epidemics has become highlighted. Spurbeck et al. [15] have evidenced that the whole genome sequencing method using simple desktop Illumina instruments can be followed for variant surveillance of SARS-CoV-2. The authors claimed that the feasibility of tracking SARS-CoV-2 at three sites, namely, neighborhood, hospital, and nursing home, is level with the ability to detect one COVID-19 positive out of 60 nursing home residents. They found that non-synonymous mutations fluctuated in the viral population using targeted wastewater-based sequencing. Despite the promising benefits of WBE in COVID-19 surveillance, it should not be considered a candidate to replace the standard clinical diagnostic tests. However, the synergy of these methods could provide more comprehensive information about the pandemic situation. In this regard, more

studies on the use of WBE in COVID-19 surveillance seem to be essential based on past and present experiences.

It is well-known that standardizing a proper analytical procedure for detecting SARS-CoV-2 in water matrices is essential to (i) monitor the SARS-CoV-2 RNA positivity, (ii) detect early warning signs, (iii) establish precautionary measures, and (iv) avoid new pandemic waves at a community level. That is why the objectives of the current review were to know the wastewater-based epidemiology surveillance and its role in quantifying SARS-CoV-2 RNA titer in multiple sources. Moreover, this review aims to understand the occurrence, distribution, and fate of SARS-CoV-2 RNA in wastewater worldwide to make this technique successful. Most importantly, the potential wastewater treatment and disinfection methods, used in a wastewater treatment plant, to assess the inactivation of SARS-CoV-2 RNA in complex matrices have been summarized. Finally, some suggestions were put forth to regulate the quality of effluents to avoid sources of pollution are considered.

2. Review Methodology

Several databases such as PubMed, Google Scholar, Scopus, Science Direct, the websites of the World Health Organization (WHO), Centers for Disease Control and Prevention guidelines, and the medRxiv server were accessed to gather relevant information, including PubMed, Google Scholar, Scopus, Science Direct, the websites of the World Health Organization (WHO), Centers for Disease Control and Prevention guidelines, and the medRxiv server. As shown in Figure 1, after collecting the obtained articles that were published with the keywords "SARS-CoV-2" and "wastewater" between 2018 and 2022, studies with inaccurate evaluations were excluded. Two independent reviewers limited the search strategy according to the purpose of the study with the keywords "wastewater treatment", "SARS-CoV-2", "transmission", "sewage" and "detection". This comprehensive research yielded 150 out of 859 results. The exclusion criteria of articles included non-English language, preprint, and duplicated articles. Furthermore, relevant articles were selected for further review and criticism, leading to an increase in the scientific values of this study.

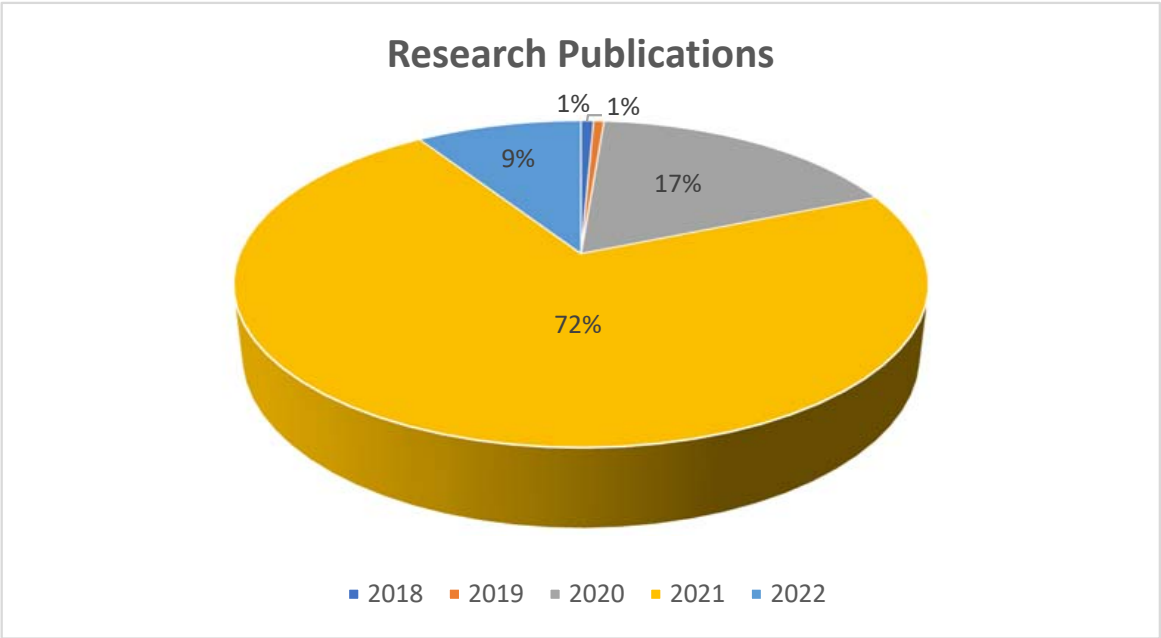

Figure 1. A great deal of research publication related to "SARS-CoV-2" and "wastewater" in the period of 2018 to 2022.

3. Detection Protocol of SARS-CoV-2 RNA in Water Matrices

The COVID-19 outbreak is far from under control, and research groups are studying routes of contamination. The potential infection route originating from contaminated waters with fecal matters and other excreta from infected people is of interest [16]. The existence of SARS-CoV-2 RNA in various sources such as STFs, pumping stations, sludge rivers, and sewer networks obtained from the treatment plants has been detected around the globe [3]. In this section, the protocols used to confirm the occurrence of SARS-CoV-2 in a few water matrices are stated.

Table 1 shows several studies on SARS-CoV-2 RNA detection worldwide. Additionally, methods for concentration are mentioned [11]. There is also evidence of detecting genetic materials of close viral SARS-CoV from Chinese hospital wastewater during the 2002–2003 SARS (severe acute respiratory syndrome) pandemic caused by SARS-CoV-1 [17]. Recently, numerous studies have been conducted on the detection of SARS-CoV-2 RNA in wastewater. The summary of the prevalence of SARS-CoV-2 RNA in the category of wastewater samples, the viral concentration, and the detection technique to identify the targeted genes of SARS-CoV-2 in the studied city and country are represented in Table 1. For instance, in the USA, some researchers [15,18–21] detected a wide range of SARS-CoV-2 RNA from 0.023 to 10^5 copies/mL through various techniques such as PEG concentration method, NanoCeram filter cartridge, ultrafiltration, and dextran. Furthermore, Nasseri et al. [22] for the first time identified the presence of SARS-CoV-2 RNA in raw and treated wastewater in Iran. They observed that more than half of the collected samples (58.34%) contained SARS-CoV-2 RNA. However, some studies [5,19,21,23–25] claimed that all samples collected from sewage in Germany, Qatar, Spain, USA, and France were contaminated with SARS-CoV-2. In contrast, Zhang et al. [26] and Haramoto et al. [7] confirmed that no SARS-CoV-2 RNA was found in the wastewater.

Table 1. The status of the SARS-CoV-2 viral detection in wastewater.

Location	COVID-19 Prevalence (per 100,000)	SARS-CoV-2 Nucleic Acid Concentration in Wastewater (RNA Copies/mL)	Sources of Sample/Month of Sampling	Total Samples (% Positive)	Genes of SARS CoV-2 Targeted for Detection	Methods for Concentration	Ref.
Ourense (Spain)	-	-	WWTP /April 2020	5 (100)	N, E, RdRP	Ultrafiltration	[5]
Buenos Aires (Argentina)	-	CT value 32–40	Raw surface water/June–September 2020	Not Available	N1, N2	PEG concentration	[27]
Pakistan	-	-	Wastewater/March–April 2020	78 (27)	ORF 1ab, N	PEG concentration	[28]
Calgary (Canada)	-	0.5 to 11,015.2	Wastewater/August–December 2020	60 (2)	N1, N2, E	5 µm PVDF filtration, 70% EtOH treatment followed by 4S-silica column for concentration	[29]
Toledo (USA)		9–110.6	WWTP/July 2020	60 (2)	N1	PEG concentration	[15]
Slovenia	-	29.65 to 38.12 Cq Up to 10,000	WWTP/June 2020	15 (66.7)	E and RdRP	Ultracentrifugation	[30]
Istanbul (Turkey)	-	9.33×10^4	WWTP/April 2020	9 (77.8)	RdRp	Ultracentrifugation, PEG8000-adsorption electronegative	[31]
Netherlands	0.1–100	2×10^3–2.2×10^6	Untreated wastewater/February–March 2020	24 (58.3)	N, E	Ultrafiltration	[32]
BneiBrak (Israel)	366–1001	-	Untreated wastewater/April 2020	26 (38.5)	E-sarbeco	Primary: PEG or Alum; precipitation; secondary: Amicon ultrafiltration	[33]
Murcia (Spain)	8.5–129	1×10^5–3.4×10^5 $<2.5 \times 10^4$	Untreated Treated wastewater/March–April 2020	42 (83.3) 18 (11.1)	N	Aluminum hydroxide adsorption-precipitation	[34]
Montpellier (France)	8	1–78	WWTP/May–July 2020	-	N1, N3	Concentration	[35]

Table 1. *Cont.*

Location	COVID-19 Prevalence (per 100,000)	SARS-CoV-2 Nucleic Acid Concentration in Wastewater (RNA Copies/mL)	Sources of Sample/Month of Sampling	Total Samples (% Positive)	Genes of SARS CoV-2 Targeted for Detection	Methods for Concentration	Ref.
Czech Republic	24–561	Cq 34–40	WWTP/April–June 2020	112 (11.6)	-	Flocculation-centrifugation	[36]
Utah (USA)	2.4–16	0.023–1.04	WWTP/April–May 2020	126 (61)	N1, N2	Centrifuged-electronegative	[20]
Wuhan (China)	-	-	WWTP/January 2020	4 (0)	ORF 1, N	PEG precipitation of centrifugation supernatant	[26]
Milan and Rome (Italy)	-	Not detected	WWTP/February–April 2020	12 (50)	ORF1 ab	Polyethylene glycol (PEG) and dextran (DEX) or PEG-dextran	[37]
Southern Louisiana (USA)	-	3.1–7.5	WWTP/January–April 2020	7 (28.6)	ORF 1a, S	Ultrafiltration and adsorption eluting using electronegative membrane	[38]
Yamanashi (Japan)	4.4	2.4	WWTP/ March and May 2020	5 (0)	N	Electronegative membrane direction RNA exaction; ultrafiltration	[7]
Southeast Queensland (Australia)	-	1.9×10^1–1.2×10^2	WWTP/March–April 2020	9 (22.2)	N	Electronegative membrane direct RNA exaction; ultrafiltration	[39]
Tehran, Qom and Anzali (Iran)	-	-	Treated & untreated wastewater	24 (58.34)	ORF 1 ab, N	PEG 6000	[22]
Doha (Qatar)	-	7889 ± 1421–$542{,}056 \pm 25{,}775$ copy/L	WWTP/June–August 2020	43 (100)	N	PEG	[24]
Pakistan	-	-	WWTP	78 (26.9)	ORF 1a	PEG/dextran precipitation of centrifuged supernatant	[40]
Paris (France)	0–2000	50–3×10^3	WWTP/March–April 2020	23 (100)	RdRP, E	Ultracentrifugation	[25]
Ottawa and Gatineau (Canada)	4.8–57.3	1.7–380	WWTP/April–May 2020	-	N1, N2	PEG precipitation	[41]

Table 1. *Cont.*

Location	COVID-19 Prevalence (per 100,000)	SARS-CoV-2 Nucleic Acid Concentration in Wastewater (RNA Copies/mL)	Sources of Sample/Month of Sampling	Total Samples (% Positive)	Genes of SARS CoV-2 Targeted for Detection	Methods for Concentration	Ref.
Arizona (USA)		10.4–993	Wastewater/August–November 2020	-	N1, N2	Ultrafiltration	[18]
Belgrade (Serbia)	-	5.97×10^3–1.32×10^4	River water/December 2020	-	N1, N2, E	Ultracentrifugation	[42]
Japan	-	4.4×10^4	Untreated wastewater/March–May 2020	17 (41.2)	N2, N3, NIID_2019-nCoV_N	PEG precipitation	[43]
Metropolitan region (Japan)	-	0.16–13	Manhole and WWTP/June–August 2020	-	N1, N2	PEG precipitation	[44]
Santa Catalina (Brazil)	-	6.3×10^5	WWTP/October 2019–March 2020	-	N1, S, RdRp	PEG precipitation	[45]
Ahmedabad (India)	1000–2700	0.78×10^2–8.05×10^2	Untreated wastewater/May 2020	19 (57.89)	ORF1ab, N, S, E	PEG precipitation, Adsorption	[46]
Frankfurt (Germany)	-	4×10^{11}–1×10^{15}	WWTP/April	2 (100)	N, S, ORF 1ab	Electronegative membrane filter	[23]
Valencia (Spain)	-	10^4–10^5	Untreated Treated wastewater/ February–April 2020	15 (80) 9 (0)	N	Aluminum flocculation-beef exact precipitation	[47]
Niteroi (Brazil)	51	4.9–8.5	WWTP/April–August 2020	12 (41.67)	N1, N2, N3	Ultracentrifugation	[48]
Milan, Turin, and Bologna (Italy)	-	5.6×10^4	WWTP/October 2019–February 2020	40 (37.5)	ORF1 ab	Dextran and polyethylene glycol, chloroform, centrifugation	[49]
Michigan (USA)	-	10^4–10^5	WWTP/April–May 2020	54 (100)	ORF, E, N	NanoCeram filter cartridge	[19]
Massachusetts (USA)	26	57–303	Untreated wastewater/March 2020	2 (100)	N1, N2, N3	Polyethylene glycol-8000 (PEG 8000)	[21]
The United Arab Emirates (UAE)	-	7.50×10^2–3.40×10^4	Treated & untreated WWTP/May and June 2020	36 (77.8)	RdRP	Ultrafiltration columns, and PEG/TRIzol	[50]

Although several studies on the event of identifying SARS-CoV-2 RNA in wastewater have been conducted worldwide, a standard procedure is lacking. The followed procedures are summarized into the following steps: (i) sampling–storing, (ii) concentration–extraction, and (iii) extraction–detection. The temperature during the first step plays an essential role in half-life and the loss of viral load in wastewater [51–53]. The lack of a protocol related to sampling and storage makes it difficult to care for viral degradation, avoid viral loss, and reduce the uncertainty in gettong the (accurate) results [54,55].

Beattie et al. [56] evaluated the impact of sample storage on the decay and recovery of SARS-CoV-2 from three wastewater treatment plant in North Carolina. They found that influent samples stored at 4 °C maintained the RNA level and detection of SARS-CoV-2 resulted most accurate titre without significant drop in concentration during a 19-day experiment. It indicates that SARS-CoV-2 concentration in wastewater is stable for more than two weeks if stored at 4 °C. Furthermore, other researchers such as Baldovin et al. [40] and Bivins et al. [57] noticed that the SARS-CoV-2 concentration in influent samples from different WWTP did not degrade significantly when stored at 4 °C for 24 h to 7 days, respectively. Therefore, sample storage at 4 °C is a robust technique allowing laboratories to store wastewater samples on site for up to two weeks without risk of SARS-CoV-2 signal degradation in its RNA detection.

The concentration method is necessary for the removal of the extra chemicals in sewage which leads to having comparatively pure viral samples in the collection. This will also enhance the cross reactivity of any chemicals used in the assay system with that of the sewage content. In this sense, several methods to concentrate SARS-CoV-2 RNA from wastewater such as polyethene glycol (PEG), adsorption-extraction using electronegative membranes, aluminum hydroxide adsorption, ultrafiltration, ultracentrifugation, precipitation, bag-mediated filtration system (BMFS), and concentrated concentration pipette have been used. Moreover, the combination of these methods is recommended for the detection of the SARS-CoV-2 viral load in sewage [7,21,34,39,46,58,59]. Zheng et al. [60] extensively investigated various methods of concentration of SARS-CoV2 RNA including ultracentrifugation , $AlCl_3$ precipitation, and membrane adsorption. They concluded that ultracentrifugation was the most feasible technique with a viral recovery efficiency of $25 \pm 6\%$. After that, $AlCl_3$ precipitation, as the 2nd best method contributed virtually half to of the RNA amount obtained in ultracentrifugation method, yielding to lower recovery efficiency [60]. However, Pérez-Cataluña et al. [61] suggested that the aluminum precipitation method coupled with automated nuclear extraction indicates an acceptable sensitivity technique to provide readily results. Another study [62] reported that although the optimized medium-speed centrifuged solids-based method had similar sensitivity vs. the ultrafiltration reference method, it included added values such as lower costs, fewer processing steps, and shorter turnaround times. Additionally, for the first time in the United States, SARS-CoV-2 wastewater concentration methods comparison was performed through BMFS, PEG, and ultrafiltration at multiple treatment plants. The results revealed that among others, BMFS is a promising method for wastewater monitoring due to its compatibility and simplicity of detection without extensive laboratory resources, and no reliance on hard to acquire consumables [63]. Similarly, the concentration step is required to enhance the viral RNA content in the collection that might have alleviated due to its reduction under dilution with sewage water or damage by chemicals or water stream. This step is a key one for the quantification of the SARS-CoV-2 thanks to a different concentration compared with normal one detected in wastewater streams by various methods either by detecting the viral genome or a particular gene as mentioned in Table 1. Various used to concentrate SARS-CoV-2 RNA in sewage streams is excellently reviewed by Cervantes-Avilés et al. [64]. They grouped the concentration methods for SARS-CoV-2 in physicochemical and only physical-based processes. In addition, Wozniak et al. [65] proposed a simple method based on acid pH separation of RNA, which was found to be the most suitable alternative to conventional RT-qPCR for SARS-CoV-2 detection.

Extraction, one of the most important steps in isolating SARS-CoV-2 RNA from an undamaged concentrated sample, may strongly affect the overall performance of detection and quantification. In this sense, numerous techniques based on extraction with organic solvents, a rotating column based on silica membrane, and using paramagnetic particles have been adopted and refined. Extraction methods include acid guanidinium thiocyanate–phenol–chloroform (TRIzol-chloroform), commercial kits based on solvent extraction utilizing TRIzol-chloroform, lysis buffer/TRIzol LS, or silica membrane-based spin column, except the paramagnetic particle's method [55,66]. For instance, Wu et al. [21] applied the TRIzol-chloroform extraction method for 24 h composite samples of raw sewage from urban WWTP in Massachusetts, USA. They found such a method to be a simple viral enrichment and RNA extraction protocol to achieve virus identification [21]. Another study [67] proposed different RNA extraction methods for specific recovery of SARS-CoV-2. They used the commercial kit Direct-zol RNA Miniprep™ as extraction for samples from influent of STF in Neuquen, Argentina. Furthermore, magnetic silica was used as a semi-automated extraction system for 24 h composite samples from different STPs in Milan, Turin, and Bologna, Italy [49]. Interestingly, in Hong Kong wastewater, different extraction methods were compared and it was concluded that the lysis-buffer-based method has higher viral recovery efficiencies as compared to the acid-guanidinium-phenol-based method [60].

Finally, the monitoring and measurement of SARS-CoV-2 RNA in environmental samples is usually executed by real-time reverse transcription-polymerase chain reaction (RT-PCR)-based methods characterized by high sensitivity and specificity [41,42,68–73]. However, they are still far from standardized globally due to the acceleration of research and trials in wastewater. The target genes should be identified to reduce false positive/negative results; in this vein, several tests have been developed to detect enveloped (E-gene) and nucleo-capsid (N-gene) genes in wastewater for SARS-CoV-2 surveillance [32,38,46,51]. The false-positive and negative results forced research groups to search for better techniques with enhanced sensitivity and improved limit of detention. Kolarević et al. [42] collected samples from the Danube River in Belgrade to quantify RNA using RT-qPCR with primer sets targeting N1, N2, and E genes. N2 primer gave positive signal in all samples from affected site. SARS-CoV-2 RNA (5.97×10^3 to 1.32×10^4 copies/L) was detected only in one sampling point. Another study designed by Navarro et al. [70] showed a multiplex RT-qPCR based method to detect different SARS-CoV-2 genes (N1, N3) and the spike (S) protein in both human and environmental samples in a simultaneous manner. The analyzed samples were collected from an STF, confirming the method's faster, cost-effective, and robustness in detecting viral genetic material in wastewaters. RT-ddPCR is another technique used to detect SARS-CoV-2 RNA. Gonzalez et al. [74] observed the limit of detection in sewage for N1, N2, and N3, at 14.6, 2, and 2.18 copies per reaction, respectively. Consequently, N2 assays were applied to analyze the SARS-CoV-2 RNA concentrations in sewage.

Wurtzer et al. [73] used an integrity-based RT-qPCR assay to observe the persistence of several forms SARS-CoV-2 RNA under several forms in wastewaters, which gives information on associated risk assessment. They confirmed that SARS-CoV-2 genomes could be noticed as infectious and non-infectious protected and non-protected conditions in wastewater samples. It is essential to highlight that the protected forms are correlated with the extensive occurrence of SARS-CoV-2 RNA in wastewaters, which may be associated with contagious risk. Canh et al. [68] investigated the applicability of three capsid integrity reagents (ethidium monoazide, propidium monoazide, and cis-dichloro diamine platinum) RT-qPCR to detect SARS-CoV-2 (murine hepatitis viral as a surrogate) in sewage. Cis-dichloro diamine platinum RT-qPCR was compared with RT-qPCR alone in wastewater samples collected in several WTTPs of the Greater Tokyo Area. They established that this technique could enhance the understanding of SARS-CoV-2 RNA positive results.

Palmer et al. [71] assessed the concentration of viral RNA through RT-PCR tests. Their results suggested that removing solids by a physical process like filtration or centrifugation

is prioritized before the SARS-CoV-2 detection in the liquid phase. However, suspended solids should be analyzed to enhance the reproducibility and confirm the SARS-CoV-2 signal. In this same tenor, D'Aoust et al. [41] studied the SARS-CoV-2 RNA in solids collected from influent post grit solids and primary clarified sludge in two STFs in Canada. N1 and N2 genes were measured through RT-ddPCR and RT-qPCR assays. The latter technique was more sensitive in the primary clarified sludge in this study. The evaluation of SARS-CoV-2 infection in sewage has been restricted owing to the rigorous requirements of biosafety level 3. Furthermore, Masindi et al. [69] assessed the spatiotemporal migration and inactivation of the SARS-CoV-2 RNA in municipal sewage, surface water resources, and potable water, using the PCR, in two STFs in South Africa. Although the viral RNA was detected in raw sewage, the SARS-CoV-2 RNA was below the detection limit in the concluding treatment steps due to the wastewater characteristics, hydraulic retention time, and temperature.

PCR-based methods have technical problems like high cost, requirement of skilled technicians, and long processing time (minimum 3 h) as well as multiple steps such as collection of samples, transport of samples into a solution, and extraction of the viral RNA [75]. Such issues have forced researchers to investigate and develop better detection and quantification techniques. Recently, many breakthroughs have been achieved, and alternative methods have been used successfully instead of conventional ones [76–79]. For instance, Lu et al. [77] explored electrochemical immuno-sensors as an option to identify viral RNA, which may improve viability compared with the conventional PCR-based approach. Pierce-Ruiz et al. [79] studied an isotope dilution mass spectrometry method (IDMS) to quantify SARS-CoV-2 antigens. This technique uses liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyze peptides of SARS-CoV-2 spike and N genes. In addition, this technique offers a total analysis time of five hours. Mao et al. [80] established an innovative paper-based device for detecting SARS-CoV-2 in wastewater, which could be inexpensive, portable, and easy to handle. Moreover, an open-source method as a rapid, affordable technique was developed based on allele-specific RT-qPCR (AS RT-qPCR) to detect and quantify the B.1.1.7 variant of SARS-CoV-2 in sewage [81].

4. Status of SARS-CoV-2 RNA in Sewage

Testing a wastewater sample is an efficient method for analyzing the presence of any molecular residue of disease-causing organisms. It can help to generate larger population-wide data during a pandemic which is suggested. It is also cost-effective and non-invasive compared to testing samples collected from COVID-19 patients. Several samples carry SARS-CoV-2 viral RNA, such as patients' feces, urine, saliva, sputum, blood, and sweat; these samples including other molecules can be found in wastewater [21,32]. Hence, an essential strategy for controlling the pandemic consists of continuous monitoring of the concentration of viral components in wastewater and other sources and complete disinfection at various levels of treatment is essential. Much research is being conducted based on the COVID-19 tailored wastewater-based approach. A nonclinical early-warning tool must be urgently designed and implemented to alert the area's residents and the area's health care system regarding the outbreak [82]. Information about COVID-19 infections through the study of SARS-CoV-2 RNA titer in wastewater is recognized as an alternative tool for early detection in populations [18,83]. As shown in Figure 2, one of the significant sources of SARS-CoV-2 production is hospitals and self-quarantine centers, which can release wastewater contaminated with SARS-CoV-2 into water matrices through contaminated urine, feces, and nasal mucus caused by sneezing. Due to improper sewage treatment in some areas, the SARS-CoV-2 RNA is transferred to the aquatic environment. Hence, strong monitoring of wastewater surveillance is needed to detect the virus in wastewater, among which RT-PCR is a common tool for detecting RNA in any type of source.

Recently, several research groups have reported their observations on the WBE approach. There are several variations in the documented data. The reports are based on data collected from various sources with little consistency due to variations in the source of

the water sample, methods of study (experimental or field), detection methods, survival number, quality of disinfectants used, treatment method and most importantly the constantly changing strains of the virus. Therefore, a systematic review and meta-analysis that assemble all this information seem difficult. Studies were not comparable because authors have designed their work for different purposes and used different methodologies related to sampling, concentration, and quantification [82]. Expression of results as a calculated value in the form of data is not found in a standard format. In some studies, the RNA titer is mentioned as a copy number, whereas some are reported as a quantification cycle/threshold cycle (C_q/C_t). RT-PCR is the common tool for detection of RNA in any kind of sample. To make this technique successful, some of the selected critical points need to be focused [84]. They are (i) for detection of SARS-CoV-2 RNA presence in sewage, (ii) to identify appropriate spots for the likelihood of the presence of COVID-19 cases, (iii) to establish an efficient method for isolation of the enveloped virus in water samples, (iv) to estimate the degree of different natural and anthropogenic conditions (pH, temperature, disinfectant, UV radiation, storage, etc.) which the virus can withstand, (v) to provide accurate clinical data to generate a systematic relevance with WBE data [3].

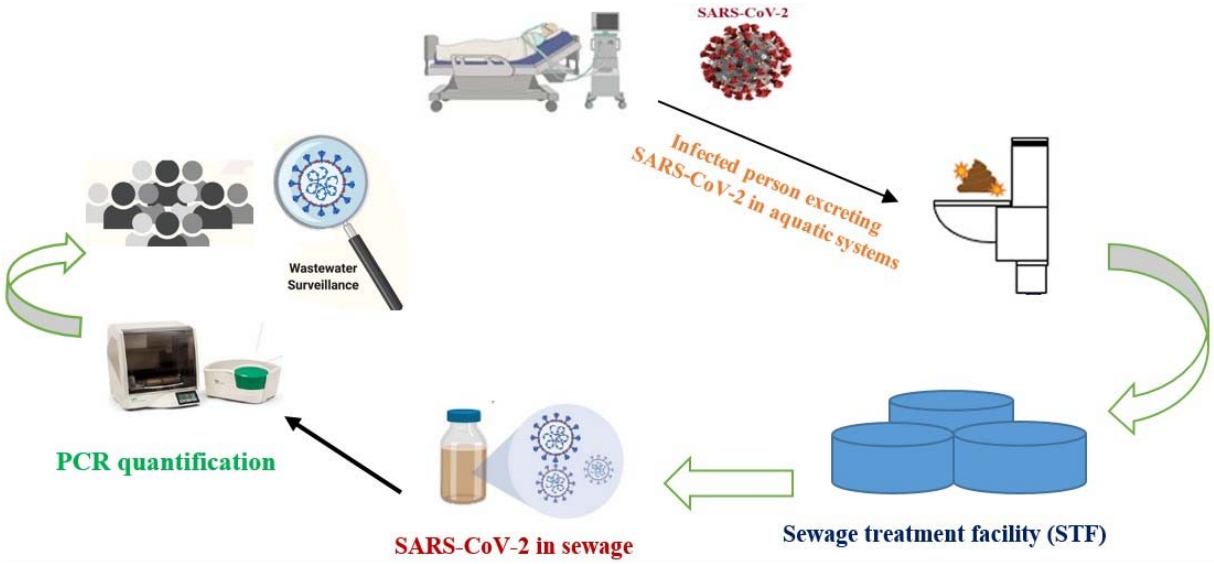

Figure 2. The fate of the SARS-CoV-2 viral RNA and its transmission to the aquatic environment.

According to many studies, SARS-CoV-2 nucleic acid persists in sewage for a prolonged time [39], which is not seen commonly in the genome of other enveloped viruses. However, untreated municipal sludge and wastewater can only preserve SARS-CoV-2 RNA, as reported in many countries, including the Netherlands, Australia, France, and the USA [21,32,39]. The most successful detection method of SARS-CoV-2 ribonucleic acid relies on an efficient separation and concentration system like ultrafiltration, ultracentrifugation, and polyethylene glycol precipitation. It was noted that the coronavirus strain could be found in sewage water one week before the diagnosis of COVID-19 positive cases and 2–3 days before being admitted to the hospital [85]. However, Wang et al. [17] proved the existence of coronavirus RNA in sewage for as long as eight days. They reported that coronavirus RNA might exist for 14 days (about two weeks) at 4 °C (inactive) and 2 days at 20 °C.

4.1. Comparison of Different Titers of SARS-CoV-2 RNA in Sewage

The sewage sample detected with SARS-CoV-2 RNA positive in various countries is found to be in variable quantities. For instance, 10 copies mL^{-1} in Montana [86], 10 to 1000 copies mL^{-1} in Detroit [19], up to 300 copies mL^{-1} in Massachusetts [21], and up to 100 copies mL^{-1} in Virginia [87]. Furthermore, Ahmedabad (India), UAE, and

Murcia (Spain) evidenced 0.06–0.350 copies mL^{-1}, 34 copies mL^{-1}, and 0.106 copies mL^{-1}, respectively [50,61,88]. At the epidemic's peak, up to 3000 copies mL^{-1} were found in sewage from Paris (France) [73]. Gallardo-Escárate et al. [89] detected up to 0.104 copies mL^{-1} only in sewage samples from a penitentiary and health care center in Southern Chillan. The highest concentration was found in a hospital sample (Slovenia) and it was of about 10,000 copies mL^{-1} sample [30].

According to the literature, it can be realized that there are very few similarities among the results in the form of RNA copy number per mL of sample. This inconsistent result of SARS-CoV-2 concentration in wastewater among countries can mainly be due to the difference in the infection rate or scale of infection in a human community. Furthermore, there is a lack of regulated standard protocol. Different laboratories are currently following several purification and concentration procedures for SARS-CoV-2 RNA analysis. Some popular methods include 20 to 800 times higher concentration yielding obtained by ultrafiltration or polyethylene glycol precipitation and aluminum flocculation/ultra-centrifugation [5,25,47]. Ahmed et al. [39] reported that using electronegative membrane filtration along with centrifugation the yield of SARS-CoV-2 RNA can be increased. RTqPCR quantification gives confusing RNA quantity results in different samples [21]. According to La Rosa et al. [37], all of their observations were negative when they used a published method taking SARS-CoV-2 RdRp and Sarbeco E gene as a target. These discrepancies in the detected viral loads with different protocols confirm that a widely standardized methodology is the need of the hour for the detection of SARS-CoV-2 RNA from wastewater.

The discrepancy in results shows the effect of storage conditions, storage time taken since collection, preparation, and transportation [32] must be correctly sorted out. Country-wise and laboratory condition-wise temperature variation should be carefully managed while designing the standard protocol from sample collection for storage and experiment. Study results of SARS-CoV-2 RNA recorded to date in a temperature interval of 4–70 °C in stored wastewater samples are presented by various research groups [39,90].

4.2. Possible Fate of COVID-19 RNA after Ingestion

When ingested the viral particles such as SARS-CoV-2 would undergo a normal digestion process in the guts of organisms. Especially in humans, the process like peristalsis, digestion in the stomach, digestion, and absorption in the intestine would be the expected fate of the virus. However, recent findings state that the viral receptor, i.e., the Angiotensin Converting Enzyme 2 (ACE-2), is also expressed in the gastrointestinal epithelial cells of the human elementary canal [91]. The expression level of the cell surface receptor ACE-2 is undoubtedly extremely high in human respiratory surfaces. However, its low level found in the elementary canal is also capable enough to infect a person, as shown by Wang et al. [91]. Once the viral particle enters the body, it can migrate to the main target organs such as the lung, heart, and kidney, which have an influential titer of the ACE 2 level.

Significant studies have proved that the fecal materials of the recovered patients (tested negative in RT-PCR from nasal or oral swab) may also shed SARS-CoV-2 RNA that can contaminate the wastewater media [92,93]. Saawarn and Hait [94] reported that the viral load in wastewater collected from treatment plants (WWTPs) ranged between 7.5×10^2 and 3.4×10^4 copies L^{-1}, which confirmed the sudden rise in epidemiology number by infection with SARS-CoV-2 in that area. In such areas, the detection of SARS-CoV-2 in wastewater prior to the peak in rising infection and severity level has been noticed. Furthermore, it has been observed that the shedding of viruses from the digestive tract lasts longer than that from the respiratory tract [95,96]. Previous studies have found that viral shedding in feces may last up to 22 days, whereas nasal–throat mixed swabs can last up to 10 days [97]. Additionally, even when respiratory swabs were negative, several studies found that viral RNA particles were shed in the feces can survive up to five weeks [94,98–100]. The prediction given by Róka et al. [101] had to be followed on a stricter basis to sanitize the patient's habitat carefully along with treatment of solid and liquid wastes, as well as management being required before they are released into (natural) reservoirs [91].

As a result, the presence of the viral genome in untreated excreta/urine/nasal mucous of symptomatic and asymptomatic patients can be monitored efficiently following the methods mentioned in Table 2. The activeness of the virus in such waste is found to be for 2 days or even more. Therefore, an extended persistence of the virus for the risk of fecal–oral transmission can be avoided [82]. Although the detection of the SARS-CoV-2 viral RNA in wastewater does not mean its activeness, a positive correlation between its detection and the spreading of COVID-19 underlines the necessity for evaluation of wastewater treatment and disinfection for development of suitable wastewater technology, for example, electrochemical oxidation.

5. Wastewater Treatment Methods to Eliminate the SARS-CoV-2 RNA

Numerous studies have detected SARS-CoV-2 in wastewater and effluents in different countries [102]. Despite its benefits and monitoring applicability for epidemiological surveillance of SARS-CoV-2 in wastewater, the virus could be a critical issue by surviving in sludge from bio-solids and being released into the environment without adequate treatment of effluents [103]. Therefore, an urgent need of wastewater treatment for removal of SARS-CoV-2 should be elucidated.

5.1. Need of Wastewater Treatment

As it is known, the viral presence is a health risk for populations with poor hygiene conditions and water scarcities [104–106]. For example, in low-income nations, there is a lack of wastewater management including discharge raw sewage to the water matrices, inefficiency of sewage treatment facilities (STFs), and failure to reduce the viral load of hospital wastewater before entering the STFs. Therefore, the virus can attach to solids in STFs and survive in sludge and sewage [107]. The survival of coronavirus in different water matrices rests on numerous factors, including viral structure, the presence of organic matter and antagonistic microorganisms, temperature, ultraviolet exposure, and pH [103,108]. The higher the concentration of organic matter or solid fraction in water matrices, the more the viral population survives due to disinfectants and extreme conditions [108]. The normal survivability of SARS-CoV-2 virus in wastewater is found to be 2–10 days at 23 °C; and the virus can survive for at least 10 days in water at 23 °C, after which it gets deactivated by 99.9% if it does not find any suitable host [109]. In temperatures lower than 4 °C, the SARS-CoVs remain infective for 14 days in wastewater, but 25 °C prevents them from being viable for more than 2 days [110]. According to de Oliveira et al. [111], SARS-CoV-2 viability was 7.7 days in river water and 5.5 days in wastewater at 4 °C. However, at 24 °C, it can only survive for 1.9 and 1.2 days, respectively [111]. Hitherto, this certainty about its survival in wastewater is unclear because of the limited investigations [112]. Due to the wide spread of the disease, it is vital to provide a limitation for SARS-CoV-2 in reclaimed water [113]. The WHO provided an interim guideline to manage wastewater and reduce the potential risks safely [37].

SARS-CoV-2 RNA was recently found in the influent to the main primal settler process but not in the effluent from the secondary treatment. The wastewater is safe for reuse and released to water bodies in terms of SARS-CoV-2 transmission [5,34,114], due to the absence of SARS-CoV-2 RNA fragments in effluents, although infectivity tests were not carried out. A study conducted by Abu Ali et al. [115] reported that the primary stage of the wastewater treatment process involved reducing the total suspended solids to the extent of approximately 50% [115]. However, complete removal of SARS-CoV-2 RNA particles from wastewater in this stage is not possible. Therefore, an alternative transmission of SARS-CoV-2 through wastewater by STFs is airborne viral transmission, especially an aeration process [102]. The COVID-19 pandemic has created a condition for facilities receiving hospital and public clinic wastewater to have higher amounts of viable virus. Zhang et al. [26] stated that the SARS-CoV-2 viral RNA from hospital sewage could spread in drainage pipelines as a secondary source if it is not appropriately disinfected. Therefore, a decentralized wastewater treatment system or in situ treatment facility is beneficial and

cost-effective in inactivating the virus and preventing its spread in communities [102,108]. Conventional treatment plants with physical, chemical, and biological processes could remove and inactivate the virus. Kumar et al. [88] assessed the inactivation of SARS-CoV-2 RNA in a conventional treatment system with a primary clarifier, a secondary process, and an aeration tank. The results showed a graduate decrease in RNA copies of the virus from the effluent of each step. However, detailed information on the removal efficiency of each process was not provided [88]. According to studies of current STFs, primary and secondary treatments are not sufficient to completely remove SARS-CoV-2 RNA, and tertiary or additional treatment is required [9]. The more important membrane-based technologies and disinfection-based strategies used to remove/inactivate the virus are presented in Figure 3. A decentralized treatment system could be a proper treatment approach in SARS-CoV-2 removal/inactivation [102,103,113]. As shown in Figure 4, various techniques have been applied so far to remove/inactivate viruses. In many parts of the world, direct natural (polluted or non-polluted) water is consumed for drinking and other purposes. When such water is contaminated with the SARS-CoV-2 viral RNA form any source, it increases the risk of the high spreading of the disease. Therefore, treatment of such water, as suggested is required to break the contamination and infection.

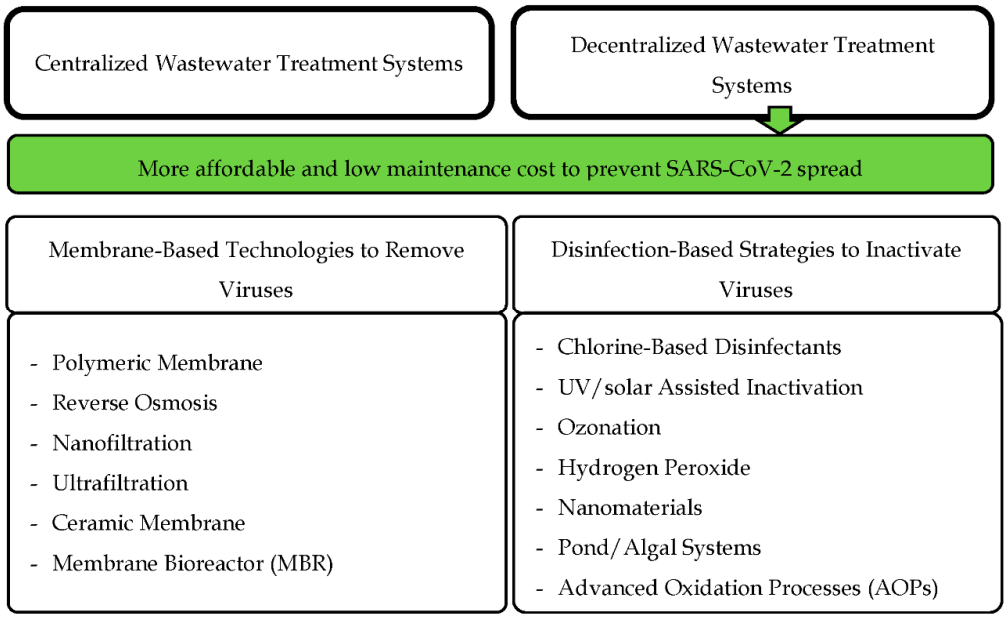

Figure 3. Proper wastewater treatment system for preventing SARS-CoV-2 spread.

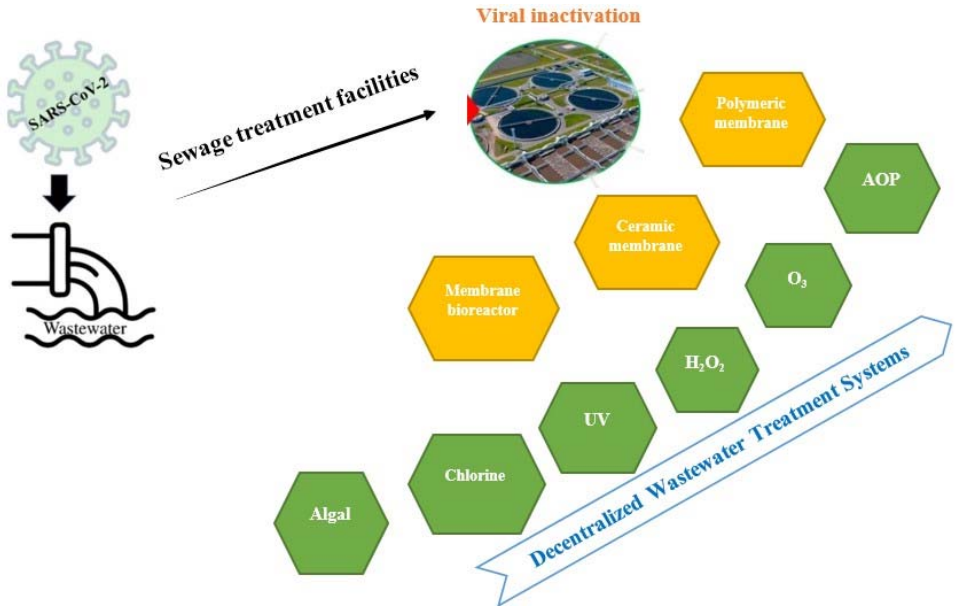

Figure 4. Application of various treatment techniques to remove/inactivate pathogens.

5.2. Membrane-Based Technologies

In membrane technology, membrane pore size is the predominant parameter influencing membrane performance in virus removal, especially when the virus particle diameter is smaller than pore size [116]. Depending on their pore size and type of pathogen, membrane technology could be applied in various steps of STFs. Membrane-based methods separate the virus using a physical barrier from water and remove them up to 0.001 m in size [107]. However, fouling of membranes can limit their implementation depending on wastewater characteristics and membrane type. To reduce fouling and, consequently, the operational costs, pretreatment of feed, periodic membrane cleaning, and chemical modification of membranes are essential to enhance their anti-fouling behavior [102]. It should be noted that despite its many advantages, membrane technology also leads to the creation of a waste stream with high concentrations of pollutants and pathogens that requires special care, which is often overlooked in the literature.

5.2.1. Polymeric Membrane

It is well known that SARS-CoV-2 has an approximate size of 100 nm [117]. Therefore, polymeric membranes (reverse osmosis (RO), nano-filtration (NF), and ultrafiltration (UF)) can be suitable for its removal since their pore size is less than 0.1 µm. Amongst them, the UF has a high potential to be a thorough obstacle to SARS-CoV-2 due to its ability to remove viral particles of 100 nm in size [102]. Using membranes in water treatment also reduces chlorine consumption during final disinfection. The UF can be applied along with a biological method for disinfection purposes. The NF can remove SARS-CoV-2 from wastewater [102]. Pendergast and Hoek [118] indicated that NF with high pressure combined with forward and reverse osmosis membranes are effective in the complete removal of SARS-CoVs [118]. However, it requires extensive studies to evaluate polymeric membrane effectiveness in SARS-CoV-2 fragments' removal.

5.2.2. Ceramic Membrane

As a pretreatment for viral removal, these membranes have been used for filtration and ozonation or coagulation [103,119]. On a pilot plant, it achieved a virus spike test using bacteriophage MS2 removal factor of 12 $\log_{10}$ [119]. According to Bartels et al. [120], virus removal successfully utilized hydrophobic ceramic capillary membranes. Zielińska et al. [121] observed that the ceramic membranes had a high removal efficiency, rejecting >96% of color and practically all TSS and turbidity. This last may help reduce and eliminate

viruses attached to such suspended particles. However, according to our knowledge, there is no report on using this kind of membrane to remove SARS-CoV-2.

5.2.3. Membrane Bioreactor (MBR)

A suspended growth biological reactor combined with membrane-based filtration can effectively remove the virus [102]. Serra-Compte et al. [9] monitored treatment lines of 16 STFs to evaluate the effectiveness of current technologies in removing SARS-CoV-2 RNA. They found that effluents from MBR and chlorination prevented SARS-CoV-2 RNA occurrence, and a log reduction of 1.97 ± 0.93 was achieved. Compared with activated sludge and a nutrient removal system, MBR was a more effective technology as a secondary treatment to eliminate the virus. In addition, the reactor design, and the operating conditions such as pH, dissolved oxygen, hydraulic retention time in MBR, and the virus's adsorption on aggregated particles on the surface and inside of the membrane, are vital factors to be considered [116]. Given the various diameters of the virus, as commented above, the membrane technology should be capable of extracting SARS-CoV-2 using the reverse osmosis (RO) option. As a result, before contemplating microfiltration and MBR [113], membrane technology for virus removal from water is a viable option. Membrane technology has been used for a long time for virus removals from wastewater and reusing treated wastewater.

5.3. Disinfection-Based Strategies

Conventional disinfection methods can inactivate enveloped and non-enveloped virus up to 99% either by oxidizing the protein layer or the structure of DNA/RNA. Research is rarely available on removing SARS-CoV-2 by various disinfection processes. To the best of our knowledge, the virus related to SARS-CoV-2 is enveloped. Therefore, it is expected to be more sensitive to disinfection [122]. However, applying high dosages of disinfection may generate toxic residual by-products in the enviromnet. Interestingly, advanced oxidation processes (AOPs), which produce in situ highly reactive oxygen species like hydroxyl radicals, emerged to inactivate the enveloped virus up to 99.99% without releasing by-products [107].

5.3.1. Chlorine-Based Disinfectants

Chlorine-containing disinfectants, such as liquid chlorine (Cl_2), chlorine dioxide (ClO_2), hypochlorite (ClO^-), chloramine, and hypochlorous acid (HClO), release free available chlorine to inactivate viruses [113]. Majumder et al. [123] reviewed existing treatment technologies used for hospital wastewater treatment containing SARS-CoV-2. The SARS-CoV-2 removal was achieved for a 10 mg min L^{-1} chlorine dose, which was a higher amount than that required for other viruses. As shown in Figure 5, Zhang et al. [26] used sodium hypochlorite (NaClO) for the first time to inactivate the SARS-CoV-2 in Wuchang Cabin Hospital for high viral loading. At first, the wastewater was pumped from toilets to the preliminary disinfection tank, and at this stage, 800 g m^{-3} NaClO was added. Then, this was repeated in another disinfection tank. Effluent was then pumped to three septic tanks. To completely inactivate the virus, the dosage of NaClO was increased from 800 (before 5 March 2020) to 6700 g m^{-3} (since 6 March 2020) for a retention time of 1.5 h. However, 332 ± 122 µg L^{-1} of trichlormethane, 1.9 ± 1.0 µg L^{-1} of tribromomethane, 5.1 ± 3.1 µg L^{-1} of bromodichloromethane, and 0.6 ± 0.5 µg L^{-1} of dibromochloromethane were detected in the effluents. Wang et al. [110] evaluated the effectiveness of ClO_2 and NaClO in inactivating SARS-CoV-1 in wastewater. The ClO_2 inactivated SARS-CoV-1 using 40 mg L^{-1} dosages with free available chlorine of 2.19 mg L^{-1} after 30 min, confirming that ClO_2 was less efficient than chlorine. In another study [124], a higher dose than 6.5 mg L^{-1} of Cl_2 and a contact duration of at least 1.5 h is suggested. However, the residual 0.5 mg L^{-1} of Cl_2, recommended by WHO as a gold standard, should be considered. In addition, free chlorine (0.2 to 0.5 mg L^{-1}) in urban wastewater treatment proved adequate to eradicate viruses quickly because viral RNA is damaged by free available chlorine from ClO^- and HClO [125]. However, chlorination may generate by-products such as chloroform,

haloacetic acids, and trihalomethanes, which are considered toxic and may cause adverse effects on living organisms and ecosystems. In this same vein, the United States Environmental Protection Agency (EPA) listed such safe chemicals against SARS-CoV-2 as sodium dichloroisocyanurate, quaternary compounds, ozone, and peracetic acid. Besides, in the case of using NaClO, treatment equipment such as containers should be made of materials that are resistant to corrosion [124]. On the other hand, the high alkalinity of hypochlorite improves the general condition of soft and highly corrosive waters. It might be employed as a small-scale viral disinfection for wastewater treatment because of its low residual toxicity, robust mobility, simplicity of handling, cost-effectiveness, and homogeneity.

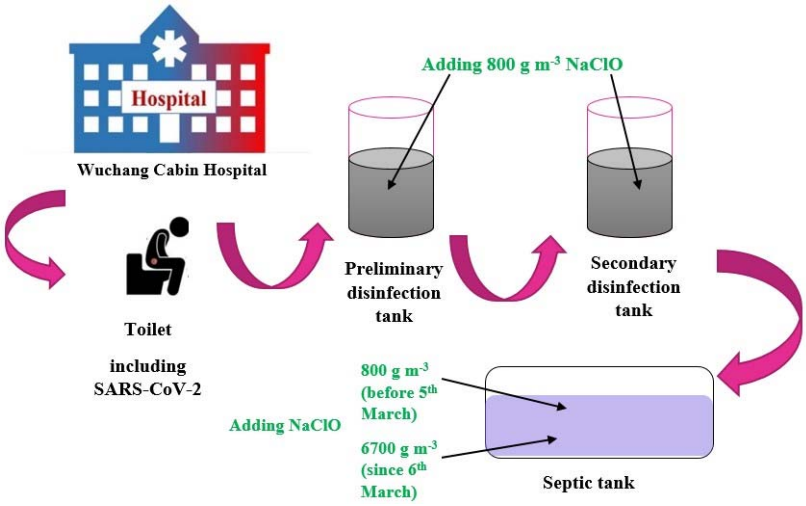

Figure 5. Application of sodium hypochlorite to inactivate completely SARS-CoV-2 from Wuchang Cabin Hospital wastewater. Modified after Zhang et al. [26].

5.3.2. Ultraviolet Radiation/Solar-Assisted Inactivation

Ultraviolet radiation (UV) with a wavelength between 200 and 300 nm could damage viral genetic materials, preventing protein synthesis [126]. The time of exposure, irradiation intensity, and characteristics of water matrices (especially suspended particles, color, and turbidity) are vital factors in UV disinfection [107]. UV disinfection is considered most efficient at a wavelength of 253.7 nm [127]. Qiu et al. [128] applied UV radiation to inactivate norovirus, reovirus, rotavirus, enterovirus, sapovirus, astrovirus, JC viral, and adenovirus at two municipal levels STFs, providing tertiary treatment removal. The treatment plant included screening and grit removal, primary and secondary clarification, activated sludge, and UV disinfection. The total infectious viral reduction was 1.46 and 1.67 log 10, and the reovirus was confirmed to be a viral index of wastewater by a reduction of 1.23 and 1.75. Cheng et al. [129] proposed an improved model to predict UV_{254} photolysis kinetics of viral genomes and viral infectivity loss. The results indicated that 3 mJ cm^{-2} UV dose was suitable for 2-log removal with a constant inactivation rate of 3.168 cm^2 mJ^{-2}. They confirmed that UV doses of 40–100 mJ cm^{-2}, conventionally used in water and wastewater disinfection, could successfully inactivate SARS-CoV-2.

Solar irradiance is a green UV-based process applied as a low-cost alternative for drinking water on small scales to inactivate pathogens and indicator organisms in wastewater treatment ponds and wetlands. Both depth and water quality are crucial factors that affect solar-assisted inactivation rates. Viral inactivation via sunlight could be (i) due to the direct absorption of photons by the virus (or nucleic acids, proteins, other biomolecules), which changes the viral structure; or (ii) via an indirect way which damages viral or cell components when photo-produced reactive intermediates are generated by absorbing photons through endogenous or exogenous components [130]. In several circumstances, UV disinfection is considered more economical than chlorine-containing disinfectants. However, UV has several disadvantages like inadequate penetration depth, energy cost,

need for lamps' replacement and disposal, and health risks. In addition, it has no residual action to prevent the recurrence of pathogens that may have survived. A way to overcome some of these limitations is the addition of another mechanism for disinfection based on the production of reactive oxygen species using different oxidants or the simultaneous use of UV radiation with different processes. Therefore, it can be efficiently used with hydrogen peroxide, ozone, or catalysts [126].

5.3.3. Ozonation

As a clean oxidizing agent, ozone (O_3) has a microbicidal impact and could be an effective oxidant against SARS-CoV-2 [131,132]. Tizaoui [131] indicated that O_3 could inactivate the virus by attacking the proteins and lipids. Most viruses may be eliminated with a standard primary ozone dosage of 3 to 10 mg L^{-1} and a response time of 10 min [133]. Although O_3 can inactivate the virus in a shorter time than other processes and discolor and deodorize wastewater, its operational costs are high. Ozonation could produce hazardous by-products, especially if bromide is present [124]. Volkoff et al. [134] demonstrated that O_3 is an effective disinfectant for SARS-CoV-2. According to their results, O_3 will primarily influence viruses' exterior structure, leading them to lose their infectiousness. However, more research is needed towards applying ozonation as disinfection on wastewaters containing SARS-CoV-2. It is advised to be used with chlorine, chloramines, or chlorine dioxide to ensure a complete disinfection [127].

5.3.4. Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a safe oxidizing agent used as a common alternative, usually at a 3% concentration. It enormously damages the various components of the virus via the hydroxyl radical's production. Despite being chlorine-based disinfectants, by-products of H_2O_2 (water and oxygen) are not considered a health threat; therefore, peroxide does not cause secondary pollution [126]. It also has an immediate effect at elevated temperatures and concentrations, and it can be stored due to its stability [135]. However, the redox potential of hydrogen peroxide is moderate; therefore, excessive amounts are needed for disinfection.

Because of its high costs as a primary disinfection method in wastewaters, the H_2O_2 is combined with UV and/or O_3 to enhance the overall inactivation performance via the production of reactive oxygen species with higher redox potential synergistic action. Ozone integrated with H_2O_2 improved the production of hydroxyl radicals in neutral and alkaline conditions [107]. Nonetheless, using H_2O_2 for large-scale wastewater treatment is insufficient, and its efficacy against SARS-CoV-2 is unproven completely [127,136].

5.3.5. Nanomaterials and Photocatalysis

In recent years, the use of nanoparticles and their activation through radiation has aroused the scientific community's interest. More specifically, when UV or visible irradiation provides higher energy than their energy gaps, the catalysts can photo-produce electrons and holes and generate reactive oxygen species, which inactivate the virus via distortion, protein oxidation, and gene damage [103].

Various materials such as metal oxides (e.g., TiO_2, ZnO), doped structures (e.g., TiO_2 doped with N^-, C^-, S^-), and engineered carbon nanoparticles (NPs) as semiconductors have shown promising results in terms of their photocatalytic activity. Nanoparticles can also combine with membrane-based technologies to inactivate various viruses with increased efficiency [107]. To inactivate bacteriophage f2 (5 log), Nano-TiO_2 P_{25} (10–25 mg L^{-1}, 0.16 mW cm^{-2} of UV intensity, membrane (pore size of 0.15 μm), and Flat-sheet polyvinylidene fluoride (PVDF) were applied in a photocatalytic membrane reactor [137]. Rahaman et al. [138] evaluated the carbon nanotube filter to inactivate viral bacteriophage in the presence of natural organic matter. The results showed that viral particle transport was improved by its attractive electrostatic interaction with multi-walled carbon nanotubes, which was inactivated via direct surface oxidation. Photocatalytic tita-

nium apatite filter as a novel material indicated 99.99% inactivation of SARS-CoV-1 after 60 min without UV irradiation [139].

5.3.6. Pond/Algal Systems

Viral elimination is accomplished in many wastewater treatment plants exclusively by natural mechanisms such as sedimentation (after virus attachment with bigger particles), predation by higher trophic level species, and solar radiation-mediated inactivation mechanisms [140]. Macro/microalgae as a novel alternative, can also be used for wastewater treatment and inactivation of the virus. The microalgae can be cultivated in oxidation ponds, membrane bioreactors, and biofilm reactors [102]. Delanka-Pedige et al. [141] applied *Galdieria sulphuraria* as an algal-based wastewater treatment system. The log reduction of Noroviral GI (1.49 ± 0.16) and Enterovirus (1.05 ± 0.32) approved the suitability of algal disinfection performance. The effluent treated with an algal system had only 14 species of virus with no pathogenic effect on humans. However, the effluent disinfected with chlorination showed 250 species. Therefore, wastewater treatment technologies include a pond and algal systems, and raceway ponds, which may reduce organic matter, nutrients, and pathogens [142].

5.3.7. Advanced Oxidation Processes (AOPs)

Other promising AOPs have emerged as sustainable disinfection methods without producing hazardous by-products [143]. Most studies have reported combining two or more treatments. Ultraviolet light-hydrogen peroxide (UV/H_2O_2) accelerated oxidation is the most used method [144]. Tu et al. [143] applied the electrochemical oxidation process as a green method to inactivate SARS-CoV-2 in aqueous solutions. The results indicated that the receptor-binding domains of the SARS-CoV-2 virus were oxidized and degraded on NiOOH formed at the anode during the electrolysis. The inactivation ratio was 95% in 30 s by applying a voltage of 5 V, and 99.99% was achieved in 5 min. Škulcová et al. [145] introduced ferrate (VI)-based tablets as potent oxidative agents for treating hospital wastewater with SARS-CoV-2 RNA, resistant bacteria, and pharmaceuticals. Potassium ferrate tablets with three types of purity could remove total RNA and DNA up to 90% and 87%, respectively, and SARS-CoV-2 fragments were removed entirely. The results indicated that ferrate tablets effectively destroyed the virus and its fragments without hazardous radical by-products. To ensure the virus-free discharge, sophisticated technologies such as AOPs or integrated processes are necessary due to the persistence of enveloped and non-enveloped viruses in treated water/wastewater after disinfection [107]. Table 2 summarizes the most recent studies using disinfection-based strategies to remove/inactivate SARS-CoV-2.

Table 2. Most recent studies on SARS-CoV-2 removal/inactivation.

Disinfection-Based Strategy	Treatment Technology	Crucial Details		Inactivation Ratio	Ref.
Chlorine-containing disinfectant	Sodium hypochlorite (NaClO)	6700 g m^{-3}	Contact time = 1.5 h V = 60 to 200 m^3	Complete removal	[26]
UV inactivation	UV_{254}	An improved model to predict SARS-CoV-2 inactivation	UV dose of 3 mJ cm^2 without attenuation in water	2-log reduction	[129]
AOPs	Effervescent ferrate (VI)-based tablets	Initial concentration of 6400 copy L^{-1}	Three different tablets: -Pure potassium ferrate of 125 mg -mass ratio 1:2:1 of potassium ferrate: citric acid, anhydrous: sodium hydrogen carbonate -mass ratio 1:4:1 of potassium ferrate: sodium dihydrogen phosphate: sodium hydrogen carbonate	-100% RNA removal -80–100% RNA removal -70–94% RNA removal	[145]
AOPs	Electrochemical oxidation	NiOOH as anode catalyst Na_2CO_3 as electrolyte	-Voltage of 5 V and time of 5 min -Voltage of 5 V and time of 30 s	-99.99% -95%	[143]

6. Conclusions and Recommendations

In low-income nations, there is a lack of wastewater management including the discharge of raw sewage to water matrices, inefficiency of sewage treatment facilities (STFs), and a failure to reduce the viral load of hospital wastewater before entering the STFs. Therefore, the virus can attach to solids in STFs and survive in sludge and sewage. The higher the concentration of organic matter or solid fraction in water matrices, the more the viral population survives due to disinfectants and extreme conditions. According to studies of current STFs, primary and secondary treatments are not sufficient to completely remove SARS-CoV-2 RNA, and tertiary or additional treatment is required. Therefore, a decentralized wastewater treatment system including membrane and disinfection-based strategies, or an in-situ treatment facility is beneficial and cost-effective for inactivating viruses, especially SARS-CoV-2, and preventing their spread in communities.

Compared with activated sludge and a nutrient removal system, MBR is a more effective technology and can be used as a secondary treatment method to eliminate the virus. In addition, the reactor design, and the operating conditions such as pH, dissolved oxygen, hydraulic retention time in MBR, and viruses' adsorption on aggregated particles on the surface and inside of the membrane, are vital factors to be considered. The hypochlorite ion might be employed as a small-scale viral disinfection for wastewater treatment because of its low residual toxicity, robust mobility, simplicity of handling, cost-effectiveness, and homogeneity. Although O_3 can inactivate the virus in a shorter time than other processes and discolor and deodorize wastewater, its operational costs are high. Ozonation could produce hazardous by-products, especially if bromide is present. The redox potential of hydrogen peroxide (H_2O_2) is moderate; therefore, excessive amounts are needed for disinfection. Because of its high costs as a primary disinfection method in wastewaters, the H_2O_2 treatment is combined with UV and/or O_3 to enhance the overall inactivation performance via the production of reactive oxygen species with higher redox potential synergistic action. Nanoparticles can also combine with membrane-based technologies to inactivate various viruses with increased efficiency. Meanwhile, AOPs have emerged as sustainable disinfection methods without producing hazardous by-products. To ensure the virus-free discharge, sophisticated technologies such as AOPs or integrated processes are necessary due to the persistence of enveloped and non-enveloped viruses in treated water/wastewater after disinfection. It should be noted that despite its many advantages, membrane technology also leads to the creation of a waste stream with high concentrations of pollutants and pathogens that requires

special care, which is often overlooked in the literature. The following suggestions can be considered in addition:

- To establish a standard method for reducing the volume to obtain the highest possible amount of RNA.
- The regular chlorination of wastewater treatment plants can inactivate a broad range of viruses and SARS-CoV-2, but the legal dose should be considered because of its side effects.
- To evaluate the degree of contamination in raw agricultural food products when reusing water for irrigation.
- Hospitals should immediately adopt advanced progressive technologies to manage the epidemiology of SARS-CoV-2 through quick approval. Wastewater surveillance of disease-causing agents in hospitals, thus, is to be urgently established, where the disinfectant rule could be easily implemented.
- In low-sanitation nations, decentralized wastewater treatment systems should be improved. Furthermore, chlorination, before the wastewater is discharged into rivers and thus, into the ocean, is the easiest possible method.
- Future work must focus on implementing the selected actions for the treatment of the wastewater released from the COVID-19 hospitals and self-quarantine centers to better regulate future waves of SARS-CoV-2.

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