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WILEY

REVIEW ARTICLE

Diving boldly into COVID-19 contaminated wastewater: Eyes at nanotechnology-assisted solutions

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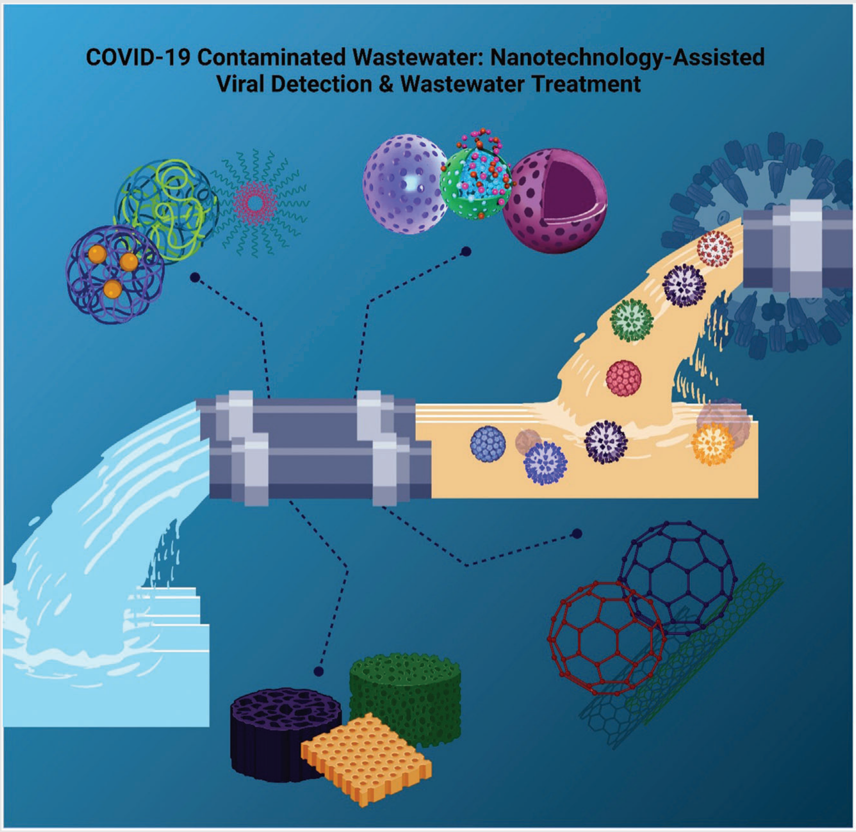
Graphical Abstract



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- Providing discussion on the role of nanotechnology in the detection and separation of coronavirus disease 2019 (COVID-19) from water.
- In-depth discussion and interrelationships between different COVID-19 detection methods.
- A clear future perspective and roadmap for scientists working on virus detection.

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Abstract

Several studies have been directed to find scalable, swift, accurate, and cost-effective strategies for detecting, monitoring, and treating coronavirus disease 2019 (COVID-19). Indeed, the lack of a fast and practical method for detecting the infected regions makes decision-making challenging to combat the critical pandemic-struck situations. The probable 'wrong', or rather inadequate, decisions not only have a boomerang effect on the economy but also can lead to an increase in the number of infected individuals, degree of hospitalization,

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and death counts. Although the current clinical methods are effective, they are costly, time-consuming, and, more particularly, inadequate because of the virus's mutation patterns. In addition, contamination of biomedical wastes with the COVID-19 virus is a matter of grave concern. Therefore, there is a perpetual need for novel methodologies to delineate the contaminated regions and determine whether those viruses contaminate the wastewater. Although several review papers have been recently published to discuss those concerns, there is a lack of a comprehensive survey of the detection and treatment of the COVID-19 virus in aqueous media. Herein, we review techniques available as spreading signifiers for detecting the COVID-19 virus in water resources and wastewater. We classify and integrate techniques into wastewater, sewage, and sludge detection and monitoring. Treatment of COVID-19-contaminated wastewater is discussed by classifying and ranking the methodologies nurtured from nanotechnology, including nanoparticle-based biosensors used in the detection and nanotechnology-based filtration systems for the removal of COVID-19 from wastewater. We also highlight the compilation of the detection methodologies in contaminated aqueous media and provide insight into the challenges associated with treating COVID-19-contaminated wastewater. The article concludes that international and robust guidelines for virus/bacteria treatment in wastewater are urgently needed to protect the environment and public health, where nanotechnology plays a key role.

KEYWORDS
virus detection, wastewater treatment, SARS-CoV-2, COVID-19, water contamination

1 | INTRODUCTION

Since the onset of the coronavirus disease 2019 (COVID-19) pandemic, there has been a reduction in air pollution and greenhouse gas emissions due to decreased transportation and industrial activities.[1,2] In contrast, the COVID-19 pandemic has caused a surge in plastic waste due to enhanced demand for personal protective equipment, yet a rise in single-use plastics from takeout and delivery services.[3,4] However, as research during the time span of the pandemic witnessed, it has become increasingly necessary to find and organize some approaches for the absolute elimination of viruses and bacteria before they develop resistance as a firewall to environmental and public health. With the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the need to curb transmission routes and find efficient treatment approaches has become even more urgent. Figure 1 illustrates the confirmed transmission routes, conditions that may affect the possibility of virus viability, and the cycle that repeats to infect healthy individuals.[5,6] Other reasons for water contamination can be attributed to bacteria and genes. Genes named antibiotic resistance genes (ARG) and antibiotic-resistant bacteria can be found in wastewaters, and these strongly affect the individuals' health and should be absolutely treated.[7] Conversely, there are more apprehensions over SARS-CoV-2 treatment than bacteria elimination. This is because of the unforeseen fate lines of this new virus. Accordingly, discovering possible transmission routes, virus distribution before and after treatment, surface absorption evaluation of SARS-CoV-2 within sewage sludge, and recognition of crucial parameters affecting the virus survival within the wastewater are crucial elements of research questions and progress priorities. Several efficient treatment methodologies, including physical, biological, and chemical protocols, have been examined.[8,9] Nevertheless, there is still an omnipresent need to enhance the treatment efficiently, utilize disinfectant methods, accelerate virus and bacteria resistance mechanisms, and disperse ARG for environmental health protection.[7,10] Typically, international and robust guidelines for virus/bacteria treatment in wastewater are urgent and should be revisited immediately.[11] Furthermore, SARS-CoV-2 (COVID-19) and some other human coronaviruses can be detected via clinical assays,

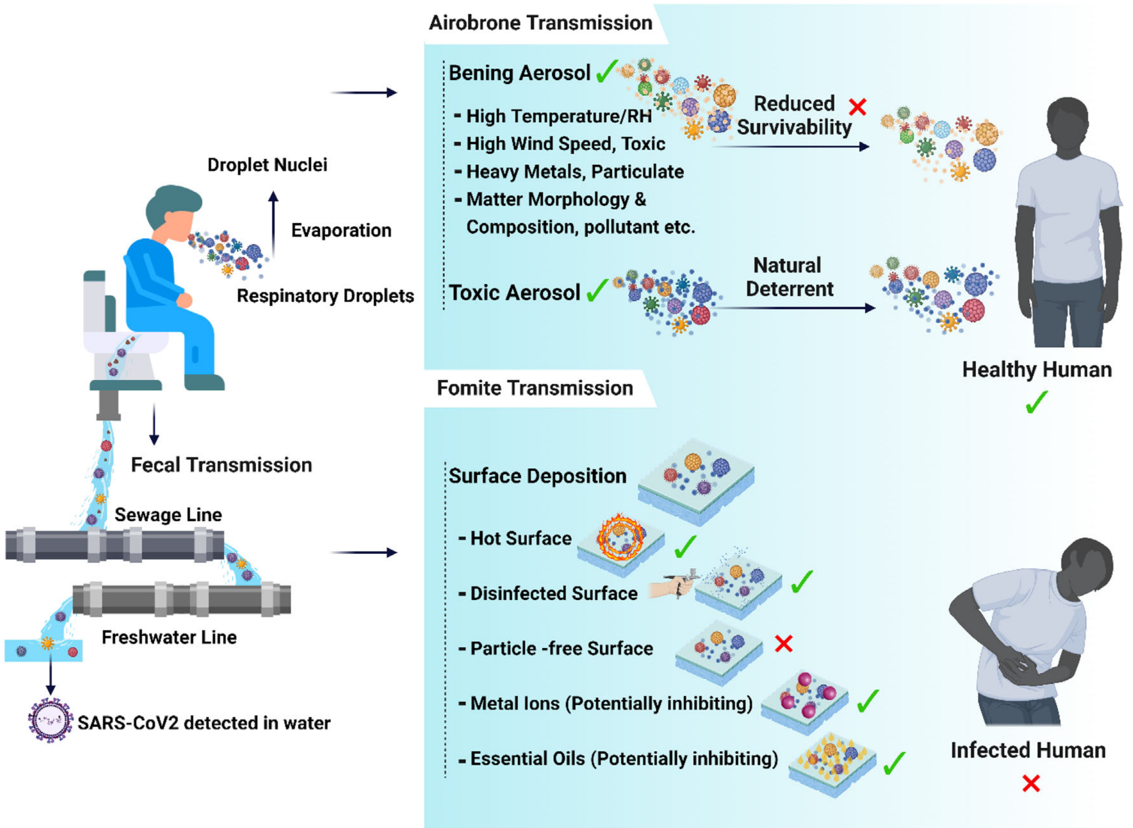

FIGURE 1 Schematic of transmission pathways and conditions that possibly increase or reduce virus viability, as well as the sustain the loop via which viruses pass on to infect people, partially adapted.[5,6]

immunosensing, biosensing, and chemical sensing. Some other computational approaches also can be utilized to detect COVID-19 viruses, predict their expansion and develop removal technologies as used in various fields of biomedical engineering.

Pieces of evidence have indicated that using wastewater as a type of indicative 'benchmark' is a practical and trustworthy procedure to be aware of the infection level in different areas around the world and elucidates paramount data about the rate of an infection increase. The mentioned achievement is worthy because it galvanizes politicians into making the best decision for locking down or the other way around. The big deal is that previous methodologies cannot answer the petition because they were chiefly designed to recognize nonenveloped viruses whose structure was profoundly different compared to SARS CoV2; the lack of answer is due to the reason that they were primarily developed to identify nonenveloped viruses with radically different structures from SARS CoV2.[12,13] Therefore, the gap lacks appropriate assessment means equal to debility in virus concentrations assessment and the efficacy of wastewater treatment evaluation. Freshly, scientists have verified that SARS-CoV-2 RNA is likely to be detected in sewage sludge using RNA concentration measurement. Furthermore, they demonstrated that increasing and abating the RNA concentration correlates with epidemic curve priming an early alarm for infection assessment in society.[14,15] This concept is well-known as WBE or sewage epidemiology.

Although few studies have reported the exact correlation between the number of COVID-19 cases with SARS-CoV-2 RNA concentration in wastewater, some relation definitely exists between these two. The practicality of this method is being streamlined due to eclectic challenges that must be conquered. However, while there is no chance to detect the disease in individuals, scientists have captured much attention to detecting community spread.[16–18] Researchers have been investigating the wastewater that originates from public drains, restrooms, and kitchens and flows into sewers. They claim that once the UK went into lockdown in late March, the level of viral RNA in the samples "plummeted," which is apparently a sign of the decline in cases brought on by social distancing. Estimations have revealed that if one person out of 10 000 gets infected with SARS-CoV-2, wastewater methods will recognize that.[19]

Coronaviruses are a clan of virus from 60 to 220 nm in terms of size, positive-sense RNA, as well as enveloped single-stranded. The root of the name of coronaviruse derives from the crown-like shape ('corona' in Latin).[20] The available knowledge about COVID subfamilies is unexampled, but Alpha-, Beta-, Gamma-, and Delta coronavirus are the main globally accepted and detected ones. Among the mentioned subtypes, Alpha- and Beta-coronavirus consisting of SARS coronavirus, Middle East Respiratory Syndrome (MERS) coronavirus, and SARS-CoV-2 introduce respiratory infection in humans.[21–23] SARS-CoV-2 as a member of the Corona family is a kind of enveloped virus encompassed by the single-stranded RNA genome, and the consequences of this virus's existence in the body range from commonplace cold symptoms to perilous acute respiratory distress syndrome. Figure 2 portrays a simple schematic of the immune system response to the virus, consisting of various cascades, and simultaneously, it depicts the virus particles.[24]

When it comes to the transmission route of COVID-19, the most straightforward route is the inhalation of aerosol/droplet. Several virus traits are unknown, but a visible abrupt change in virus longevity is observed as a function of temperature. In previous studies,[25,26] virus persistence is reported regarding virus type, water sample type, and temperature. For instance, a sharp reduction in SARS-CoV2 longevity up to the level in which the virus is undetectable after four days at room temperature compared to 14 days in 4°C and up to 2 days in 20°C.[27,28] Moreover, other reports suggest that UV-B/UV-A light availability, organic matter levels, and predation can seriously affect the virus properties.[29,30] SARS-CoV2 RNA was first discovered in untreated wastewater in Spain by Randazzo et al. Before local officials reported the first COVID-19 cases, SARS-CoV-2 RNA was found in wastewater. In addition, the presence of microorganisms and bacteria can leave a trace of virus viability.[31]

Moreover, reports show that in addition to solution pH, oxidants make CoV really sensitive and unstable.[22,32,33] Although the exact mechanism is unclear, enveloped viruses seem much more vulnerable to chlorine-based disinfectants than nonenveloped ones.[34] Table 1 reports the CoV subfamilies. Within this table, we have collected the Centers for Disease Control's pandemic reports, and the influential factors on virus viability, with the way they affect it in parallel, are also gathered. Moreover, Figure 3A depicts the synergistic effects of UV light and chlorination on virus inactivation.[32] Figure 3B shows the collection of variables that are conducive to changes in virus viability.[35]

Briefly, comprehensive characterization of virus traits leads to achieving conceivable information that endorses the decision makers to make smart decisions about focusing on the suitable sources and targeting the regions to intervene. Such lines of action not only halt this aggressive disease but also enhance local economies and probably hinder the intense financial damages. Apart from using wastewater detection methodologies for cost economizing and faster estimation, other driving forces emphasize the importance of this method. For instance, lower-income and middle-income countries, like many African and Asian nations, are seriously suffering from financial and technical issues.[38–40]

Several articles have addressed the detection of SARS-CoV-2 and the disinfection process of drinking water, or monitoring and treatment of different types of wastewater containing COVID-19, ranging from detection to epidemiological modelling.[9,41] Each review dealt with the subject from a particular angle. For instance, Singh et al. and Fu et al. separately reviewed surveillance of wastewater as a non-invasive, cost-effective, and early warning epidemiological method to detect the genetics of SARS-CoV-2 for precise epidemic management.[42,43] Mandal et al. reviewed the topic and summarized that COVID-19 was found in different kinds of wastewater and can be inactivated by a disinfection method.[18] La Rosa et al. reviewed the COVID-19 presence and concentration in water environments.[22] They indicated that future research must adapt the methods usually employed for sampling and concentrating enteric and non-enveloped viruses from water sources to the enveloped ones. According to Bhatt et al., the occurrence, and potential treatment approaches for the removal of viruses from wastewater were reported, however, the detection methods were not indicated, and processes to fully treat COVID-19 were not dealt with adequately.[8] Further, Paul et al. reviewed the impact of environmental variables on the transport and fate of SARS-COV-2 in aqueous media, such as temperature, UV exposure, organic matter, disinfectants, and adversarial microorganisms.[30] Some researchers also highlighted the behaviour of viruses in aquatic environments, soil, sewage sludge, and other solid materials.[9]

Viruses originate from sewage water, drinking water distribution systems DWDS, and SWSS and infect drinking water supply systems. Chen et al. systematically compared conventional and emerging disinfection technologies and they reported the virus in drinking water, but no reference was made to wastewater treatment to protect public health.[44] Rummaging around in the literature, we could not find a succinct review paper that integrated and classified all aspects of COVID detection in wastewater. However, bearing in mind the unexampled COVID-19 spreading and lethal traits, an integrated review with a mechanistic point of view is certainly warranted. Moreover, we could not find a review paper considering the contribution of nanotechnology and nanomaterials to the detection and treatment of COVID-19 in wastewater. With these perspectives in purview, the main objectives of this review article are anchored in the classification and

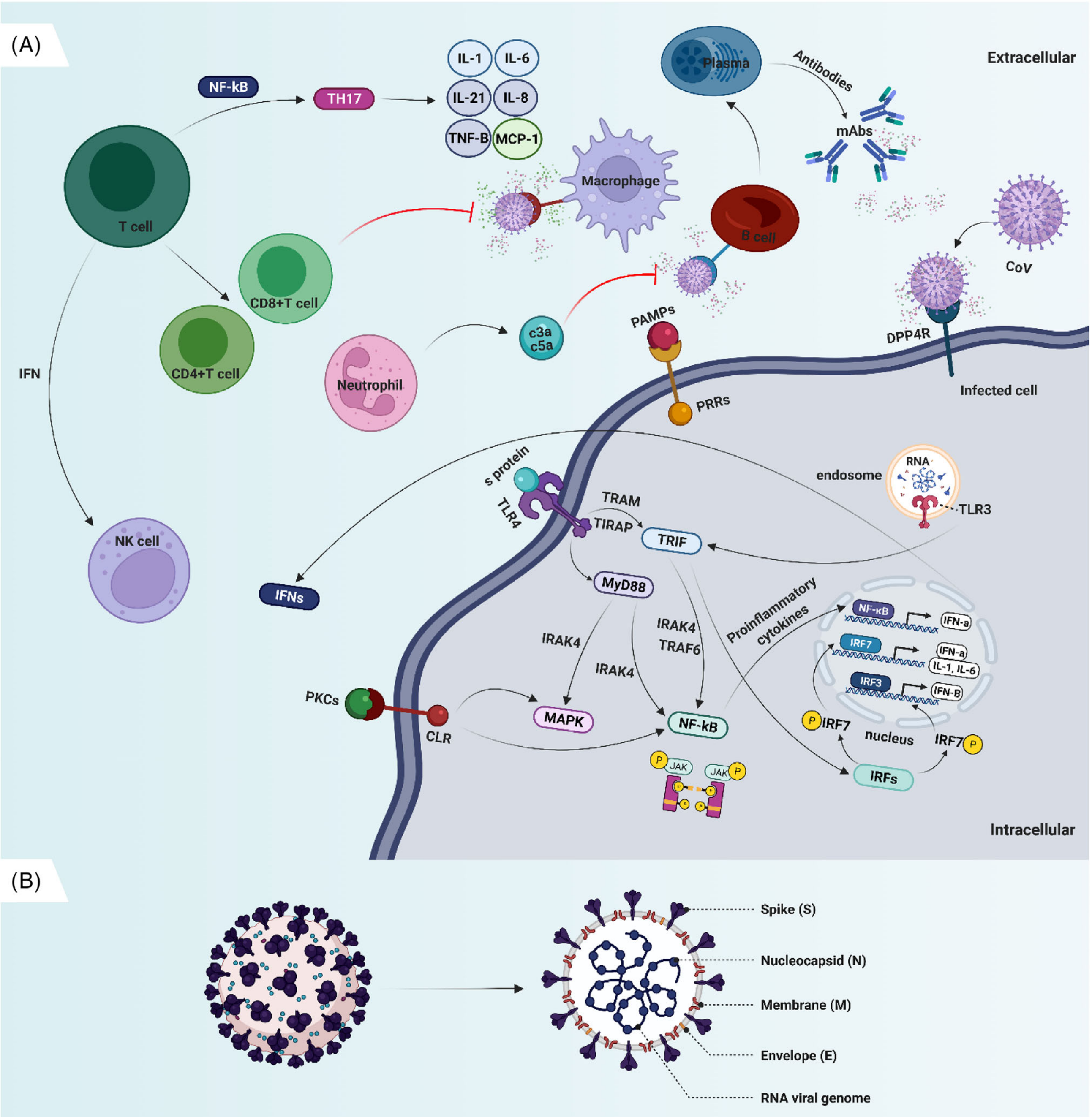

FIGURE 2 A very simplified schematic of (A) the immune system response when runs into coronavirus infection. In this process, macrophages, CoV antigens, and T cells are seriously involved. As an essential key role, T cells get activated, which is conducive to the massive release of cytokines. The following steps involve CD8 T cell activation and other essential signalling pathways. Among these pathways, the MyD88-dependent signalling pathway is one of the essential ones which brings about a massive release of immune mediators. (B) Enveloped virus particles comprised a spike, membrane, nucleocapsid, enveloped, and genome RNA. Adapted and redesigned.[24]

integration of possible ways to detect and treat COVID-19 in wastewater as well as the contribution of nanotechnology to these processes. Several questions needing answers have been designed in this regard, and they are:

1. Is wastewater apt to infect humans? Better formulated, is there any hazard for human infection by wastewater?
2. What treatment methods, and does wastewater threaten humans even after treatment?
3. Is it likely to discover the polluted areas utilizing methods of recognition of viruses in wastewater?
4. Are the obtained results from wastewater assessment consistent with clinical tests? Better stated, are the results trustworthy to anticipate the polluted areas?

TABLE 1 The collection of coronavirus families and variables leaves essential traces on virus viability (CDC = Centers for Disease Control).

Virus family/ variables	Characteristics/names	Comments	Refs.
CoV family	gamma CoV	"More severe symptoms or death compared to other variants," according to the CDC	[21–23]
	beta CoV A	"May unfurl swifter than other variants," according to the CDC	
	beta CoV D		
	beta CoV B		
	beta CoV C		
	alpha CoV	"Spreads far swifter than other variants" and "likely to bring about more people to be sicker and to die," according to the CDC."	
	delta CoV	"Likely to induce severe symptoms than other variants," according to the CDC	
Influential factors in viability	Temperature	Undetectable after four days at room temperature compared to 14 days in 4°C and up to 2 days in 20°C	[21,27]
	UV-B light	UV LED with a peak of ~286 nm can be considered a fighting tool against human Coronaviruses	[36]
	UV-A light		
	Presence of microorganisms	Bacteria/microorganisms affect your body's response to the virus	
	Presence of bacteria		
	Solution pH	Stability is maximum at pH = 6.0 incubated at both 4 or 33°C	[37]
	Addition of oxidants	It makes virus sensitive and unstable	[22,32,35]
	Addition of chlorine-based disinfectants		

5. How much time deviation exists between the pandemic pinnacle in different regions predicted by wastewater and clinical methodologies?
6. How and to what extent does COVID-19 detection and treatment in wastewater contribute to nanotechnology?

In addition, scientists proved that common virus concentration methods are mostly improper in discovering enveloped viruses. Using new methodologies to recognize SARS-CoV-2 RNA in water recourses was recently reported in the Netherlands, Australia, France, and the USA. Regarding the practicality of wastewater assessments, Hart (researcher in the Biodesign Center for Health Engineering) declares: "*However, when preceded by a population-wide screening of wastewater, the task becomes less daunting and more manageable.*" We can, in one go, monitor an entire community for the presence of the new coronavirus. Hence, we have no way to discover the polluted areas, estimate the prevalence, recognize the genetic deviation and diversity, and predict what will go on in further steps. This article also discusses the urgent need to find effective approaches for the elimination of viruses and bacteria before they become resistant and pose a threat to public health. With the outbreak of COVID-19, there is a growing concern about finding efficient treatment approaches and curbing transmission routes. The article highlights the importance of WBE or sewage epidemiology, which can be used as an indicative benchmark to monitor infection levels and provide crucial data about the rate of an infection increase. The article also discusses the challenges and opportunities of using RNA concentration measurements to detect SARS-CoV-2 in sewage sludge and the correlation between the number of COVID-19 cases with SARS-CoV-2 RNA concentration in wastewater. The article suggests that nanotechnology-assisted viral detection and wastewater treatment may be a solution to the problem, which explores the potential of different treatment methods.

2 | THE DETECTION METHODS AND JUXTAPOSITION

Reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR), adsorption-elution techniques (electropositive or electronegative microfilter), polyethylene glycol (PEG) precipitation, gel electrophoresis, amplicon sequencing, ultrafiltration, aluminium flocculation as well as plaque-forming units (PFU) are the avenues all of which have been opened for virus detection since long.[45–47] To the best of our knowledge, many of the above-mentioned techniques are not employed for the novel SARS-CoV-2

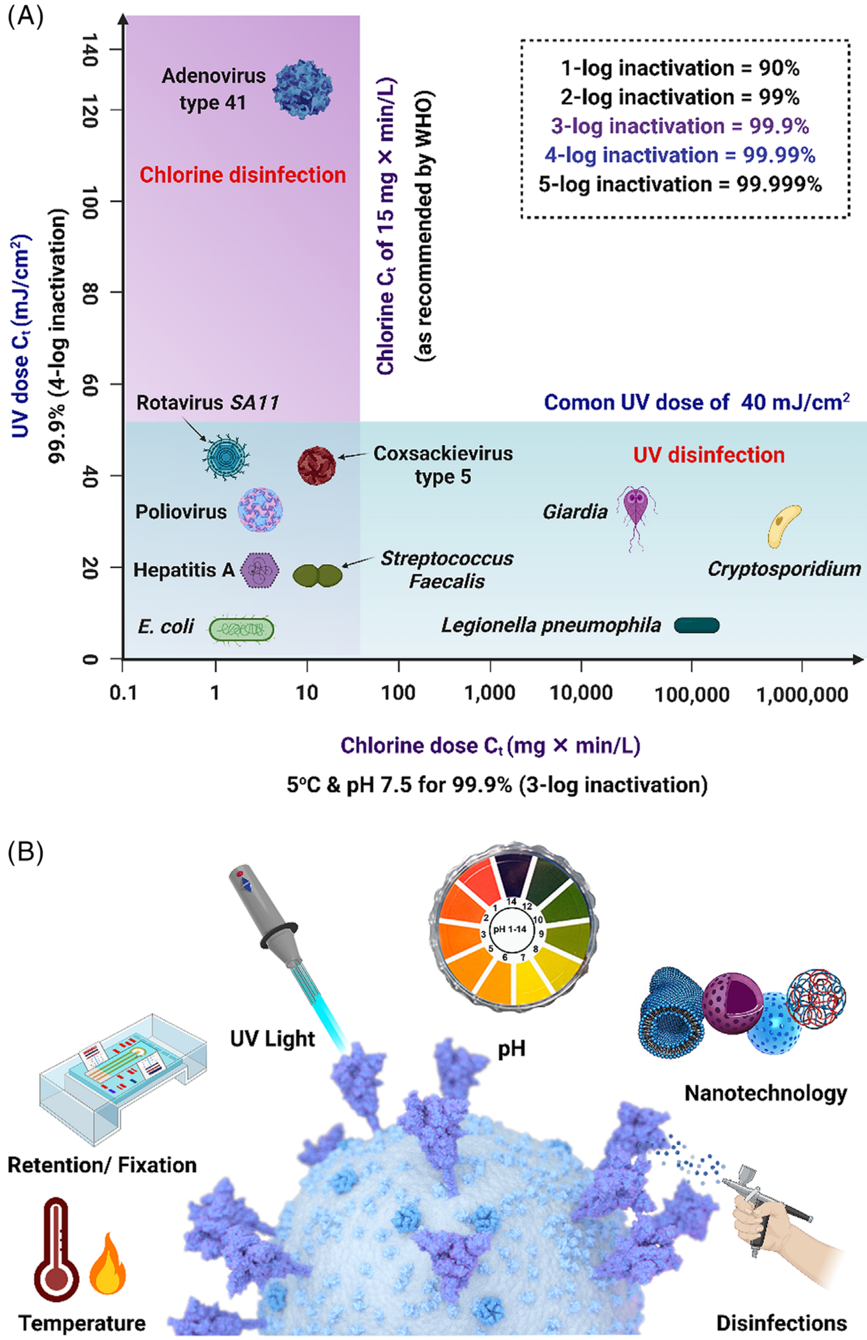

FIGURE 3 (A) The parallel influence of ultraviolet (UV) light and chlorination on coronavirus. It can be inferred from the picture that chemical disinfection (chlorination) and non-chemical disinfection have great performance in virus inactivation. Changes in virus activation at different chlorine doses with a constant UV dose are reported simultaneously with the changes in virus activation at different UV doses with a constant chlorine dose. (Scheme recommended by World Health Organization, WHO).[32] (B) The fundamental parameters have essential effects on coronavirus viability.[35] It was adapted and redesigned from the mentioned reference.

detection due to some blind spots. The adsorption-elution technique is an instance of a method that is improper for enveloped viruses. The efficiency of adsorption-elution technique is reported to be less than 10% for enveloped virus recovery. Hata et al. reported that a variety of PCR-based assays were used to detect SARS-CoV-2 RNA in the samples, and PEG precipitation is claimed to be one of the most cost-effective techniques for detecting virus and protein recognition.[48] Gel electrophoresis, amplicon sequencing, ultrafiltration, and aluminium flocculation are addressed as inexpensive and easy to set up, but unfortunately, they are actually time-consuming, thus hindering their usage in the face of such a fast-evolving pandemic. Combining some of these methods with RT-qPCR analysis is utilized to optimize the results.[16,46,49,50]

In some studies, three parallel droplets of digital PCR (ddPCR) (Ultrasensitive assays based on PCR) assays (N1, N2, and N3) have been utilized for better detection of SARS-CoV-2 with fewer uncertainties.[51,52] However, PFU can properly estimate virus concentration, but it is prolonged and difficult. Furthermore, most quantitative molecular approaches cannot measure viral infectivity.[53,54] Chiefly, RT-qPCR is the most frequent or maybe better stated as "the only used" technique for virus detection

TABLE 2 Comparison of coronavirus detection methods, their advantages and limitations, the limit of detection (LOD), accuracy, and volume of sample required for measurements.

Methods	Advantages/ disadvantages	The limit of detection (LOD) (Viral copies/ reaction)	The accuracy of methods	The sample volume required	Refs.
RT-qPCR	RT-qPCR is the most frequent/swift rate/ not suitable for real-time surveillance	1–10 viral copies/ reaction	High, and it is able to detect the virus even in asymptomatic individuals	3–10 μl of viral RNA	[55]
Adsorption-elution techniques (electropositive or electronegative microfilter)	Improper for enveloped viruses/efficiency is less than 10% for enveloped virus recovery	100–1000 viral particles/ml	Relatively high	3–9 ml	[56]
PEG precipitation	Cost-effective technique	10^4–10^5 viral particles/ml	Moderate	5–10 ml	[46,49]
Gel electrophoresis	Inexpensive and easy to set up/time-consuming	10^6–10^7 viral particles/ml	Low	3–10 ml	[16]
Amplicon sequencing	Time-consuming/not suitable for mortal pandemic	10^3–10^4 viral particles/ml	High	3–10 μl	[46]
Aluminium flocculation		10^3–10^4 viral particles/ml	Moderate	3–10 ml	[48]
Ultrafiltration	It needs to be used in parallel with other ones	10^4–10^5 viral particles/ml	Moderate	5–10 ml	[57]
Plaque-forming units (PFU)	Very slow and intricate	1.0×106 PFU/ml	33%	Sevral ml	[58]
Quantitative molecular approaches		Not able to measure viral infectivity			[53]

because of the swift rate, albeit not suitable for real-time surveillance.[55] Table 2 compiles the advantages and disadvantages of current methods for virus detection within the wastewater.

Various methods have been employed for virus detection, including RT-qPCR, adsorption-elution techniques, PEG precipitation, gel electrophoresis, amplicon sequencing, ultrafiltration, aluminium flocculation, and PFU. However, some of these methods are not suitable for enveloped viruses, are time-consuming, or cannot measure viral infectivity.[55] RT-qPCR is the most commonly used technique due to its swift rate, but it is not suitable for real-time surveillance. The use of digital PCR assays and the combination of multiple methods with RT-qPCR have been suggested for better detection. Indeed, further research is needed to develop efficient and cost-effective methods for real-time monitoring of SARS-CoV-2 in wastewater.

3 | CORONAVIRUS DETECTION IN WASTEWATER

To confirm the existence of various COVID-19 target genes, WBE investigations were conducted by Arora et al. using samples from various WWTPs and hospital wastewater samples and targeting the virus genes within untreated wastewater using the RT-qPCR technique.[59] The considered genes for detection were the S gene, E gene, open reading frames 1ab (ORF1ab) gene, RNA-dependent RNA polymerase (RdRp) gene, and N gene, all of which endorsed the study to detect the SARS-CoV-2 within the wastewater correctly. The noteworthy point in this study was the wastewater temperature that had been retained above 40°C. This seems to be a massive step toward withdrawing uncertainties. Moreover, they underscored the practicality of sodium hypochlorite for treating raw wastewater.[59] In one study, a beta-coronavirus strain (OC43) with a close lineage to SARS-CoV, SARS CoV-2, and Middle MERS-CoV was inserted under advertent investigation to find the answer to the following questions. 1) SARS CoV-2 stability presuming within various circumstances, and 2) whether assessing the virus concentration is authentic using dead-end hollow-fibre ultrafiltration (D-HFUF) technologies. Their results revealed that most viral losses might happen when 1-h passes the incubation at room temperature, and the results of cold storage and freeze-thaw cycles were totally different. Indeed, they speculate that protein denaturation occurs in higher temperatures due to extracellular enzyme activity increase.

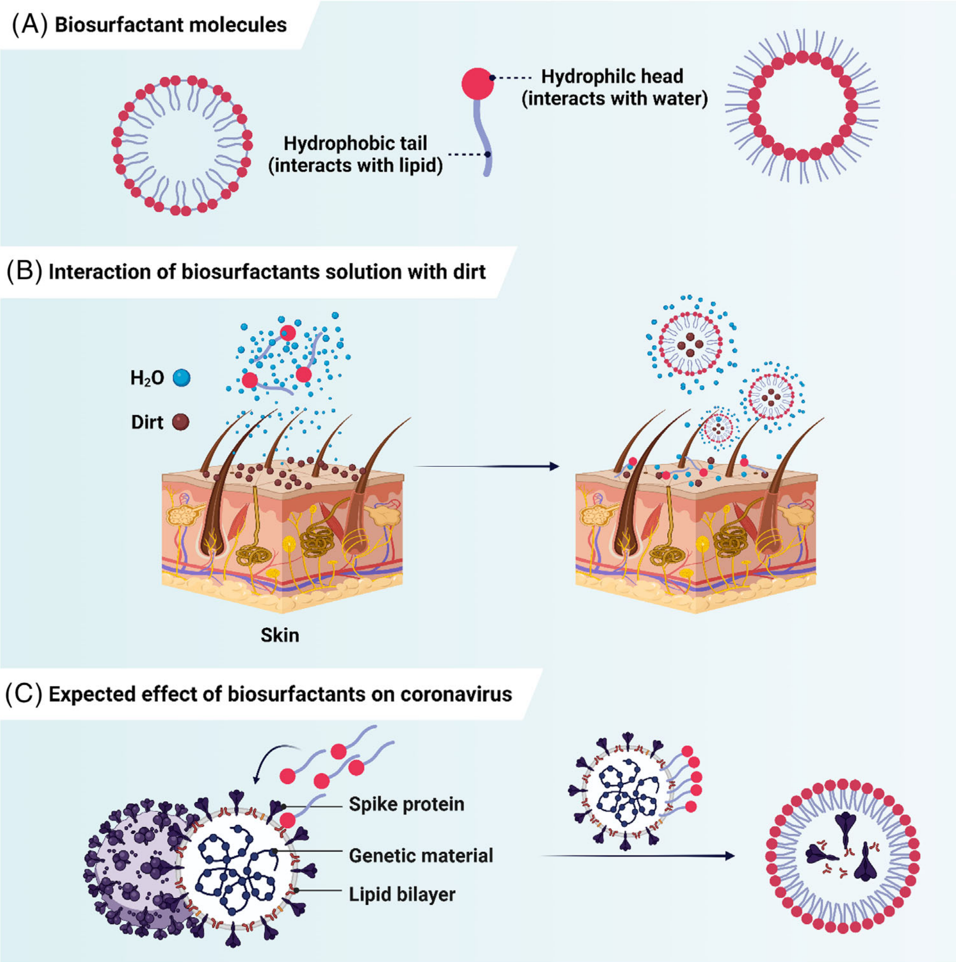

FIGURE 4 Based on the figure, (A) Biosurfactant molecular structure, (B) The interaction between dirt, and (C) Biosurfactants and the anticipated influence of the biosurfactant on coronavirus are depicted and adapted and redesigned.[60]

Also, they hypothesized that adding polysorbate surfactant utilizing protein coating processes has a functional role in postponing the OC43 degradation at room temperature. Using the D-HFUF method, they presumed the enveloped virus concentration in large volumes of wastewater (>1 L) before and after the primary treatment process in about 25 min.[12] Figure 4 shows the biosurfactant structure, biosurfactant interaction with dirt, and the final influence of the biosurfactant.[60]

To confirm the existence of various COVID-19 target genes, WBE investigations were conducted by Green et al. using samples from various WWTPs and hospital wastewater samples.[61] In addition to quantifying SARS-CoV-2 concentration, proposed a scalable technique to detect the virus.[61] Their analysis revealed that RNA detection was possible by utilizing ultracentrifugation technology summed with 50% sucrose cushion addition. The frail part of their study was the small sample size (about 20 ml) within about 8 h.[61] Medema et al. utilizing the ordinate speed of centrifugation, could purify the genomes.[62] Additionally, adding some electronegative membranes enhanced the authenticity of their results.[63] Other laboratories may be unable to replicate their strategy because their methodology was costly and limited in terms of equipment.[16] Nonetheless, the ultracentrifugation methods are reported as the simplest ones that prepare the output results for small volumes (11 ml) of wastewater in about 1 hour considering the assumption that the number of carriers—symptomatic or non-symptomatic be correlated with the quantity of SARS-CoV-2 genomes found in wastewaters.[64]

Aluminum-driven flocculation is another new strategy to recognize the virus's existence in wastewater. Besides, tangential flow filtration followed by PEG precipitation is a practical method, but drawbacks such as problematic scaling-up, time-consuming detection process, and expensive equipment restrict their applicability.[46,49] A time-course quantitative analysis of wastewater samples for pandemic anticipation was proposed by Wurtzer et al.[65] utilizing the RT-qPCR method. Interestingly, increasing the genome units within the wastewater was totally in concordance with the growth of the number of infected people reported by accurate sources. On the other hand, decreasing the genomes was parallel with abating the reported number of infected individuals after the lockdown.[64] Randazzo et al. utilized RT-qPCR and aluminium hydroxide adsorption-precipitation concentration methods, and their result could detect the infection weeks before the time that clinics reported the first case! Therefore, they believe their suggested avenue can be used to determine the onset time of the "next wave". In order to convince the inconsistencies, their hypothesis is based on climate conditions and hydraulic retention time in sewers.[31,66]

Another team took advantage of the RT-qPCR technique to analyze whole genome sequencing and phylogenic-related results in treated and untreated wastewater. Showing negative results for treated water and positive results for raw water signifies the effectiveness of their procedures. Noteworthy, some viral RNA copies exist at a low concentration, but it was under the sensitivity threshold. This team claims that because only 32% of symptomatic people are tested in hospitals, this approach proposes a perfect means for estimating infected individuals.[67] Using human-specific crAssphage plus the addition of 50% sucrose cushion, Green et al. could extract the nucleic acid the estimate the number of COVID-19 cases. They suggest that the ratio of log10 (SARS-CoV-2): log10 (crAssphage) has a connection with the estimation. As a bright point in their research, we can address the rate of their tests. Indeed, they can operate 60 samples in 24 h which is considerable. For the inconsistencies, their hypothesis is the rapid decay of SARS-CoV-2 compared to crAssphage within the water.[61] Ahmed et al. took advantage of utilizing two parallel methods to detect SARS-CoV-2 RNA within the wastewater. Combining the RT-qPCR technique and electronegative membrane approach could greatly constrain the reported numbers of infected individuals with their estimation. They selected an approximate range of 3×20 s at 8000 rpm centrifugation to homogenize the samples. Moreover, their unique method to make a bridge between the RNA copies and the infected people was a mass balance for every single day, which is hereunder determined by Equation (1)[16]:

$$\begin{aligned}&\text{Persons infected} =\\&((\text{RNA copies/liter wastewater})\,(\text{liters wastewater/day}))/\\&((\text{g feces/(person–day)})\,(\text{RNA copies/g feces}))\end{aligned}\tag{1}$$

Finding the reason for inconsistency among results obtained from wastewater assessment and the clinical consequences, Trottier et al. suggested some hypotheses. Although they believe these seem to be more of only presuming, their assumptions make sense. They recommend that these discrepancies are all relevant to tourist accommodation, people how to move to their second houses, people who are in the rudimentary stages of the disease which their symptoms have not emerged, underestimation of disease progression stem from statistics as well as the deviation of the virus spreading pattern in different geographical regions.[68] Leibowitz et al. have suggested an excellent allegation for the inconsistencies between the estimation and the actual number of infected. Evaluating the viral stocks without any enzymatic pretreatment brings about overestimation. Indeed, they believe that pre-purification or enzymatic pretreatment is essential to prevent overestimation.[69]

Two interesting viewpoints have been suggested for subsequent studies as new detection methodologies to decrease uncertainties. Noteworthy, Mao et al. proposed a paper-based technique for SARS-CoV-2 in wastewater detection as a promising tool that can be printed with a wax printer.[70] They believe all detection processes can be integrated into only one inexpensive paper-based device. The following great notion is that membrane bioreactors (MBRs) as a potent facility to diminish viral loading in wastewater treatment.[71,72]

Nanotechnology can potentially be used in conjunction with other wastewater treatment methods to improve COVID-19 mitigation efforts in several ways. First, nanoparticle-based biosensors can be used to detect the presence of SARS-CoV-2 in wastewater. A novel fluorescence sensing array based on lanthanide-doped carbon nanoparticles is presented, which effectively detects SARS-CoV-2 in wastewater and bargains a possibly cost-effective method for epidemiologic surveillance to mitigate the spread of COVID-19.[73] Sahu et al. reviewed the latest advances on targeted implementation of nanoparticle-based colourimetric methods in detecting various substances, including SARS-CoV-2, in various fields such as environmental science, virology, and biomedicine, with a particular focus on the physicochemical properties and applications of gold and silver nanoparticles and their potential for future development.[74]. Second, nanotechnology-based filtration systems can be used to remove SARS-CoV-2 from wastewater. For instance, nanofiber membranes made of materials such as graphene oxide, titanium dioxide, or carbon nanotubes (CNTs) have shown promise in removing various types of viruses from wastewater.[75] Nanotechnology-based disinfection methods can also be applied to decompose SARS-CoV-2 in wastewater. For example, silver nanoparticles have been shown to be effective in disinfecting wastewater by disrupting the virus's membrane and inhibiting its replication.[76] Additionally, the development of water treatment technologies that may be utilized to address water-related issues can be aided by the application of nanometals (silver and gold) and nanomembranes.[77] Nanomaterials can be employed to treat the water to avoid microbial growth. Many organic and synthetic nanomaterials have demonstrated strong antibacterial capabilities in a variety of processes, such as the photocatalytic generation of reactive oxygen species supporting cell components and viruses (e.g. TiO_2, ZnO and fullerol) destruction. Moreover, nanotechnology can be employed to improve the effectiveness of water filters. To provide secure water for consumption, clay filters were made and coated with colloidal silver and strengthened with husk. For emergency relief efforts, these filters were mass-produced, and based on the anti-virus properties of silver nanoparticles, fullerenes and CNTs can potentially be utilized in the water treatment process of COVID-19-contaminated wastewater.[77]

Recent studies have utilized various methods to detect SARS-CoV-2 in wastewater. Most studies have used RT-qPCR techniques to detect COVID-19 target genes, and some have used ultra-centrifugation and aluminium-driven flocculation methods. The studies suggest that increasing genome units in wastewater are parallel to the number of infected people, and decreasing genome units correspond with decreasing infected individuals after lockdowns.[78] Some other studies have shown negative results for treated water, but positive results for raw water, indicating the effectiveness of the procedures. However, there are still limitations in terms of small sample sizes, costly equipment, time-consuming processes, and scaling-up difficulties. Therefore, further research is required to improve the scalability, and reliability of the examined and under-design methods.

Nanotechnology can increase the accuracy of COVID-19 detection in wastewater by providing highly sensitive detection methods. Detection at a level with extremely low concentrations of the virus is correspondingly possible. One approach is to use nanoparticles functionalized with specific biomolecules that can bind to the virus. For example, gold nanoparticles can be functionalized with antibodies to attract viruses. When the virus is present in the wastewater, it will bind to the nanoparticles, causing a change in the optical properties of the nanoparticles. This change can be measured using a technique called surface-enhanced Raman scattering, which can detect the presence of the virus at very low concentrations. Another approach is to use nanofiltration membranes that can selectively capture and concentrate the virus from the wastewater. These membranes can be functionalized with specific biomolecules that can bind to the virus, allowing for efficient capture and concentration. This approach can improve the sensitivity of the detection method by increasing the concentration of the virus in the sample, making it easier to detect. Overall, nanotechnology can provide highly sensitive and specific methods for detecting COVID-19 in wastewater, which can be a valuable tool for monitoring the spread of the virus in communities and helping to identify outbreaks early.[77,79,80]

Nanotechnology has the potential to play a significant role in wastewater treatment to mitigate the spread of COVID-19 and has also the potential to be a crucial factor in treating wastewater and preventing the spread of COVID-19. There are several ways that nanotechnology can be used in wastewater treatment. Nanofiltration membranes can be used to filter viruses and other tiny particles out of wastewater. These membranes have small pores that can trap viruses, including the SARS-CoV-2 virus. This method can be used to prevent wastewater from contaminating water sources and limit the spread of the virus. Nanotechnology can also be used to disinfect wastewater and eliminate viruses and pathogens. Nanosensors can monitor the concentration of the virus in wastewater and track its spread, providing real-time data. Nanoparticles can also improve the bioremediation of wastewater by providing a surface for bacteria to attach to and grow, effectively breaking down organic matter in the wastewater and removing viruses and contaminants. Overall, nanotechnology provides a range of tools and approaches that can help reduce the risk of transmission of COVID-19 in communities.[81–84]

While nanotechnology offers promising solutions for COVID-19 detection and wastewater treatment, there are also some challenges to be considered. One major challenge is ensuring the safety of nanomaterials and minimizing their potential impact on human health and environmental contamination. Additionally, the cost of producing and implementing nanotechnology-based solutions may be high. There may also be issues related to the scalability of these technologies and their integration into existing wastewater treatment systems. Finally, there may be regulatory hurdles to overcome, such as ensuring that the nanotechnology-based solutions meet safety and efficacy standards.[85–88]

4 | CORONAVIRUS DETECTION METHODOLOGIES IN SEWAGE, SLUDGE OR FAECES SAMPLES

Peccia et al. reported that the virus detection procedure using sewage. Primary sewage sludge from raw wastewater consists of a solid matrix encompassing various human virus strains. It can prime a dense mix of coronaviruses that are reported to have concentrated SARS-CoV-2 RNA three times greater than that of raw wastewater. Interestingly, the detection time is 2–4 h, visibly shorter than previous reports. Noticeably, this technique helps figure out the onset of COVID-19 shedding sooner than clinical warnings.[89] Ampuero et al. proposed a new methodology to recognize the circulation of the SARS-CoV-2 virus within the sewage. This group utilized the combination of ultracentrifugation associated and RT-qPCR to provide a precise tool before treatment and after heading away from two stages of treatment (they detected SARS-CoV-2 in untreated and treated wastewater samples obtained from two treatment plants). Their results indicated that increasing the number of complicated cases in hospitals is precisely parallel with increasing the genome copy numbers in polluted areas of Santiago, Chile.[90]

Wu et al. aimed to compare the number of confirmed cases by a hospital with the results obtained from the PCR product by direct DNA sequencing that can signify the number of infected individuals utilizing the RT-qPCR. Oddly enough, they reported that the viral titers were increasingly more than the confirmed reported number by hospitals. Their interpretation of the deviation is set on account of patients without symptoms who have not been referred to the hospital. With all these in mind, they assert that their output is unrelated to the clinical reports' hesitation. In more explicit terms, they acknowledge that this inconsistency does not necessarily mean that clinical results are incorrect. Noteworthy, using equipment for time-integrated sample collection and the scalability of their method are the outstanding features of this study.[91] Using the RT-PCR technique, sewage samples in 7 cities of the Netherlands were collected for subsequent investigation. Their detection was 2 specific kinds of genes: protein gene (E) and nucleocapsid protein gene (N1-3). Their analyses revealed that looking over the SARS-CoV-2 circulation in society can be achieved using high-sensitivity sewages. They also related that 10^7 RNA copies/g of faeces are observed at the pandemic's pinnacle, decreasing to 103 RNA copies/g when the number of reported clinic cases diminishes.[62]

A real-time RT-PCR diagnostic panel, a detective technique in sewage samples, can recognize the polluted areas as soon as possible before intense spreading and is reported as a sensitive and practical tool.[92] Bar-Or et al. utilized PEG or alum precipitation techniques to detect viral particles within the sewage. To remove sediment and large particles, they used first centrifugation, which caused more concentrated samples. Their research's outstanding feature was the use of two parallel methods for viral isolation. Furthermore, their hypothesis for convincing the inconsistencies in the existing differences in sewage composition and sample processing can affect the consequences of viral genome detection.[50]

Not many reports have been published on recognizing SARS-CoV-2 within sludges despite many critical driving forces proving the necessity of coronavirus recognition within sludges. To name but a few, sludge can be applied as a soil conditioner on agricultural land or be landfilled. Two specific kinds of sludges exist primary sludge (PS) and waste-activated sludge (WAS). PS refers to settling the solid ingredients under the action of gravity and will ultimately consist of about 1%–2% solids by weight. WAS comprising 0.6%–0.9 % solids by weight will be processed using specific treatment techniques. Kocamemi et al. aimed to detect the SARS-CoV-19 within the PS, and the WAS using PEG 8000 adsorption procedure with a concentration-based result. Their findings indicated that the copy number of viruses changes between 1.17×10^4 to 4.02×10^4 per litre.[92]

One report shows positive RT-PCR tests in the stool samples have been observed despite negative nucleic acid in throat swab specimens. Zhang et al. devised an experiment in which three infected children were under investigation. Their cases were mild, and they did not run into arduous conditions. Howbeit, their throat swab specimens revealed negative consequences. Two weeks after diagnosis, they could induce faecal-oral transmission of SARS-CoV-2. This is why we need to know deeper details about viral faecal infection.[93] Apart from wastewater and sewage, one of the most dubious resources for virus transmission is the plumbing system. Gormley et al.[17] have provided some practical pieces of advice to minimize the possibility of transmission by way of the plumbing system. First, never ignore a foul smell in your wash areas. Second, utilize a functioning U-bend to fit all the existing water appliances within the home. Third, ensure you do not lose any water trap seal within the inserted U-bend. Fourth, if you infer that the wastewater pipework has disconnected, repair it as soon as possible. Fifth, use glue or a trap to repair the leakage in the pipework. Sixth, repetitively check the status and working conditions of the whole system, especially if you live in a tall building.[17,94] Also, to detect SARS-CoV-2 in faeces, environmental researchers have combined three methods simultaneously. Centrifugal ultrafiltration, PEG precipitation, and aluminium hydroxide flocculation are utilized for virus recovery up to 65%; moreover, they claimed that enveloped viruses, such as coronaviruses, can be concentrated effectively. The viral stock must be accurately measured for recovery calculations.[57]

An important question that arises now is whether conventional treatment plants can eliminate the virus or not. In fact, all three ORF1ab, N, and S genes of SARS-CoV-2 have been eliminated after treatment but not completely removed.[20,94] Therefore, we must be extremely cautious about not accidentally letting infection enter the supply and distribution network of drinking water via row sewage, which will allow infection into homes. In addition, the formation of biofilms will impact the water supply and significantly threaten human health.[95,96] Furthermore, we are roughly relieved about Greywater (i.e., water from sinks and showers). Although saliva and sputum in these sources can exceedingly induce infection, the presence of detergents, soaps, and other disinfectants highly lower the viral concentrations.[97,98]

The previous part discussed various studies on coronavirus detection methodologies in sewage, sludge, or faeces samples. The studies indicate that sewage and faecal samples provide a precise tool to recognize the circulation of the SARS-CoV-2 virus within a population. The detection time is shorter than clinical warnings, which helps in identifying the onset of COVID-19 shedding sooner. The studies further suggest that using equipment for time-integrated sample collection and the scalability of the method are outstanding features. However, a few reports have been published on recognizing SARS-CoV-2 within sludge, despite many critical driving forces proving the necessity of coronavirus recognition within sludge. Some studies show positive RT-PCR tests in the stool samples have been observed despite negative nucleic acid in throat swab specimens. Furthermore, the plumbing system is considered one of the most dubious resources for virus transmission. Practical advice is also provided to minimize the possibility of transmission through the plumbing system.

5 | CORONAVIRUS DETECTION MODELS

Another team aimed to examine the virus concentration in wastewater resources. Their results could predict the changes in the number of infected people 4–10 days sooner than the clinical reports. Interestingly, they could infer viral shedding dynamics using their prepared model. Ultimately, they discovered that the inconsistency between their modelling results with the clinical cases refers to asymptomatic or mildly symptomatic cases that will be revealed in further days. Their model suggests a viral shedding function which is novel among the previously reported models. They presume that to reduce deviation in this model, they can utilize normalization with the human faecal indicator.[91] Only one specific model has factored in the influence of climate on the prediction output. A prediction model is primed to determine the effect of various flow rates on concentration in the metropolitan area of A Coruña with Atlantic weather (chiefly rainy days in autumn and winter). Although this model is specified in the mentioned area, entering the flow as a variable is noteworthy even if applying the mentioned model to other regions needs reformulation.[28]

Wu et al. recommended a dynamic model (scalable approach useful for modelling the SARS-CoV-2 pandemic) of virus shedding in wastewater.[91] Their result showed an early peak before the onset of diseases. At least, their model seems to support identifying the COVID-19 transmission trends. Considerably, utilizing wastewater-based methods caused Barcelona to be aware of the pandemic one month ahead of the first case was reported.[99] Ye et al. created a model that aimed to compare the survival and partitioning behaviour of murine hepatitis virus (MHV), ϕ6, MS2, and T3 in raw wastewater. MHV and ϕ6 have enveloped viruses, and MS2 and T3, are nonenveloped bacteriophages. Their findings revealed that 26% of the enveloped viruses could absorb solid particles within the wastewater compared to 6% for nonenveloped bacteriophages. This trait causes enveloped viruses to be infective for days. Moreover, the absorption and inactivation percentages are reported for both enveloped and nonenveloped viruses (Figure 5).[100] This is the reason enough why we have to obtain more comprehensive data about wastewater treatment to compile the various traits of viruses in different circumstances.

Similarly, Vallejo et al. primed another nonparametric and even simple parametric model, which was practical in estimating the number of infected people by COVID-19 as a function of the viral load. Using new parameters such as vaccination rate in their model made it unique in terms of accuracy.[101] Another group prepared another mathematical model for modelling the SARS-CoV-2 spreading dynamics, inspiring politicians to pinpoint the best intervention time. Despite the previous wastewater model to estimate the pandemic, this one assesses the RNA concentration as an infection signifier within municipal sewage sludge. Their output suggests that SARS-CoV-2 RNA concentration can provide superior results for the pandemic but about three to five days earlier (over tracking hospital admissions, consistent with purely reported statistical time series analysis), analogous to previous results.[14] As one of the most important researches, Hart and Halden proposed a computational model that analyzes the feasibility, economy, and enumeration of active Coronavirus within the wastewater. Their suggested model is significantly global because it can analyze all the above-mentioned in Wuhan, Milan, Madrid, New York City, Tehran, Seattle, Detroit, and New Orleans. To achieve the mentioned parameters, we have to prim three significant variables, of which temperature is the most paramount. Undeniably, the cost of this type of estimation is cheaper millions of times over again than when we test all the individuals. Interestingly, they symbolize cost-effectiveness and time management by using their model to make it more sensible. They take this example: If we have a country with an 83 million population and 100 000 clinical assays per day, it takes at least three months of incessant testing.[102] It is preferable to underscore that apart from money and time, many human resources and, in parallel, many infected people will be saved.

Research on coronavirus detection models has been ongoing, and various teams were developing models to

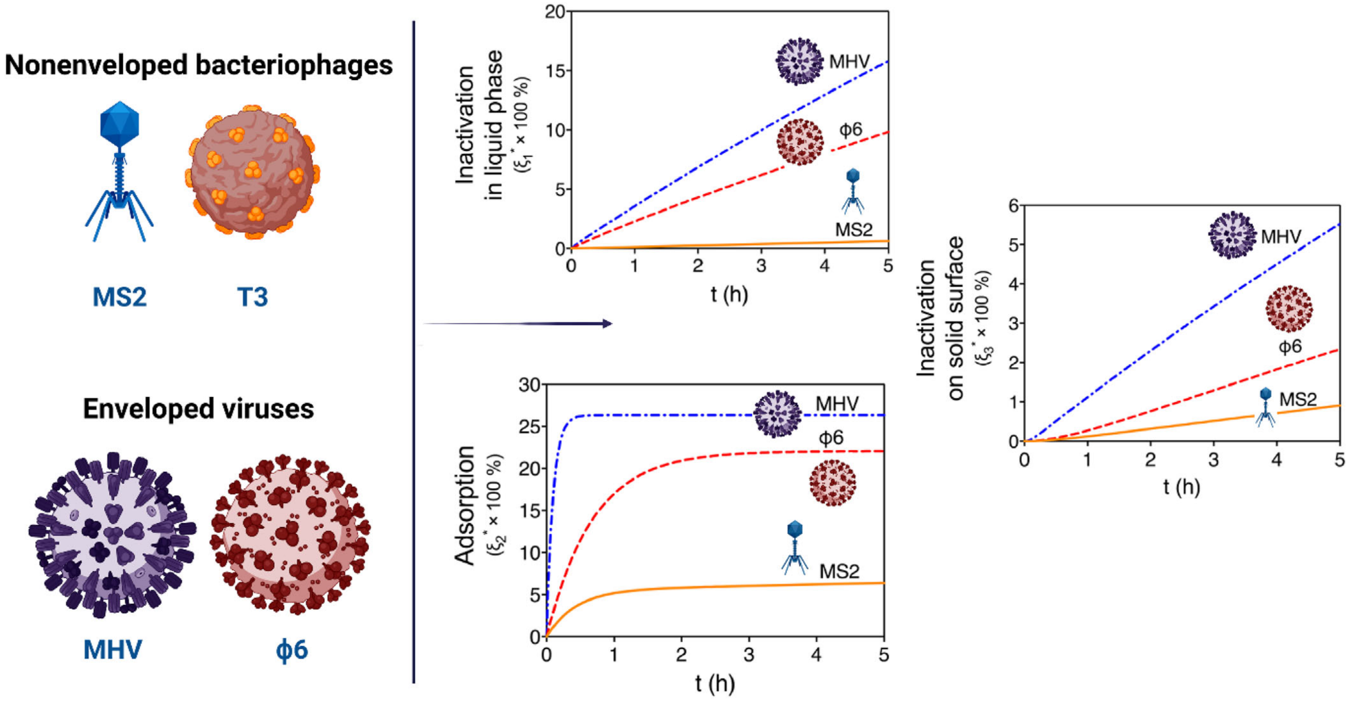

FIGURE 5 Comparison of nonenveloped bacteriophages and enveloped viruses' behaviours regarding survival and partitioning within the wastewater. As observed, enveloped virus adsorption to the solid phase of wastewater is 26%, compared to 6% for nonenveloped. Also, the percentage of virus inactivation is reported for both enveloped/non enveloped viruses within the liquid/solid phase of wastewater.[100]

predict changes in the number of infected people, infer viral shedding dynamics, and estimate the number of infected people as a function of the viral load. These models have proven to be effective in identifying COVID-19 transmission trends and have even enabled cities like Barcelona to become aware of the pandemic one month ahead of the first reported case.[103] However, there are still shortcomings in these models, including their inability to account for asymptomatic or mildly symptomatic cases and the need for more comprehensive data on wastewater treatment. Suggestions for further research include utilizing normalization with the human faecal indicator, factoring in the influence of climate, and analyzing the feasibility, economy, and enumeration of active coronavirus within the wastewater. Such research can save time, resources, and lives while effectively managing the COVID-19 pandemic.

6 | TREATMENT OF COVID-19 CONTAMINATED WASTEWATER

The critical aim of wastewater treatment is to eliminate organic matter and remove nutrients (S, N and P), suspended solids, and soluble contaminants from wastewater.[104–108] At all events, the wastewater treatment processes consist of the physical processes (primary treatment), biological processes (secondary treatment), flocculation sedimentation, sand filtration, and membrane filtration efficient as the physiochemical process (tertiary treatment) for pathogen removal from wastewater. Additionally, based on,[109] it is important to highlight that the approaches, all of which use sand filters for infection removal in the handwash-wastewater treatment of COVID-19, will become more efficient if the use of biological processes and/or physical filtration is made.[109,110] Moreover, reports have claimed that pathogenic elimination using a biochemical process is mainly effective in bacteria removal than viruses.[111] Thus, the higher efficiency of virus removal needs disinfection, which consists of ozonation, UV, chlorination, hydrogen peroxide, and solar radiation, and should be a final process in the wastewater treatment plant. To clarify whether wastewater treatment has efficacy for SARS-CoV-2 removal,[65] discovered that SARS-CoV-2 RNA concentrations in the treated wastewater were 100 times lower than in untreated wastewater in Paris. Besides the exact mechanism of treatment, virus viability data and viral particle reports are not declared utterly therein.

Prado et al. estimated the pathogenic removal efficiency in wastewater treatment which is established as the number of viruses removed from any process using Equation (2).[112]

$$\text{LogReduction Value (LRV)} = \log_{10}(C_b) - \log_{10}(C_a) \quad (2)$$

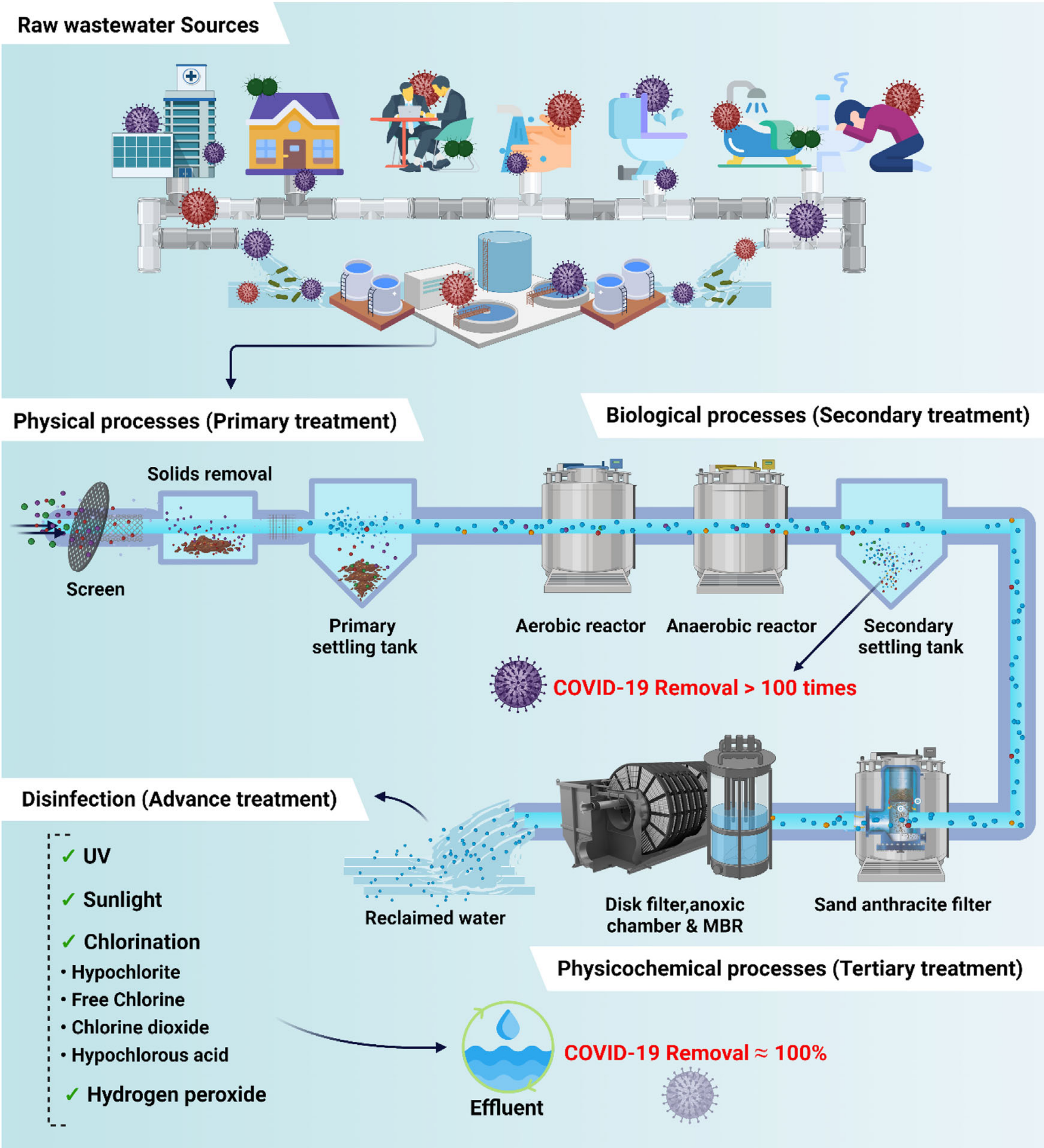

FIGURE 6 Visualization of combining whole processes for virus removal in wastewater treatment plants partially adapted from.[8,27,112,113]

where C_b and C_a stand for the viable virus number before and after the wastewater treatment.

Figure 6 shows the visualization of combining whole processes for the removal of viruses in treatment plants of wastewater.

6.1 | Physical processes

Among the various practical approaches to physical wastewater treatment, sedimentation adsorption and filtration have been exploited for partial viral pathogens elimination. Researchers reported that compared to wastewater solids settlement, virus particle adsorption during sedimentation is the primary mechanism of virus elimination.[114] Numerous other studies have proposed that sedimentation is the fundamental mechanism of virus concentration reduction within the wastewater. Researchers observed that the conventional wastewater treatment process accomplished 0.65–2.85 (LRV) for various types of viruses in the sedimentation phase.[115] Similarly, the LRV was achieved at 1.4–1.7 for groups of viruses containing enteroviruses, rotaviruses, and noroviruses during the stage in which phase settlement happens.[116] Other physical treatments, such as ionizing radiation utilizing gamma rays, non-ionizing radiation utilizing ultraviolet light, photodynamic oxidation and heat, have been utilized during this pandemic.[9]

Noteworthy of concern, various studies have researched the utility of filtration processes to eliminate viruses. The filtration process can be called membrane and sand filtration, specifically slow sand filtration.[117–119] Slow sand filtration holds significant potential for virus removal from drinking water, rainwater, and wastewater treatment. In addition, it is worth noting that the various viral and bacterial pathogens (such as *Escherichia*, *Echovirus*, *Salmonella* and *Escherichia coli*) were efficiently removed by the slow sand filtration process.[120]

6.2 | Biological processes

The biological processes have depended on the cultivation of microorganisms such as bacteria, fungi, or algae to convert organic matter to nitrogen gas and other environment-friendly (benign) biodegradation products under aerobic and anaerobic conditions in wastewater treatment.[121,122] According to Feng et al., granular sequencing batch reactors, MBRs, moving bed biofilm reactors, biological aerobic and anaerobic digesters are the most popular technology systems. It is worth mentioning that most studies have concentrated on sequencing batch reactors and MBRs.[104] Researchers have suggested the process in a pilot-scale granular sequencing batch reactor to evaluate water quality for reusing as irrigation for agricultural purposes.[123] In order to monitor virus removal efficiency, evaluation of pathogens (*Salmonella*, *Giardia lamblia*, *E. coli*, *Clostridium perfringens*, *Cryptosporidium parvum*, *Adenovirus*, *Enterovirus* and Enterovirus *Somatic Coliphages*) was performed. The obtained LRV was 3, 2, and 3 somatic coliphages, adenovirus, and enterovirus, respectively. Moreover, other amounts of 4, 3, and 1 for *Escherichia coli*, *Giardia lamblia*, and *Clostridium perfringens* were successful. For *Salmonella* and *Cryptosporidium parvum*, thorough removal happened in a granular sequencing batch reactor,[123,124] confirmed that the algal-based wastewater treatment using *Galdieria sulphuraria* was successfully applied to treat viral viruses including somatic coliphages (3.13 LRV), F-specific coliphages (1.23 LRV), enterovirus (1.05 LRV) and norovirus GI (1.49 LRV) in full-scale at Las Cruces, New Mexico, USA. The MBR combines two parts in one stage, including membrane filtration and a biological reactor. It has been a promising avenue for obtaining virus removal efficiency from wastewater samples.[125] For efficiency assessment of virus removal within MBR, the LRV reports show 6.3 for adenoviruses, 4.8 for noroviruses, and 6.8 for enteroviruses.[126] In contrast, Zhou et al concluded that the efficiency of virus removal, including enteroviruses, noroviruses, and rotaviruses, could not be observed in an MBR.[116] The consequences of biological treatment revealed that this type of treatment avenue during the COVID-19 pandemic decreased dissolved organic carbon (DOC) and UV_{254}. On the contrary, it boosts dissolved organic nitrogen (DON) and specific ultraviolet absorption. Additionally, biological treatment increases the DON/DOC ratio and bromide/DOC ratio.[127]

6.3 | Physiochemical processes

The physicochemical process is a simultaneous coagulation-flocculation and filtration process utilized in municipal wastewater treatment,[128] stated that few studies had estimated the impact of physicochemical parameters on the viral abundance in wastewater treatment plants. Similarly, the recently observed results indicated the limited utility of the physicochemical variable as an indicator of enteric virus existence in the effluents from biological procedures. In contrast, the temperature influenced the decrease of viral abundance detected in the treated samples.[112] These results could be expected as numerous studies propose an increase in water temperature could favour the indicated decrease of viruses in these environmental conditions.[129] Furthermore, only the inflow rate was the variable that was positively associated with the viral abundance present in the secondary settling tank. Additional studies are needed to study the impact of physicochemical processes on the operational parameters in wastewater treatment plants, particularly pilot-scale and full-scale research, where the variables of significant concern in the treatment performance will be better controlled.

6.4 | Disinfection processes

It has been thought that conventional wastewater plants can fully remove the viruses in water effluent. However, the ultimate result of treatment of coronavirus fate within the wastewater needs to be the topic of further research.[130] Thus, the effluent water needs more disinfection for complete virus removal as an indicator of a successful consequence in the dual fight against the COVID-19 virus and water scarcity. The disinfection process mainly consists of chlorination, ozonation (O_3), ultraviolet irradiation (UV), and sunlight-mediated wastewater disinfection. Moreover, disinfection could be used as a standalone or combined process. Figure 7 shows the order of different critical approaches for urban water treatment (sedimentation, deep bed and membrane filtration and disinfection) and their effect on virus inactivation and the route of virus transmission via pipelines.[44,131]

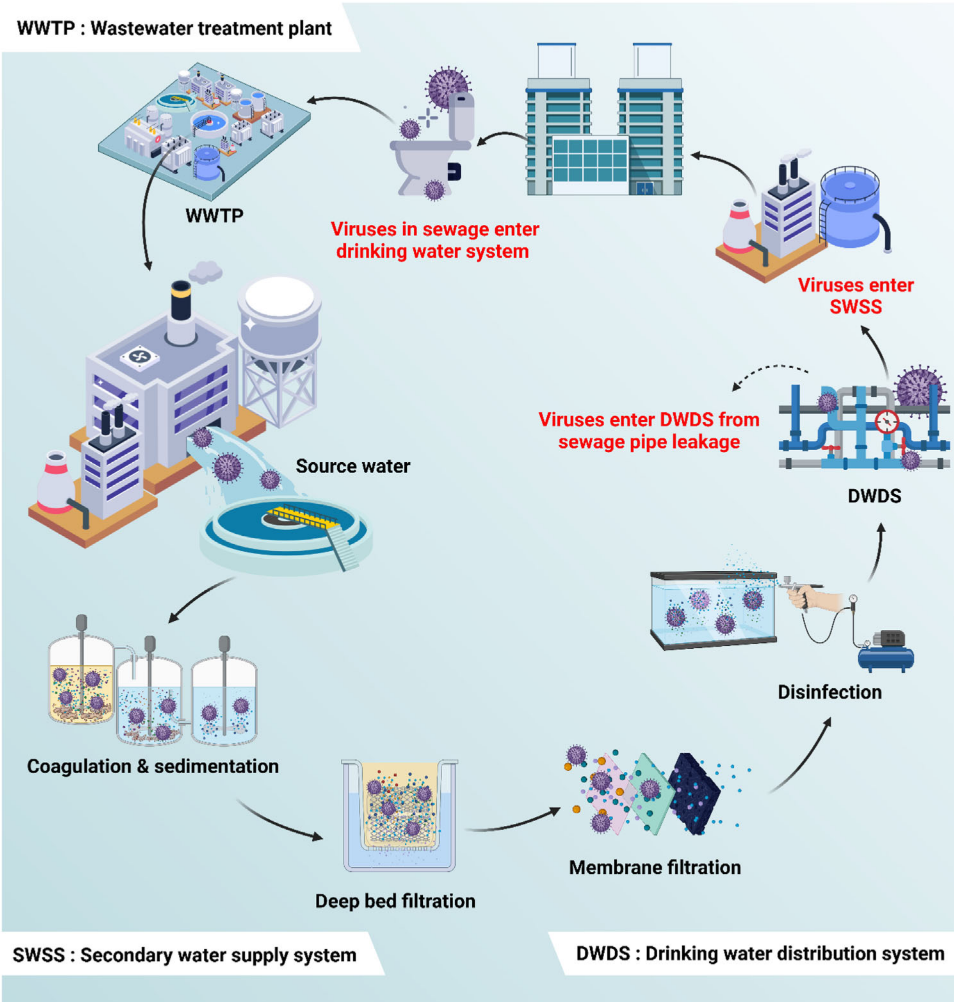

FIGURE 7 Here, the order of different critical approaches for urban water treatment (consisting of sedimentation; deep bed, and membrane filtration as well as disinfection) and their effect on virus inactivation as well as the route of virus transmission via pipelines are depicted.[44]

6.4.1 | Solitary process based-disinfection

The essential solitary process based-disinfection are chlorination, ozonation, and UV treatment.

1. Chlorination: As the most profitable avenue to counter viral pollution, the chlorination process, which chiefly consists of free chlorine release (hypo-chlorous acid (HOCl) and hypochlorite ion (ClO-)), is addressed by researchers.[113] On the ground of that report, free chlorine sources can be referred to as elemental chlorine, sodium hypochlorite, chloramines, calcium hypochlorite chlorine dioxide, and chloro-isocyanurates. Moreover, hypochlorite strongly oxidizes organic matter, while microbial organisms are primarily removed by un-dissociated HOCl.[113,132] Understanding the diversity of chlorine and its relative concentration during disinfection in wastewater seems to be essential. Adenoviruses establish an excellent resistance to chlorination. Researchers observed that the activated sludge treatment followed by chlorination was successfully implemented to treat various viruses, including Torque Teno virus and Norovirus genogroups II which were limited in the effluent from full-scale wastewater in the northern part of Pisa, Italy.[28] Bhatt et al. reported that the coronaviruses had demonstrated high sensitivity to chlorine. Thus, it could be indicated that the inactivation of Coronavirus using the chlorination process is feasible.[8]
2. Ozonation: Ozone (O_3) is a massive antiseptic oxidizing agent presenting a great microbicidal influence on the inactivation of viruses and bacteria. Wigginton and Kohn observed that O_3 destroys viruses via an attack on the viral protein.[133] Kataki et al. reported that the microbes' inactivation is done when O_3 acts on the cytoplasmic membrane's configurations.[113] Thus, O_3 is

a powerful disinfectant that enhances the biological water quality in a shorter period at low concentrations and with higher efficacy. For more effective disinfection by O_3, Gehr and Nicell observed that the prospective O_3 demand through inorganics or organics matter and suspended solids need to be estimated. Therefore, the ozonation process needs to be applied at the optimum point during treatment due to its instability.[134] Yao et al. reported that O_3 treatment with a higher concentration of O_3 and at a high temperature causes deactivation of SARS-CoV-2 transmission. An escalation of O_3 concentration from 48.83 to 94.67 μg m^{-3} and temperature drop from −13.17°C to −19°C were attributed as an explanation for the reduced spread of SARS-CoV-2 infections.[135]

3. UV treatment: UV radiation has garnered substantial interest in water treatment because of its high efficiency in pathogens removal in the recent decade.[111] Activated sludge followed by UV disinfection was successfully utilized to remove viruses such as Rotavirus, Sapovirus, Astrovirus Adenovirus, Enterovirus, and JC virus in full-scale municipal wastewater in Alberta, Canada. Under UV radiation, the virus's ability to reproduce or infect diminishes on the ground of deterioration caused to the virus' genome or protein.[113] Hijnen et al. reported that wastewater treatment involving exposure to radiation from a UV lamp (100–280 nm) destroys considerably enteric protozoans, including Giardia sp., Cryptosporidium sp., and the microbial bacterium pathogens.[136] Furthermore, UV lamps release dense radiation between the 240–260 nm range. In this range, nucleic acids of the viruses absorb the emitted energy.[113] Besides these pathogens, Biasin et al. suggested that the UV-C is practical in its ability to inactivate the viruses such as SARS-CoV-2. Therefore, the inactivation of SARS-CoV-2 engendered by monochromatic UV-C occurred from 254 nm irradiation and used various illumination doses (3.7, 16.9, and 84.4 mg cm^{-2}) with viral titers ranging from 0.05, 5, and 1000 multiplicity of infection.[137] Moreover, reports show that the DUV-LED of (280 ± 5 nm) has been rapidly effective in SARS-CoV-2 inactivation by 87.4% reduction within 1 s in the infectious viral titer and a quasi-ideal 99.9% decrease for 10 s of UV irradiation.[8] The recent results by Gerchman et al. suggest that the sensitivity of human Coronavirus is comprehensively dependent on the amount of UV LED wavelengths. For instance, UV LED with a peak wavelength of ~286 nm seems to be a great 'weapon' against human Coronaviruses threat.[36]

Bhatt et al. observed various advantages for the disinfection process characterizing UV radiation, for example, a short time is required in the process without any by-product formation.[8] In addition, UV radiation is safer than dealing with chlorine derivatives during the process. In contrast, UV radiation is limited in its use due to high infrastructure costs and high energy consumption during the implementation of this disinfection method.[138]

6.4.2 | Combined disinfection processes

Researchers reported that combined disinfection processes such as ozonation, UV treatment, and chlorination accomplished 99.99% inactivation of faecal coliform after treatment. Furthermore, the results revealed that using ozonation enhanced UV transmittance to up to 20–30% within the water. The combination of O_3 and UV irradiation primes strong and practical disinfection performance without any necessity for the secondary disinfection process.[113,142] Fang et al. reported that O_3 achieves 0.3 LRV of coliphage in tap water compared to 4 LRV when O_3/UV processes are combined.[143] The aforementioned disinfectants show some potential in terms of virucidal advantage. In order to understand the fighting ability against the types of viral COVID-19 pandemic, it is important to investigate deeper wastewater treatment processes, including systematic monitoring of their performance concerning their respective dosage.

Moreover, the impact of temperature, pH, and other environmental parameters must be considered essential vital process-related phenomena was needing more elucidation. Future studies must be conducted to uncover the survival time of SARS-CoV-2 Coronavirus after disinfection.[32] Table 3 compares the characteristics of wastewater treatment processes in virus removal.

The treatment of COVID-19-contaminated wastewater is crucial for protecting public health and preventing the spread of the virus. Physical, biological, and physiochemical processes have been employed for the removal of pathogens from wastewater. Physical processes such as sedimentation adsorption and filtration have been effective in partially eliminating viral pathogens, while slow sand filtration has been effective in removing various viral and bacterial pathogens. Biological processes have utilized microorganisms to convert organic matter to benign products under aerobic and anaerobic conditions. While different processes have shown some efficacy in removing pathogens, further research is needed to optimize these processes and determine their viability in eliminating the SARS-CoV-2 virus from wastewater. Additionally, studies need to focus on virus viability and particle reports to develop more effective and efficient processes.

TABLE 3 Characteristics of technologies applied in virus removal during wastewater treatment processes. WWTPs are for wastewater treatment plants.

Biochemical process	WWTP Location	Type of wastewater sample	Analysis method/ biomolecular analysis	Types of viruses/L	Log of virus removal Rate	Refs.
Membrane bioreactor	Full-scale, Traverse City, USA	Settled solids	Real-time quantitative	Human enterovirus	5.1	[126]
				Norovirus genogroup	3.9	
				Human adenovirus	5.5	
Activated sludge followed by chlorination	Full-scale, Pisa, Italy	Urban sewage	QIAamp DNA kit and the QIAamp RNA mini-kit (Qiagen-Germany)	Torque Teno virus	2.10 ± 0.53	[28]
				Norovirus genogroups II	2.16 ± 0.42	
Activated sludge followed UV disinfection	Full-scale, Alberta, Canada	Municipal wastewater	Real-time quantitative and Integrated viral cell culture	Norovirus	1.08 ± 0.50	[111]
				Rotavirus	1.64 ± 0.39	
				Sapovirus	1.77 ± 0.39	
				Astrovirus	1.80 ± 0.54	
				Adenovirus	2.81 ± 1.06	
				Enterovirus	2.02 ± 0.36	
				JC virus	2.28 ± 0.52	
Algal-based wastewater treatment using *Galdieriasulphuraria*	Full-scale, Las Cruces, New Mexico, USA	Conventional wastewater	Culture- and nucleic acid-based quantitative methods and shotgun metagenomic sequencing techniques.	Somatic coliphages	3.13 ± 0.34	[124]
				F-specific coliphages	1.23 ± 0.34	
				Enterovirus	1.05 ± 0.32	
				Norovirus GI	1.49 ± 0.16	
Wastewater pond system	Arani, Bolivia	Wastewater	Single Agar Layer and *E. coli* strain ATCC 15597	F+ coliphage	0.3	[114]
	Punata, Bolivia				0.7	
Pond system	Full-scale WWP Sant Gregori, Spain	Municipal raw sewage treatment	Double Agar Layer and Salmonella typhimurium WG-49	Somatic Coliphage	3.9	[139]
				Somatic Coliphage	2.9	
				B. fragilis phage	1.3	
				B. fragilis phage	1.5	
				F+ coliphage	3.4	
				F+ coliphage	2.2	
Three-pond serial waste stabilization pond system	Pond system Northwest France	Waste Stabilization Pond	Real-time reverse transcription-PCR	Norovirus GI	1.8	[140]
				Norovirus GII	1.6	
On-farm ponds wastewater	Ponds system, Accra, Ghana	Wastewater using vegetable irrigation	Analyzed membrane filtration pore size, mixed cellulose ester HA filters (Millipore), plating filters on agar, lasting filters on mEI agar, and colony-forming units	Somatic coliphages	2.0	[141]
				F+ coliphages	1.5	

7 | CHALLENGES AND FUTURE RESEARCH PRIORITIES

Even though data on the detection of COVID-19 in water resources and wastewater are increasing daily, several challenges hinder swift progress in this field (Table 4). On the other hand, highlighting these challenges would pave the way for critical topics for future investigations. Moreover, some hypotheses considered in existing data analyses would need to be revisited to paint a more realistic picture of reports on the relevant datasets compiled from hospitals, along with a more appropriate COVID-19 contaminated wastewater evaluation. They can be categorized but are not limited to the following items:

1. The hazard of losing the virus signal exists when assessing the concentration.
2. Some of these approaches need a prolonged sample processing duration of about 24–48h, which is improper in terms of span and practicality. For instance, PEG precipitation spans more than an entire day, which is improper since limiting the spread of COVID-19 depends only on a swift conclusion.
3. Typically, 50–250 mL sample evaluation will attenuate detection quality and sensitivity. Indeed, one should devise more sensitive methods to process much larger volumes, leading to far more quantifiable virus data.
4. The recent approach that tests people from person to person is no longer useable because of various sophisticated immunological analyses, limited accessible centres, day-to-day virus spreading during sample collection, handling large polluted and unpolluted samples, and very costly detection. Therefore, finding a far more appropriate detection methodology is quasi-obligatory.
5. How can these introduced strategies factor in the effect of flows in virus concentration? In order to illustrate this point, it can be envisioned as a situation when

TABLE 4 Compilation of existing issues, major challenges, and hypotheses for inconsistencies in regard to controlling levels of the virus during wastewater treatment

Issues	**Virus concentrations assessment**
	Wastewater treatment evaluation
	Finding out about alarms during a pandemic
	Discovering the intensity of infection in society
	Finding the virus longevity in different temperatures
	Finding the virus longevity in light
	Finding the virus longevity in the presence of microorganisms and bacteria
	Finding the virus longevity in various pHs
	Cost economizing and minimizing the damages in lower-income and middle-income countries
	The efficiency of raw wastewater treatment
	Stability presuming within various circumstances
	Analyze whole genome sequencing
	Time-integrated sample collection
	Halting virus transmission via plumbing system
	Discovering the viral shedding dynamics
Major challenges	The hazard of losing the virus signal
	Sample processing duration with the techniques[56]
	Processing larger volumes leading more quantifiable virus data
	Factoring in the effect of flows in virus concentration assessment in wastewater[28]
	Being reusable in agriculture industries or not[9]
	The time duration of free viral RNA has persistency[146]
	Spread the virus through Aquatic environments, lakes, rivers, and ponds
	Revealing the virus survival time for wildlife[23,147]
	Minimizing the number of assumptions in computational models[46]
	Detecting both viable and non-viable SARS-CoV-2 in wastewater based on half-life time[149]
	Discovering the utility of infected water in desalination (solar stills)[35]
Hypothesis for inconsistencies	Climate condition and hydraulic retention time in sewers[31,89]
	Rapid decay of SARS-CoV-2 in wastewater[61]
	Tourist accommodation influences the amount of sewage[68]
	Asymptomatic people (not reported by clinics)[91]
	Lacking enzymatic pretreatment[69]

on one rainy day, the virus concentration will subside while the number of cases is unchanged or has increased. This can mean that the flow rate study in a specific geographic region must be carried out.

6. More investigations are justified regarding running inspections on whether the polluted wastewater with coronavirus subfamilies is reusable for agricultural activities or not. Moreover, the nexus of the interaction of the virus with solid particles like those in sewage and the virus's survival has to be unravelled. It may end up on lands and crops, which can incept new risks for public health due to virus migration through soil or market garden produce. This is why studying the fate of viruses within this area is really crucial.[9,144]
7. Being unaware of the time duration in which free viral RNA has persistence appears to be an issue. Some studies suggest that the time duration changes between less than 1 h to 2 days in wastewater and seawater, respectively. Remaining indifferent to the dynamics of viral behaviour can lead to misunderstandings about the proper prevention politics to think of, design, and eventually implement.[145,146]
8. Aquatic environments, including lakes, rivers, and ponds, can be sources of infection and breeding points, eventually endangering the natural habitats of animals. This is why we believe there must be well-designed, systematic, and continual investigations to elucidate the exact key roles of aquatic environments in the viral transmission chain. For example, one of

the factors for lake-related and river-related viral contamination (HCoV-HKU1, HCoV-229E, HCoV-OC43, MERS-CoV, HCoV-NL63, SARS-CoV-2 and SARS-CoV) is reported as inadequate treatment of sewage effluents.

9. We need fresh studies to reveal that the virus is surviving time in wildlife. Indeed, if there is the possibility of a virus spreading through the birds or other animal faeces, it will have some bearing on the surviving time. Observations have demonstrated that the virus will survive at room temperature in human faeces for 96 h, but for animals, it remains an unanswered question,[23,147] especially in the presence of microorganisms that can inactivate the virus.
10. The prepared computational models have relied on many assumptions (e.g. dynamics of viral shedding/persistency in the sewer), making them oversimplified. In order to have a more accurate and realistic model, more complex investigations are required but not at the expense of essential subprocesses involved in the whole sequence of microbiological and environmental events.[46]
11. More useful techniques are needed to detect both viable and non-viable SARS-CoV-2 in wastewater based on virus half-life, which is estimated to be between 4.8 and 7.2 h at 20°C in hospital wastewater.[102] On the other hand, on the premise that the virus will degrade into other products after death, more thorough and smart strategies are required to probe and document this issue.[148,149]
12. Nanotechnology can potentially be scaled up for large-scale COVID-19 detection and wastewater treatment. However, there are some challenges that need to be addressed, such as the high cost of nanomaterials, the potential toxicity of nanoparticles, and the need for specialized equipment and expertise to produce and use them effectively. Additionally, the effectiveness of nanotechnology-based approaches needs to be precisely assessed in large-scale trials before they are implemented on a wider scale. Despite these challenges, there is great potential for nanotechnology to provide effective solutions for COVID-19 detection and wastewater treatment, and ongoing research and development in this area are likely to yield important advances in the coming years.
13. Nanotechnology-based approaches for COVID-19 wastewater treatment may have potential environmental impacts to be investigated prior to being implemented. One concern is the release of nanoparticles into the environment, which may lead to unintended ecological consequences such as toxicity to aquatic organisms or accumulation in the food chain. The production and disposal of nanomaterials used in wastewater treatment can also pose environmental impacts, including energy use, waste generation, and water consumption. While some studies have explored sustainable approaches to minimize potential risks, it is crucial to carefully evaluate the environmental impacts of nanotechnology-based wastewater treatment and prioritize the development of responsible solutions.[150]
14. The issues are not restricted only to health problems. The reality is that desalination (solar stills) works at low-temperature, which coincides with the virus viability. Indeed, survival of the SARS-CoV-2 in various water matrices is high between 4 and 37°C. Hence, utilizing solar stills during the pandemic period is threatening and calls for more investigation.[35]

The ensuing pool of information can be expected to equip better decision makers to reach plausibly more adapted and effective decisions and strategies to control and contain the blends of socio-economic impacts and their damage. Those decisions can also be expected to help craft a priority-based action plan for enforcing mitigative and remedial actions in the most virus-afflicted regions so that their local economic and financial recovery is prompt.[151–153]

Additionally, nanotechnology has the potential for diverse applications in response to the COVID-19 pandemic beyond wastewater treatment. For instance, nanoparticles can be utilized to create vaccines and drug delivery systems, in addition to the detection of the virus using rapid diagnostic tests. Furthermore, nanotechnology can be employed in air filtration systems and the development of antimicrobial surfaces to limit virus transmission. Developing protective clothing and equipment for healthcare workers should be considered as another application realm of nanotechnology. All in all, ongoing research should examine applications of nanotechnology in various fields of COVID-19 pandemic control.[154]

8 | CONCLUSIONS

The *sine qua non* need to explore and understand mechanisms underlying the contamination of water resources under the influence of COVID-19 incorporation is fully tangible in the today's global conjuncture of water stress and water scarcity situations. Immediately, this defines the necessity for detecting and treating COVID-19-contaminated water. However, there is a need to deepen our knowledge about the detection and juxtaposition of COVID-19 in wastewater applying modern and highly efficient methodologies. For instance, one of the main challenges that remain 'unattended' is the possibility of defining a detailed procedure to investigate the number of infected individuals in a population arising from the COVID-19 genome concentration through nanotechnology-based wastewater analysis. At least, fundamental concepts and approaches can be defined and/or assessed herein.

In this review, we attempted to classify and integrate all related techniques used for the detection/monitoring of SARS-CoV-2 in sewage, sludge, and wastewater. Moreover, the treatment processes of COVID-19-contaminated wastewater were discussed by comparing the advanced methodologies used for undertaking such treatments. In conclusion, we understood that in wastewater treatment plants, one should preferably avoid the secondary treatment phase in which COVID-19 would be present. In particular, in small-scale water supply plants that may pose serious difficulties it seems essential to establish several facilities. In the context of the COVID-19 pandemic, it may be an intelligent strategy to combine biochemical and disinfection processes to gain advanced treatment procedures to enhance the selective removal of target viruses. Concomitantly, some conventional process equipment can be deployed to monitor COVID-19 in order to reduce the higher exposure that may emanate from pipe network leakage. Based on the challenges of wastewater treatment processes, we may also need more investigation for treating COVID-19-hit wastewater to prohibit the dissemination of waterborne sources of diseases. Indeed, combining biochemical and disinfection technologies could constitute a proactive process to prohibit the spread of the virus in aqueous environments, which can eventually enable health authorities to implement effective and efficient methods to confront the outbreak and damaging effects of the virus.

There are a number of forgotten angles to be explored and dealt with in the use of nanotechnology for COVID-19 wastewater detection and treatment. One area of research would be developing intelligent nanomaterials and nanosensors with enhanced sensitivity and accuracy to detect viruses and other pathogens. This may lead to earlier detection of COVID-19 in wastewater and better mitigation of virus spread. Additionally, researchers are willing to explore more effective and efficient nanotechnology-based disinfection methods. Nanotechnology could also support the recovery of valuable resources like energy and nutrients from wastewater, which would make wastewater treatment more sustainable and cost-effective. Ongoing innovations and research projects grounded on nanotechnology should be likely to end in multifaceted developments in COVID-19 wastewater detection and treatment.[155–157]

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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