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Approaches applied to detect SARS-CoV-2 in wastewater and perspectives post-COVID-19

Pabel Cervantes-Avilés a,*, Iván Moreno-Andrade b, Julián Carrillo-Reyes b

a Tecnológico de Monterrey, Escuela de Ingeniería y Ciencias, Vía Atlíxicyayotl 5718, Reserva Territorial Atlíxicyayotl, Puebla, Pue, CP 72453, Mexico
b Laboratory for Research on Advanced Processes for Water Treatment, Instituto de Ingeniería, Universidad Autónoma de Mexico, Blvd. Juriquilla 3001, Querétaro, CP 76230, Mexico

A R T I C L E   I N F O

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A B S T R A C T

Currently, SARS-CoV-2 has been detected in the influent of wastewater treatment plants (WWTP), pumping stations, manholes, sewer networks and sludge of WWTP and facilities of countries as France, Spain, Italy, Netherlands, United States, Australia, Ecuador, Brazil and Japan. Although this virus has been detected in the wastewater streams, there is no robust method for its detection and quantification in wastewater. This review compiled and analyzed the virus concentration approaches applied to detect the SARS-CoV-2, besides to provide insights about the methodology for viral concentration, limit of detection, occurrence, persistence, and perspectives post-COVID-19 related with the implications of the virus presence in wastewater. The SARS-CoV-2 detection in wastewater has been related to virus concentration methods, which present different recovery rates of the virus. The most used viral concentration methods have been the polyethylene glycol (PEG) for precipitation of viral material and the ultrafiltration at molecular weight level. After viral concentration, the detection and quantification of SARS-CoV-2 in wastewater are mainly via quantitative reverse transcription polymerase chain reaction (RT-qPCR), which is the clinical assay adapted for environmental purposes. Although in some experiments the positive control during RT-qPCR is running a surrogated virus (e.g., Mengovirus or Dengue virus), RT-qPCR or reverse transcription droplet digital PCR (RT-ddPCR) targeting the gene encoding nucleocapsid (N1, N2 and N3) of SARS-CoV-2 are highly recommended to calculate the limit of detection in wastewater samples. Current results suggest that a rigorous methodology to elucidate the positive cases in a region from genomic copies in wastewater is needed.

1. Introduction

The disease caused by the new coronavirus SARS-CoV-2 is officially called COVID-19, which has caused a pandemic worldwide with deep impacts on aspects of human life [1]. Currently (October 1st, 2020), there are 33,842,281 confirmed cases for COVID-19 around the world, and 1,010,634 accumulated deaths in 188 territories [1]. The high rate of infected people, the long time that takes for the human host to develop antibodies against the virus [2], and the mortality rate [3] are characteristics of the COVID-19, which are not common in some other viral diseases.

Detection of SARS-CoV-2 in the sewer networks is essential in the initial stages of community spread. Moreover, early detection in a community reduces the rate of contagion and prevents over-saturation of health systems. At this time, the most common tests to diagnose a person with COVID-19 are chest computed tomography (CT) and quantitative reverse-transcription polymerase chain reaction (RT-qPCR). This last test is based on the multiplication of nucleic acids present in the virus, which make the test accurate due to it confirms the genetic presence of SARS-CoV-2 in a person, a medium or an object. Moreover, applying RT-qPCR can be quantified the genomic units of SARS-CoV-2. This assay is performed to detect different regions of the SARS-CoV-2 genome in human fluids such as rhino-pharyngeal, oropharyngeal, as well as fecal samples, surfaces and wastewater. The genetic regions of SARS-CoV-2 detected include the envelope protein gene (E gene), nucleocapsid protein gene (N gene), or RNA-dependent RNA polymerase gene (RdRsp gene) [4,5]. Comparative assays among different primer-probe sets from those genes showed to be highly specific with no false-positive, but with different sensitivities regarding to the quantitative presence and fluids [6]. The reverse transcription
droplet digital PCR (RT-ddPCR) is another technique which has also been applied to quantify the SARS-CoV-2 in wastewater [7–9]. In terms of sensitivity, Falzone et al. [10] found that the ddPCR technique has shown higher sensitivity and specificity compared to RT-qPCR for the diagnosis of COVID-19 in false-negative rhino-pharyngeal swab samples with low viral load.

The persistence of SARS-CoV-2 in humans includes its presence in fluids such as urine [11] and feces [12–14] that arrive to the wastewater streams by these excretions. In a recent study, it was reported that SARS-CoV-2 presence is more common in feces than in urine [15], hence stool can be considered as main contributor of genomic units of the virus in the wastewater streams. Indistinctly to the source of the excretion of the virus, its detection in wastewater streams via surveillance of wastewater is an opportunity to track the pandemic in a covered area by monitoring the sewer networks, which may potentially prevent a fast spread of the COVID-19 [16,17]. In fact, surveillance of wastewater streams can be considered as an early warning tool. This approach is known as wastewater-based epidemiology (WBE), which has been proposed for surveillance of viral diseases such as poliovirus, and to track the use of illicit drugs in a determined area [18,19]. Currently, nearly a dozen research groups around the world have performed tests to detect the presence of SARS-CoV-2 in wastewater [20]. Their findings have confirmed the presence of the virus in wastewater, and in some cities, the SARS-CoV-2 has been detected in wastewater before confirming the first case by the health authorities [21].

From the wastewater treatment point of view, the road map will include the next milestones: i) The development of robust methodologies for the detection of SARS-CoV-2 in wastewater, ii) Application of wastewater-based epidemiology (WBE) as a COVID-19 monitoring tool, iii) Analysis and response for possible impacts on the WWTP operation (process and technology), and iv) Development of protocols to response to the new diseases (including virus or bacterial pandemics). This review is focused on the first milestone, but the WBE and wastewater treatment post-COVID-19 are also reviewed.

The systematic detection of the SARS-CoV-2 in wastewater is needed to set the WBE, which can provide useful information on the level of spread of COVID-19 in a community. In addition to the research needs on the subject of detection of SARS-CoV-2 already compiled by Kitajima et al. [17], and research needs about of SARS-CoV-2 in the public health already reported by Farkas et al. [16], there is a basic research need for selecting the most suitable method for viral concentration in wastewater prior its detection by molecular techniques based on PCR. The concentration of virus in wastewater samples provides higher resolution when a molecular technique for detection is applied. Several studies reported, via formal peer-reviewed publication, the detection of SARS-CoV-2 in wastewater and related their findings to the epidemiological behavior. However, the methodologies applied to detect the virus have not been standardized in a robust one. Hence, to generate a robust method for SARS-CoV-2 detection in wastewater, the methodologies applied for viral concentration before its detection must be analyzed in formal studies. Moreover, facing the new normality, perspectives about the presence on the virus in wastewater in a post-COVID-19 scenario are also demanded. This review compiles and analyze the SARS-CoV-2 approaches and protocols for viral concentration, detection, occurrence and persistence in wastewater in order to provide insights and best practices, as well as needs and perspectives for the wastewater treatment post-COVID-19.

2. Detection of SARS-CoV-2 in wastewater

One of the major challenges in SARS-CoV-2 detection and quantification in wastewater samples is the lack of an optimized and standardized protocol [22]. However, there are overall considerations in the protocols already applied for the detection of SARS-CoV-2 in wastewater. These considerations can be categorized as steps for virus detection in wastewater, which include the sampling timing and conditions, virus inactivation (e.g. heat treatment, pasteurization of the sample), the correct use of protective equipment for virus handle, grit and solids removal in some samples, concentration of the virus, detection of the virus, and quantification of the genetic units of the virus. All these steps will be discussed in this section.

2.1. Sample collection, handling and storing of the wastewater samples

According to the population-based health management analysis proposed by Thompson et al. [23], the upstream sampling of multiple locations may provide higher spatial resolution related to positive cases per inhabitants, when compared to downstream sampling (e.g. influent of WWTP), which presents higher travel time of the wastewater and may present signal degradation. Although sample collection could be random through collection of single samples at an arbitrary time, the timing of sampling has influenced the detection of SARS-CoV-2 in wastewater. Sherchan et al. [24] found that the sampling method is critical, and that composite samples of 24 h may provide the most reliable daily average of viral concentration in wastewater. Moreover, sampling protocols should consider the inactivation time of the coronavirus in wastewater, that could be between 2.3 and 3.5 days at 23 °C [25]. However, the temperature during collection and preservation may play an important role in virus degradation. In recently reported studies, the preservation temperature of collected samples for has been 4 °C or -20 °C (Table 1), without a clear trend toward lower RNA signals when the samples are stored at 4 °C for almost two weeks [26]. Ahmed et al. [27] demonstrated that the SARS-CoV-2 RNA signal is unlikely to degrade significantly at the temperatures of storing (4 °C) for at least 10 d. Nevertheless, the decay in RNA signal is influenced by the temperatures (15 °C, 25 °C and 37 °C), water matrix (tap water or wastewater) and thermal treating of the samples by autoclaving [27]. Sherchan et al. [24] processed wastewater samples stored at -80 °C between 1 and 2 months before the pandemic, which tested negative since first case was reported a month later. Although the majority of samples are processed within 12 h after collection, reprocessing stored samples under different temperatures is needed in order to determine the potential bias in the detection and sensitivity during storage. Regarding the handling of samples potentially containing SARS-CoV-2, they should be handled according to the subsequent use of samples. For example, since infectious SARS-CoV-2 is classified as a biosafety level 3 (BSL-3), culturing of samples that potentially contain SARS-CoV-2 requires BSL-3 guidelines [4], nevertheless, procedures that concentrate viruses, such as precipitation or membrane filtration, can be performed in a BSL-2 laboratory with unidirectional airflow. Although there is no evidence that infectious SARS-CoV-2 is present in wastewater streams, samples can be submitted to heat treatment (e.g. 30 min at 60 °C) before opening containers to increase the safety of the workers who perform the analysis [28,29], since this procedure can inactivate the potentially infectious agents present in the samples. The effect of heat treatment of wastewater samples (e.g., pasteurization or autoclaving) on RNA signal intensity of coronaviruses is a topic with few insights and contradictory findings, that represents a research need. While the high persistence of RNA signal intensity of SARS-CoV-2 over time (up to 10 d) in autoclaved wastewater samples has been attributed to changes in the physicochemical characteristics of wastewater after the autoclaving them [27], the recovery rate of RNA of coronaviruses can decrease after heat treatment due to its weak resistance and high sensitivity to the environmental factors such as temperature [30] and chlorine ions [31], as well as to the presence of RNases which are present even in autoclaved samples [32]. In addition, special care must take into account during virus inactivation and virus concentration, some BSL-3 precautions are recommended. Some studies removed the grit and solids from the matrix before and after viral RNA concentration and isolation from wastewater samples, which mainly consisted in two ways for removing larger and dense particles from wastewater (Table 1): filtration (0.45–0.20 μm) [33] and centrifugation [8,24,28]. It should be highlighted that when filtration and
Table 1

| Concentration | RNA recovery | Quantification | Reference |
|---------------|--------------|----------------|-----------|
| Centrifugation (229 g, 1 h) | QiAamp Viral RNA Mini kit (Qiagen) | RT-qPCR | [29] |
| Adsorption and precipitation | Nucleo-Spin RNA virus kit (Macherey-Nagel GmbH & Co) | RT-qPCR | [21] |
| pH adjustment (6.0 and AlCl₃) | RNeasy PowerWater Kit and RNeasy PowerMicrobiome Kit (Qiagen) | RT-qPCR | [27] |
| pH adjustment (3.5) | AccuPrep® Universal RNA Extraction Kit | RT-qPCR | [34] |
| pH adjustment (3.5) | QiAamp Viral RNA Mini Kit (Qiagen) + QIAcute automated platform (Qiagen) | RT-qPCR | [35] |
| pH adjustment (3.5) | ZR Viral RNA Kit (ZymoResearch) | RT-qPCR | [24] |
| Sample treatment (56 C, 30 min) | NucliSENS miniMAG (bioMérieux) + OneStep PCR Inhibitor Removal Kit (Zymo) | Reverse Transcription + nested PCR, RT-qPCR | [28] |
| Centrifugation (4654 g, 30 min) | RNeasy PowerMicrobiome Kit (Qiagen) or BioMerieux Nuclisens kit (BioMerieux) | RT-qPCR | [8] |
| Centrifugation (4750 g, 30 min) | Qiamp Viral RNA mini kit (Qiagen) | RT-qPCR | [33] |

N.D. Not Defined; > 70 % of studies report a 4 °C temperature of sample preservation, and processing time in less than 24–48 h after sampling.

centrifugation are applied, the biomass retained in filters or pellets can be analyzed to quantify the presence of RNA, which will allow to consider that value for full quantitative purposes.

2.2. Concentration of the virus in wastewater

The viral concentration is a key step for detection and quantification of SARS-CoV-2 in wastewater due to its concentration decreases by dilution in wastewater streams. The approaches applied for this purpose are multiple and include the one used for poliovirus concentration based on two-phase separation method [36], precipitation [21], centrifugation [37,38], ultracentrifugation [8,38], conventional filtration [33] and filtration by negatively charged membranes [39], and a combination of these approaches (Table 1). Although there are efficient methods to concentrate enveloped virus such as coronaviruses from wastewater [40] and tap water [41], the specific efficiency in recovering SARS-CoV-2 may differ under different conditions. Therefore, it is highly recommended to determine the recovery rate of the virus for the concentration method applied. In this review, the methods for SARS-CoV-2 concentration are grouped in two categories, the physicochemical based and the strictly physical based methods (Fig. 1). The physicochemical based methods incorporate the use of chemical reagents in some steps of physical separation for the extraction and concentration of the virus. Chemical reagents include the combined use of the polymer polyethylene glycol (PEG) with NaCl, dextran or alum, in order to perform a liquid-liquid fractionation (aqueous two-phase technique), with potential for the extraction, separation, purification, and enrichment of viruses. The miscibility of solu- tions containing polymers results in the formation of two phases, separating the solute selectively in one phase [42]. The use of Al(OH)₃ and AlCl₃ with pH adjustment by addition of HCl has been addressed in order to precipitate the viral content due to the modification of surface charge in the virus. In general, the incorporation of chemicals reagents considers reaction and incubation time, which even can be for overnight periods for phases separation (Fig. 1). Finally, the extraction and amplification of genetic information are performed in the extracted or precipitated phase. According to the reported studies, most of the physicochemical approaches for virus concentration take more time (up to 15.5 h) and they involve more steps than the strictly physical methods. Although the physicochemical based approaches can include some physical steps such as, filtration and centrifugation, their effectiveness for virus concentration is attributed to the chemical adsorption [36,43]. The strictly physical based methods for viral concentration mainly applied a combination of centrifugation and filtration or ultracentrifugation and resuspension (Fig. 1). Although the reported physical based protocols for SARS-CoV-2 concentration take less than 1.4 h to be completed, centrifugal ultracentrifugal with cut-off up to 100 kDa, units for centrifugation of high volume, equipment for ultracentrifugation or ultrafiltration units may be required.

Some studies have tested the effectiveness of methods for viral concentration of SARS-CoV-2 in wastewater. Ahmed et al. [27] found inconsistent results in SARS-CoV-2 concentration and recovery when they compared the electronegative membranes with the ultrafiltration tubes with a cut-off at 10 kDa of molecular weight (MW). In the same study, it was used murine hepatitis virus (MHV) as surrogate of SARS-CoV-2 and it was found that adsorption-extraction methods for
viral concentration could recover up to 65 % of MHV [43]. It is important to highlight that MHV and SARS-CoV-2 are enveloped viruses from the same genus (*Betacoronavirus*) [44], share similar genomic structure and envelope glycoproteins spike [45]; hence, the recovery rates could be close between both viruses, even though, as the recovery rate depends on the wastewater matrix, high recoveries may not be observed at all times, thus, variations on the recovery are expected. Randazzo et al. [21] validate the Al(OH)$_3$ adsorption-precipitation method using Porcine Epidemic Diarrhea Virus (PEDV) and Mengovirus (MgV), reporting recovery efficiencies of 11 % of both virus in influent samples. In the same sense, using Mengovirus as surrogate virus, no significant loss of RNA was reported for heat-treating the wastewater samples [28]. Medema et al. [8] validated the purification and concentration steps using the ultrafiltration method (100 kDa membranes), reporting recovery efficiencies of 11 % of both virus in influent samples. In the same sense, using Mengovirus as surrogate virus, no significant loss of RNA was reported for heat-treating the wastewater samples [28]. Medema et al. [8] validated the purification and concentration steps using the ultrafiltration method (100 kDa membranes), reporting recovery efficiencies of 11 % of both virus in influent samples. In the same sense, using Mengovirus as surrogate virus, no significant loss of RNA was reported for heat-treating the wastewater samples [28]. Medema et al. [8] validated the purification and concentration steps using the ultrafiltration method (100 kDa membranes), reporting recovery efficiencies of 11 % of both virus in influent samples. In the same sense, using Mengovirus as surrogate virus, no significant loss of RNA was reported for heat-treating the wastewater samples [28].

### 2.3. Detection and quantification of the virus

The method more used to detect and quantify the SARS-CoV-2 in wastewater has been RT-qPCR (Table 1), which is part of the second generation of PCR. Although this method can detect the regions ORF1a, ORF1b, E and N of the SARS-CoV-2 genome, N or nucleocapsids have been the most used target during assays after RNA extraction of viral concentrated from wastewater [8, 46]. The commercial availability of nucleocapsids (N1 and N2) primer-probe sets has facilitated their use during detection assays in wastewater, even when some reagents for COVID-19 detection have limited availability to conduct tests [20]. Nucleocapsid region has also been used to determine the limit of detection (LOD) during quantitative analysis, that has ranged from 2 log$_{10}$ [7] to 4.45 log$_{10}$ copies per liter of wastewater [21]. These quantitative results for SARS-CoV-2 have been validated theoretically for LOD regarding to the spiked concentration. In addition to the quality controls to measure the recovery efficiency during the RNA concentration and isolation steps, some studies have included other controls during the quantification, as internal reference and indicators of PCR inhibition, like Sketa 22 asa (Oncorhynchus keta), and β-actin [39]. As well, human crAssphage was used by Green et al. [47] (pre-print) as internal control for normalizing SARS-CoV-2 concentration in wastewater samples. Similarly, pepper mild mottle virus has been used as internal control for detection of SARS-CoV-2 [35, 48]. Since positives and internal control assays follow different targets, even with different nucleocapsids, the determination of limit of detection of SARS-CoV-2 in wastewater is unclear and not rigorous in terms of analytical protocols. The use of noninfectious SARS-CoV-2 or a positive fecal samples in wastewater in order to set the limit of detection and quantification of SARS-CoV-2 in wastewater could be an approach that increase the reliability of the quantitative methods [21].

Besides RT-qPCR, RT-ddPCR could be used to detect and quantify SARS-CoV-2 in wastewater Gonzalez et al. [7]. This last tool has presented higher sensitivity in human samples than RT-qPCR [10, 49]. Gonzalez et al. [7] used a RT-ddPCR assay and determined theoretical LOD in wastewater of N1, N2, and N3, which were 14.6, 2, and 2.18 copies per reaction, respectively. The N2 assay results were used in the work performed by Gonzalez et al. [7] as they were the most sensitive, and presented SARS-CoV-2 concentrations in wastewater samples between 10$^9$ and 10$^8$ copies per 100 m L$^{-1}$ during the 21-week study in wastewater streams divided as physicochemical based (green boxes) and physical based virus concentration (orange boxes) methods. Blue circles were assigned to the steps with reported time to perform them. Red circles were assigned to steps without time reported to perform them. Concentration time is the sum of the time reported in all steps. The concentration time of SARS-CoV-2 can vary depending on the concentration method and the specific conditions of the wastewater. For example, using adsorption-precipitation with Al(OH)$_3$ (green boxes), the concentration time can be around 5.2 hours. On the other hand, using physical methods such as centrifugation (orange boxes), the concentration time can be around 1.0 hours. These methods are effective in concentrating the virus particles from wastewater samples. The concentration time is an important factor in the efficiency and effectiveness of these methods. Additionally, it is important to consider the quality controls and validation steps to ensure accurate and reliable results.
Southwestern Virginia. In another study, Falzone et al. [10] performed a comparison between RT-qPCR and ddPCR sensitivity in human positive rhino-pharyngeal swab samples and found that sensitivity of ddPCR permitted the identification of positive signals in undiluted samples and in the 10-fold dilution, demonstrating that ddPCR is effective in the detection of very low viral load. Although RT-ddPCR has been more sensitive in rhino-pharyngeal samples [10,49], research is needed to compare the sensitivity of various methods, including RT-qPCR and RT-ddPCR in wastewater samples. Table 2 shows the advantages and disadvantages of RT-ddPCR and RT-qPCR.

3. Occurrence of SARS-CoV-2 in wastewater

The virus that causes COVID-19 has been detected in the wastewater of several cities and regions around the world (Table 3). Although the majority of samples were collected at the influent of the WWTP, sampling points in the sewer network, manholes, hospital wastewater and pumping stations were also monitored to delimit the scope of the study. The sampling dates were different for all experiments but ranged from February 3rd to April 25th. For samples collected in some municipalities of Murcia, Spain, such as Lorca, Cieza, and Totana, positive results of SARS-CoV-2 in wastewater alerted COVID-19 cases 12–16 days before first cases were declared [21]. The same was reported for Amersfoort, Netherlands, where wastewater samples on March 5th resulted positive, few days before the first case in the city was confirmed [8]. These findings strongly suggest that surveillance of the untreated wastewater streams may contribute to monitoring the pandemic. However, the limited availability of the reagents for COVID-19 detection should be considered to avoid a shortage of the reagents for clinical purposes. Nonetheless, it is important to mention that reagents used for research are commonly tag as “research-use only” and they should not be utilized for clinical diagnose.

Although the SARS-CoV-2 has been detected in untreated wastewater, this virus has also been detected in 2/18 samples of treated effluent after wastewater passed throughout the activated sludge process as secondary treatment; moreover, all samples after tertiary treatment, a disinfection process is recommended for those systems with only secondary treatment to prevent the circulation of SARS-CoV-2 titers in secondary effluent water.

The presence of SARS-CoV-2 RNA in the primary sludge and waste activated sludge was recently detected in 7 WWTP of Istanbul, Turkey [54] (pre-print). Titers of SARS-CoV-2 RNA in primary sludge and waste activated sludge ranged from $1.17 \times 10^4$ to $4.02 \times 10^5$ per liter of sludge. These concentrations may vary depending on the solids concentration in samples and on the sources of the samples. For example, the of SARS-CoV-2 RNA copy number detected in the suspended solids of raw wastewater [55] were lower than those reported previously for waste sludge [54] (pre-print). Westhaus et al. [53] filtered 45 mL of raw wastewater and compared the SARS-CoV-2 RNA copy number present in the wastewater phase and in the pellet that resulted of the ultrafiltration by 10 KDa. The experiment indicated that the solid phase of samples of raw wastewater are one log unit higher of SARS-CoV-2 RNA copy number per mL (25 copies mL$^{-1}$) than to the aqueous phase of the influent sample (1.8 copies mL$^{-1}$). Cheung et al. [56] detected SARS-CoV-2 RNA in fecal matter and found median fecal viral load of $5.1 \log_{10}$ copies per mL in patients with diarrhea, while for patients without diarrhea was 3.9 $\log_{10}$ copies per mL. These results support that the titers of the virus could remain in the fecal matter and other suspended solids present in the wastewater. These findings also suggest the presence of titers of SARS-CoV-2 in the disposed sludge from WWTPs; hence, further disinfection of the waste sludge could be needed before sludge disposal while occurrence studies of the virus in the waste sludge are still under research. Nevertheless, comprehensive studies related to the fate of SARS-CoV-2 RNA in waste sludge, digested sludge or biosolids [57], as well as studies determining whether or not the virus in the sludge may be infectious are also needed.

4. Persistence of SARS-CoV-2 in wastewater

The environmental conditions (e.g., temperature, humidity, particulate material, among others) related to the SARS-CoV-2 spread have recently considered in some studies [22,25,58,59], since they affect the persistence of infectious SARS-CoV-2 and its RNA signal. The physicochemical conditions of the wastewater streams may influence the persistence of infectious SARS-CoV-2 and its RNA signal [60]. Some ions in the wastewater may alter the membrane protein of SARS-CoV-2 as ions altered the membrane of the SARS-CoV, which is a virus from the same subgenus (sarbecovirus) as SARS-CoV-2 and has hydrophobic and selective (for monovalent cations) membrane protein [61].

Although SARS-CoV-2 has been less resistant than bacteria to chlorine concentration in water [46], some coronavirus can persist in wastewater up to 3.5 days at 23 °C [25]. Wang et al. [31] suggest that SARS-CoV-2 persists in fecal samples for 3 days and in urine samples for 17 days, and that SARS-CoV-2 is more vulnerable to disinfectants than E. coli and Φ2 phase. Although this virus from the same subgenus than SARS-CoV-2 may persist for some time in water, the potential transmission of infectious SARS-CoV-2 through wastewater streams has not been confirmed [8,46]. It means that the detection of SARS-CoV-2 in wastewater does not imply that the virus is able to infect humans [62]. Since the transmission is linked to the viability of the virus in wastewater, research focused in virus stability in the environmental matrices, distinguishing infectious and noninfectious virus, and determination of the persistence factors of SARS-CoV-2 in wastewater are needed.

Regarding waste solids, SARS-CoV RNA has been detected in the feces of infected individuals. SARS-CoV RNA levels showed a maximum in fecal samples up to 14 days after the onset of the illness (in one case, fecal samples tested positive for the virus for up to 73 days after the illness onset) [63]. Therefore, it is probable that sewage sludge from an area with infected people can contain SARS-CoV-2 RNA [15]. However, the survival of SARS-CoV-2 in biosolids would be very low at

| Technique | Principle | Advantages | Disadvantages |
|-----------|-----------|------------|--------------|
| RT-qPCR   | Amount of amplified product is comparative to the fluorescence signal intensity. The sample is quantified using the reaction cycle threshold and standard curve [50]. | Extensive dynamic range, wide application range, low cost [50], rapidity of diagnosis, can be used to screen large numbers of samples, a run-time of approximately 90 min [51] | Amplification efficiency is susceptible to PCR inhibitors [50] |
| RT-ddPCR  | Generates microreaction units with water-in-oil and microfluidic technology. The nucleic acids are randomly subdivided into water-in-oil droplets that undergo PCR separately [50]. | No external calibration curves are needed, and it may be less sensitive to inhibition and suboptimal PCR efficiency, high sensitivity, better resistance to PCR inhibitors, greater precision in quantification [52, 50] | Narrow dynamic range and high cost [50] |
temperatures higher than 20 °C [25]. Actually, there is no evidence for transmission of SARS-CoV-2 via solid waste [64].

5. Wastewater-based epidemiology of the SARS-CoV-2

The study of the several compounds in wastewater, such as illicit drugs and biological agents as Hepatitis A virus and Norovirus, have provided early warnings of outbreaks [18,62]. For this reason, the quantification of SARS-CoV-2 in wastewater affords the ability to monitor the prevalence of the virus among the population via WBE [39], which is a promising strategy for monitoring the spread of SARS-CoV-2 (or resurgence). The basic principle of WBE is that sewage can be used as a key-factor will be the development of a robust protocol for SARS-CoV-2 detection in wastewater, focusing on the effective concentration methods before the subsequent RNA extraction and RT-qPCR detection [67].
WBE has demonstrated the feasibility of being used as an inexpensive technique to monitor and manage the health and behavior of human populations. Simulations with data from 2017 obtained in 13,940 WWTP of USA demonstrated the potential sensitivity of WBE for generating skewed data [68]. WBE has also been used as a tool to monitor drugs consumption or exposure to environmental and food contaminants. For example, it has been applied to estimate tobacco consumption in seven regions of Spain, reporting the differences in regional consumption. However, no conclusive correlation was found between those values and the prevalence data taken from two different national surveys, nor sociodemographic and health data [69]. WBE can be a powerful tool for specific cases as the COVID-19 using WWTP data but need to be complemented with other indicators to assess the disease accurately, including the correct characterization of the sewage system (maps, flows, etc.) and the existing clinical results.

Few studies present consistent results of COVID-19 surveillance using WBE. For instance, in Southeastern Virginia, USA, the RNA data from weekly samples of nine WWTPs presented spatially and temporally, with regional loading estimates over time, were considered as viable way to describe the occurrence and trends (onset) in SARS-CoV-2 infection [7]. However, the lack of a reliable SARS-CoV-2 stool shedding rate is the current limitation in the use of WBE to estimate total infection within a community [7]. The authors recommend future work to examine the lead-lag association between SARS-CoV-2 wastewater data and regional confirmed clinical data. With a similar aim, a technical bulletin of SARS-CoV-2 RNA surveillance from sewage at Belo Horizonte, Brazil, correlated the reported COVID-19 cases, with the estimated cases, using the typical viral load from sick individuals and the genomic viral copies from sewage [70]. The trend of RNA concentration in sewage allowed to infer that prevalence of COVID-19 was higher than the confirmed cases by local health authorities. Furthermore, percentage of infected people were estimated by regional maps, delimited by the 15 monitored sewage sub-basins, where concern areas were identified due to the increasing RNA concentration in the sewage [70]. Another study in the New Haven, Connecticut, USA metropolitan area, showed that SARS-CoV-2 RNA concentration in primary sludge of WWTP wastewater treatment facilities, was up to 8 days ahead from the SARS-CoV-2 positive test results by reporting date [71].

6. Perspectives for the wastewater treatment post-COVID-19

The presence of SARS-CoV-2 in wastewater generates concerns for the WWTP in the future: i) Occurrence of new pollutants in WWTP, ii) Efficient disinfection and sanitary concerns for the presence of the virus in WWTP, iii) Wastewater management to protect public health and environment, and iv) Implementation of WWTP results in the WBE for monitor of COVID-19.

The development of new drugs and treatments for the COVID-19 (e.g., Remdesivir, hydroxychloroquine (HCQ), chloroquine (CQ), among others), will increase their occurrence in wastewater treatment facilities. CQ and HCQ compounds could be risky for the environment and should be classified as harmful to aquatic organisms [72,73]. In the case of CQ, there are not reports about the occurrence or fate of this compound in the conventional wastewater treatment plants. However, Lindroos et al. [74] reported the degradation of CQ as a pharmaceutical compound model, with efficiencies higher than 98 % in 20 h, using a membrane bioreactor.

For the case of anti-viral drugs, the European Medicines Evaluation Agency has recommended for compassionate use of the Remdesivir, although information on the related environmental risk, such as eco-toxicity and degradability in the environment, is lacking [72]. This means that research related to the persistence of this drug in wastewater will be an important topic to consider facing the new normality. It is also necessary to evaluate the role of the SARS-CoV-2 as an environmental contaminant and its relation to other viral contaminants. Water management should consider the presence of enteric viruses such as SARS-CoV-2 to protect public health though the water supply and the environment.

The SARS-CoV-2 is a serious concern for the irrigation industry, particularly for the wastewater stakeholders of the world. The irrigation with treated wastewater has low risk, since. Gundy et al. [30], for the case of SARS-CoV, reported that survival of the coronaviruses in wastewater is between 2–4 d, and survival in primary wastewater was only slightly longer than secondary wastewater, probably due to the higher level of suspended solids that offer protection from inactivation. Regarding SARS-CoV-2 prevalence in wastewater, half-life mean was 0.64 day, and 4.3 days required for 99 % reduction of infectious virus titers [60]. It should be noted that in developing countries, the unregulated use of wastewater for irrigation needs to be considered for the WBE. In this case, methods for irrigation with wastewater without proper treatment may be advised to operate with caution to avoid contact of SARS-CoV-2. Currently, there is no evidence confirming the survival of SARS-CoV-2 virus after the disinfection process for both drinking water treatment plant and centralized WWTP [21,24,75]. However, a recent study performed by Zhang et al. [76] reported the presence of SARS-CoV-2 RNA in septic tanks after disinfection with 800 g m⁻³ of sodium hypochlorite in medical wastewater. Since medical wastewater may concentrate the SARS-CoV-2 RNA, disinfection of treated effluent in medical WWTP should follow specific guidelines that include the concentration of SARS-CoV-2 RNA copy number in such water matrix.

The existing disinfection guideline for centralized disinfection of the World Health Organization [75], suggests a concentration of free chlorine ≥ 0.5 mg L⁻¹ after at least 30 min of contact time at pH < 8.0 [75] in order to ensure a complete inactivation of SARS-CoV-2 in all the centralized treatment systems. As it was reported for the medical WWTP, the disinfection process must be tested and optimized for the SARS-CoV-2 efficient inactivation depending on the wastewater source. The disinfection process in the conventional WWTPs (e.g. adsorption, advanced oxidation processes, ozonation, chlorination, ultraviolet light, and membrane from ultrafiltration to reverse osmosis) applied previously to inactivate other coronaviruses can be applied, but better understanding of the efficacy of such technologies for SARS-CoV-2 inactivation is needed [77]. Membrane technology has been applied from long time to remove the virus from water [78]. Currently, membrane technology has been combined with catalytic electrospun [79] and electrocoagulation [80] for virus capture, control and remediation from water at lab scale experiments. There are also approaches that focus in the adsorption of the virus through cell culture [81] and polysaccharide-based resins [82]. However, protocols during drinking water treatment focused on disinfecting from SARS-CoV-2 are missing [83]. The disinfection step in WWTP and drinking water treatment could be the key-process to assure the removal or inactivation of SARS-CoV-2 in wastewater and drinking water, respectively.

The implementation of WBE will be a key-tool for surveillance, assessing, and managing the pandemic. Besides the enhancements in virus concentration and molecular analysis for the detection of SARS-CoV-2, the WBE will employ computational analysis and modeling to examine the feasibility, economy, opportunities and challenges of enumerating active coronavirus infections locally and globally [59]. WBE can contribute to solve the pressing problem of insufficient diagnostic testing and provide a cheap and early warning method for future COVID-19 outbreaks [22]. WBE can also be used as a tool for the decision of the post-isolation phase (restarting activities and finishing the isolation if the population), reinstatement of isolation facing a rebound or seasonal re-emergence, as earlier alert-tool without random-diagnostic testing [84], and as a pre-screening tool to better target clinical testing needs in communities with limited resources [7]. WBE may represent the only viable means of effective surveillance for low-income regions [59], such as Latin-American, Caribbean, Central and South Africa regions, which are emerging as the epicenter of the pandemic [1]. In addition, the majority of these countries present low
coverage in sanitation facilities [34]; hence WBE can also be addressed by monitoring surface water such as urban rivers.

Interdisciplinary studies that include epidemiological, environmental, social, and economic interaction, as well as transformative innovation are necessary to create strategies to new challenges in the new normality, including the current second wave for some countries. Finally, the improvement of the sensitivity of techniques for the detection and quantification of the virus in wastewater, especially the concentration methods and PCR amplification, and their international validation, are key factors to implement the WBE.

CRediT authorship contribution statement

Pabel Cervantes-Avilés: Conceptualization, Investigation, Formal analysis, Validation, Writing - original draft, Writing - review & editing. Iván Moreno-Andrade: Investigation, Formal analysis, Validation, Writing - review & editing. Julían Carrillo-Reyes: Investigation, Formal analysis, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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