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Capacity of existing wastewater treatment plants to treat SARS-CoV-2. A review

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ABSTRACT

Water is one of many viral transmission routes, and the presence of Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) in wastewater has brought attention to its treatment. SARS CoV-2 primarily transmits in the air but the persistence of the virus in the water possibly can serve as a secondary source even though current studies do not show this. In this paper, an evaluation of the current literature with regards to the treatment of SARS-CoV-2 in wastewater treatment plant (WWTP) effluents and biosolids is presented. Treatment efficiencies of WWTPs are compared for viral load reduction on the basis of publicly available data. The results of this evaluation indicate that existing WWTPs are effectively removing 1–6 $\log_{10}$ viable SARS-CoV-2. However, sludge and biosolids provide an umbrella of protection from treatment and inactivation to the virus. Hence, sludge treatment factors like high temperature, pH changes, and predatory microorganisms can effectively inactivate SARS-CoV-2.

1. Introduction

Viruses are continuously present in wastewater but are not routinely monitored since most of them are non-pathogenic and naturally disappear during the treatment process. (Tran et al., 2020; Hata et al., 2021). Given the onset of the COVID19 pandemic, monitoring of wastewater for this novel coronavirus has taken on a new urgency. Wastewater based epidemiology (WBE) was originally applied as an anonymous method for detecting community usage of illegal drugs (Zuccato et al., 2005), and has since been proposed as a new approach for the epidemiological toolbox (Lorenzo and Pico, 2019). Polio is a silent epidemic and in 2013–2014 polio virus was detected in Israel through WBE and allowed rapid mobilization of vaccine (Brouwer et al., 2018). WBE can indicate the presence of infected people in a city, town, or housing complex before the outbreak of a pandemic (Mandal et al. 2020; Scott et al., 2021). Wastewater sampling for COVID-19 can potentially identify the regions where the disease incidences are increasing but undetected via individual screening (Ahmed et al., 2020; Randazzo et al., 2020; Wang et al., 2020; Hata et al., 2021).

Domestic wastewater typically contains a diverse array of human enteric viruses, which potentially can cause infections in water reuse applications (Delanka-Pedige et al., 2020). The prevalence of harmful viruses in addition to SARS-CoV-2 such as polio virus and hepatitis viruses has been shown in wastewater effluent causing risk to public health (Hewitt et al., 2011; Osuolale and Okoh, 2015). United State Environmental Protection Agency (US EPA, 2020a) contaminant candidate list (CCL) describes in its 3rd contaminant list four major groups of viruses: 1) Adenovirus: mostly causing respiratory illnesses, 2) Calciviruses: mostly causing gastrointestinal illness (including norovirus), 3) Enterovirus: mostly causing respiratory illness (including polio, coxsackie and echo-virus), and 4) Hepatitis A virus: mostly causing liver diseases and jaundice (including fever, joint pain, diarrhea, and acute liver failure) (US EPA, 2020b). Coronavirus is a new virus in wastewater, and its presence and mitigation treatment methods need further investigation to ensure the generation of virus-free treated water. However, the US EPA (2020b) is claiming that standard treatment and disinfection methods are effective against coronavirus without research-based evidence to support that claim.

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Municipal wastewater contains several and wide range of different viruses which are generated and shed by sick people. More than 100 types of pathogenic viruses from human and animal origin end up in municipal wastewater (Qiu et al., 2015). Viruses are shed from people in levels ranging from 10^5 to 10^{13} virus particle per gram of stool and majority of viruses is not pathogenic (Bosch et al., 2008; Tu et al., 2008; Hewitt et al., 2011). Major transmission pathways of viruses include person to person transmission, food and water contamination and fomites (Godfree and Farrell, 2005). Enteroviruses are associated with asymptomatic infections and various clinical disorders like febrile illness, meningitis, encephalitis, paralysis and neonatal enteroviral sepsis (Costán-Longares, 2008). Other chronic diseases such as juvenile dermatomyositis, schizophrenia, and primary Sjogren's syndrome have been linked with these viruses (Melnick, 1996; Pallansch and Roos, 2001; Tang and Holmes, 2017). In the US; human enteroviruses cause 10–15 million symptomatic infections each year (Strikas et al., 1986). Adenovirus can cause gastroenteritis, conjunctivitis, respiratory diseases, and chronic systemic infections in immune suppressed patients (Jong, 2003; Selvaraj et al., 2006; Kuo et al., 2010). Hepatitis A virus (HAV) is the main cause of hepatitis and its transmission is via fecal-oral route either by person to person contact or contaminated food or water (De Paula et al., 2001).

COVID19 is caused by SARS-CoV-2, which is a severe respiratory syndrome coronavirus 2 (WHO, 2020). It is an enveloped single stranded RNA virus and causes respiratory infections in humans (Cui et al., 2019). Most of the environmentally persistent viruses are non-enveloped ribonucleic acid (RNA) viruses except adenovirus and polyomavirus JC which are non-enveloped DNA viruses (Qiu et al., 2015).

Conventional wastewater treatment systems are not efficient enough to remove all the pathogenic microorganisms (Simmons and Xagoraraki, 2011; Zhang and Farahbakhsh, 2007). A number of pathogens like *Cryptosporidium parvum, Giardia lamblia*, and a variety of enteric bacteria and viruses have been detected in conventional secondary treated wastewater and subjected to tertiary treatment (Zanetti et al., 2006). Tertiary treatment methods and disinfection processes can lessen the health risk but most methods target bacteria like chemical disinfection, UV treatment and ozonation etc. (De Luca et al., 2008; Chen and Wang, 2012; Cromeans et al., 2010; Li and Mitch, 2017). Residual by-products of chemical disinfectants and their persistent nature have diverted attention towards the development of new and alternative wastewater treatment systems such as membrane bioreactors (MBRs), algal wastewater treatment systems, and microbial fuel cells, etc. (Wert et al., 2007; Chen and Wang, 2012).

There are a number of research articles about the occurrence, and detection methods for coronavirus in water and wastewater (Carducci et al., 2020; Rosa et al., 2020; Kitajima et al., 2020; Mandal et al. 2020; Tran et al., 2020; Chen et al., 2021). However, the question that existing wastewater treatment plants are able to treat wastewater and not becoming a secondary source of resurgence is still unanswered. In the current paper, the aim is to evaluate the capacity of existing WWTPs to remove coronavirus and potential for viral resurgence via wastewater that does not undergo advanced treatment. In this review the discussion is based on the following questions; 1) How can SARS-CoV-2 be detected in water and wastewater samples? 2) To what extent can conventional and advanced wastewater systems treat SARS-CoV-2? 3) What disinfection methods are useful against inactivation of SARS-CoV-2? 4) What is the role of WBE in detecting SARS-CoV-2 and potentially other pathogenic viruses in water and wastewater?

2. Removal of coronavirus in wastewater treatment plants

Conventional wastewater treatment plants commonly consist of primary sedimentation, secondary aerobic treatment and chlorination of effluents before final reuse or discharge into surface water bodies. The influent of the wastewater treatment plants is one of the most important points of sampling for the WBE to determine the prevalence of viruses in a targeted area. Infected patients shed viruses in feces and urine in wastewater 5–8 days before the appearance of symptoms (Nemudryi et al., 2020). Katayama et al. (2008) investigated six wastewater treatment plants in Japan to monitor the presence of viruses over a year and observed seasonal impact as well. Samples were collected monthly. Noroviruses were present in summertime, with a concentration level 100 times greater than what was reported in winter. Adenovirus was present throughout the year. The reduction of viruses in the effluent of conventional treatment plants is almost constant and independent of concentration of influent. Release/overflow or untreated discharge of wastewater into recreational waters can be a source of infection. Virus removal is higher in secondary treatment plant which can be attributed to the adsorption of activated sludge and suspended solids (Gerba et al., 1980). Virus survival in wastewater is highly temperature dependent. High temperatures denature viral proteins and increase extracellular enzyme activity which reduces virus survival rates (John and Rose, 2005).

Organic matter and bacteria present in wastewater reduce the virus survival rates as adsorption and antagonistic bacteria remove viruses from treated water. Solvents and detergents in wastewater also remove viral envelop and reduce the viral load. The study observed a 99.9% reduction in 2–3 days (Gundy et al., 2009). Longer hydraulic retention time (HRT) favors removal by adsorption and high virus loads can be detected in the sludge. Anaerobic treatment (AT) of sludge removes pathogens by denaturing proteins and nucleic acids and a study observed 5.9 $\log_{10}$ removal of bacteriophage (Sassi et al., 2018). SARS-CoV-2 is a protein enveloped virus hence $6\log_{10}$ removal/inactivation during biosolids treatment is possible in AT (Amoah et al., 2020). Hasan et al. (2020) reported none of the samples of treated effluent from different WWTPs in the UAE was tested positive. These results suggest that implemented wastewater treatment methods can remove SARS-COV-2 from wastewater and produce high quality treated water.

The survival of viruses in the primary effluent is higher than the secondary effluent as the primary treatment is only sedimentation, where viruses attached to solids are in protected, while in secondary treatment protozoa, antagonistic bacteria predates on viruses, and adsorption on sludge can be removed as biosolids. High temperatures in the summer and conventional WW treatment systems can reduce viral survival rates and the danger of infection from the effluent water. Fig. 1 represents the fate of viruses in wastewater treatment systems.

Membrane separation is an advanced treatment of municipal wastewater for water reclamation and reuse. It can remove pathogenic microorganisms, such as protozoa, helminthes, bacteria, and viruses. Farahbakhsh and Smith (2004) studied microfiltration of viruses from wastewater. The results suggested that clean membrane virus removal is lower compared to foul membrane. Coliphage size (63 nm) is smaller than membrane pore size (0.2 μm), so it can pass through the membrane when permeate flux is high or membranes are clean. The authors concluded that inertial impaction caused by adsorption forces is the main mechanism of virus removal. An apparent factor in the virus removal is its isoelectric (pI) point. It is related to the pH at which a molecule carries no electrical charge. Van Voorthuizen et al. (2001) observed bacteriophage MS-2 at pH 3.9 and pH 7 has an isoelectric point and maximum removal of 5 log retention and 4.3 log retention respectively, in the presence of salts using hydrophobic microfiltration membranes. Virus removal using membranes requires optimization of parameters since the fluctuation in salt concentration, type of wastewater, and pH can change the rate of virus removal. Coliphages and bacteriophages can be removed efficiently in membrane filtration, but human viral genomes cannot be efficiently removed. Ottoson et al. (2006) observed 0.5 log additional efficiency and in total 1.7 log using membrane filtration with secondary treatment. Biofilm development enhances virus removal from wastewater.

MBRs are advanced treatment systems which replace the secondary settling process in the conventional activated sludge process. Membrane pore sizes range between 0.03 and 0.4 μm and can remove most

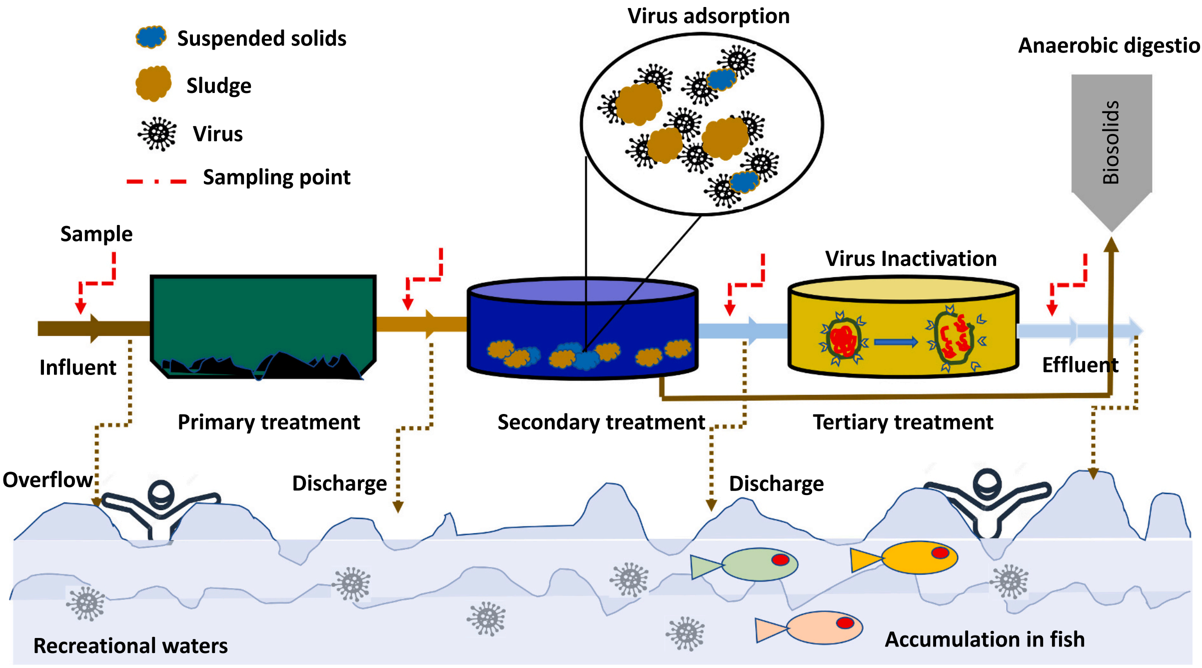

Fig. 1. Fate of virus in wastewater treatment plant and WBS.

microorganisms, but the majority of viruses are even smaller than the membrane pore size (Ottoson et al., 2006; Simmons et al., 2011; Purnell et al., 2016). Adenovirus is the most abundant human virus present in WW treatment plant effluents, and it can serve as an indicator of viral contamination (Hata et al., 2013; La Rosa et al., 2010). MBRs can remove higher quantities of adenovirus (3.4–4.6 log removals) as compared to the conventional treatment systems (1.3–2.4 log removal) (Haramoto et al., 2007; Hewitt et al., 2011; Katayama et al., 2008; Kuo et al., 2010; Simmons et al., 2011; Simmons and Xagoraraki, 2011); however, the choice of treatment system has no effect on the diversity of viruses in effluents of MBR and conventional systems (O'Brien et al., 2017).

Algal raceway ponds are other effective wastewater treatment methods capable of reducing organic matter, nutrients, and pathogens. Delanka-Pedige et al. (2020) studied the removal of four human viruses (Somatic coliphages, F-specific coliphages, Enterovirus, Norovirus) and compared their removal efficiencies with conventional WWTP. The results suggested that the virus removal in non-chlorinated effluents of algal ponds is comparable to chlorinated effluent of conventional WWTPs. However, the viral diversity in the effluents of an algal system is very low, 14 comparable to 250, and they are mostly non-pathogenic. These are promising results in terms of non-pathogenic effluent, and it requires minimal post disinfection. Pond systems, rock filters, and constructed wetland (CW) filters can remove viruses from 1, 1.7 and 2 $\log_{10}$ (Williams et al., 1995; Alcalde et al., 2003). The major mechanism suggested by different studies is predation by higher trophic level organisms and direct or indirect sunlight inactivation. Nano-flagellates, ciliates, and protozoa can consume virus-like particles and decrease their abundance in water (Manage et al., 2002; Bettarel et al., 2005; Miki and Jacquet, 2008; Battistini et al., 2013; Verbyla and Mihelcic, 2015). Still, the association of viruses with higher trophic level microorganisms needs further investigation. Biomass growth on plant surfaces in constructed wetland (CW) also assists in the virus removal (Jackson and Jackson, 2008).

Symonds et al. (2014) compared two wastewater treatment systems in terms of virus removal efficiencies: 1) facultative pond (FP); and 2) up-flow anaerobic sludge blanket reactor (UASB). The results suggested that effluents of both WWTS do not comply with the WHO health target of $<10^{-4}$ disability-adjusted life years (DALYs) lost per person per year. The effluent of FP reported 1–2 $\log_{10}$ reduction of viruses whereas the UASB effluent reported 2.5–4.5 $\log_{10}$ reduction of viruses. Thus, WWTS are inadequate systems for treating viruses to safe levels, and their release into recreational waters or used in agricultural irrigation before virus inactivation could be a source of secondary transmission. Therefore, inactivation of the virus before it reaches to WWTPs is necessary. Hospital wastewater contains 5–15% more pollution and could be the source of viruses. It should be treated with ozonation, chlorination or advance oxidation pre-treatments before it enters common WWTP (Khan et al., 2021). Coronavirus associated with sludge escape inactivation techniques but cannot survive sludge treatment processes due to high temperature, pH changes, and predatory microorganisms (Chen et al., 2021). It is suggested that solids and sludge of wastewater treatment plants should be treated through sludge treatment techniques before disposal.

3. Inactivation of coronavirus with disinfection methods

Virus inactivation during tertiary treatment can stop the transmission of infections. Heat, UV, hypochlorous acid, singlet oxygen, chlorine dioxide, ozone, and free chlorine can all be used as inactivation methods for viruses. Each inactivation method has a specific mode of action. Chlorine and UV treatment cleave protein capsule (Fig. 1) and inhibit viral bonding with host cell by destroying nucleic acid. Hypochlorous acid makes viral genome nonreplicable and leads to the inactivation of viruses (Wigginton et al., 2012; Wigginton and Kohn, 2012). Eight different viruses from the contaminant candidate list were inactivated by adding chlorine and monochloramine in water. Inactivation greatly varies between types of viruses and disinfectants. For example, a greater variation of inactivation by monochloramine was observed than with free chlorine. Monochloramine was less effective in inactivation of human adenovirus 2 (HAdV2) and echovirus 11 (E11) while most effective for echovirus 1 (E1). Some viruses need longer exposure time for inactivation (Cromeans et al., 2010). Young et al. (2020) measured genome decay using different disinfectants in Coxsackievirus B_5 and Echovirus 11. Inactivation does not mean genome decay.

Different disinfection methods damage the viral genome to different extents. When using ozone, the genome damage was higher than inactivation while UV_{254} showed a correlation between genome decay and inactivation. Several studies have reported coronavirus inactivation using UV. UV is more effective with enveloped viruses than non-enveloped viruses (Shirbandi et al., 2020). A study by Eickmann et al. (2020) observed infectivity of SARS-CoV, Crimena-Congo hemorrhagic fever virus (CCHFV) and Nipah virus (NIV) using UV-C irradiance of 0.2 (J/cm^2). Hashem et al. (2019) observed complete inactivation of MERS-CoV using UV A in Saudi Arabia. Darnell and Taylor (2006) conducted a study and observed heat and UV C treatment can inactivate SARS-CoV. Darnell et al. (2004) observed in another study the complete inactivation of SARS-CoV using a combination of UV C 254, heat, formalin glutaraldehyde, and extreme pH. Wang et al. (2005) studied inactivation of SARS-CoV with free chlorine and chlorine dioxide. The data showed 0.5 mg/L of free chlorine while 2.19 mg/L of chlorine dioxide in wastewater ensures complete inactivation. It was observed that some disinfectants had better and faster inactivation efficiency for SARS-CoV; however, the type of wastewater treatment system, temperature, season, suspended solids, organic matter, and exposure to sunlight are other factors that affect the inactivation process. Virus surveillance in wastewater and desirable future measures.

Asymptomatic people are a huge source of viral shedding in municipal wastewater. Other sources include public places like airports and patient waste from hospitals. As a result, SARS-CoV-2 will persist in the pipeline network and will become a secondary source of infection if the wastewater containing it's viral shedding is untreated (Zhang et al., 2020a; Zhang et al., 2020b). Therefore, it is important to implement the following measures: 1) use and proper disposal of personal protective equipment (PPE) during patient and waste handling; 2) disinfection of hospital wastewater before entry to the drainage network; 3) monitoring of SARS-CoV-2 in wastewater treatment plants; 4) decentralized treatment of wastewater; and 5) monitoring of residual disinfectants in wastewater to protect from future ecological losses. Zaneti et al. (2020) was the first to publish a quantitative risk assessment for sewage plant workers. Three scenarios were designed (moderate, aggressive, and extreme) in terms of percentage of the infected population, and the viral load ranged from 1.03×10^2 to 1.31×10^4 genome copies/mL. Only the moderate level was below the WHO benchmark of a tolerable limit, which affirms that wastewater is a transmission pathway of SARS-CoV-2. Daugthon (2020) described an urgent need to develop WBE methods for SARS-CoV-2 in wastewater to protect public health via early detection within a community. It is necessary to develop a scale of infection spread assessment, introduce sensors for rapid WBE, and improve the sharing of data between states and countries is required along with guidelines for poor countries who do not access to modern technology. The possible route of fecal oral transmission and human to pets and wildlife transmission must also be assessed (Franklin and Bevins, 2020; Heller et al., 2020). Table 2 summarizes SARS-CoV-2 concentrations reported in wastewater during surveillance in different parts of the world.

Along with WBE standardized testing, optimized WWTP needs attention as well. Zhang et al. (2020a) suggested sodium hypochlorite (800 g/m^3) is effective in treating hospital wastewater at source; however, residual chlorine decline in the piping network and slow release of SARS-Cov-2 is possible. High organic matter and suspended solids reduce the free chlorine concentration in water, and the residual byproducts of chlorination are environmentally harmful. It is necessary to maximize the removal of solids, OM, and use alternative disinfection methods like UV. Algal wastewater treatment systems, membrane bioreactor treatment, and ultrafiltration using membranes (Ottoson et al., 2006; O'Brien et al., 2017; Delanka-Pedige, 2020) are some of the methods that have proven effective in virus treatment, yet specific treatments should be optimized for SARS-CoV-2, and its regular monitoring should be included in routine wastewater testing parameters. In addition, the results of regular monitoring should be publicly available on municipal, state EPA, and WWTP websites. Overflows and leakages during wastewater transmission to WWTP should be monitored. Testing should be done to recreational waters and water treatment sources. Solid waste management and biosolids management facilities also needs testing and standard protocol for management and treatment of SARS-CoV-2.

4. Virus detection methods and devices from wastewater and threshold values

Since the spread of COVID-19 pandemic, virus detection methods have been improved significantly. Table 1 summarizes most commonly used methods reported in literature. Major steps of the virus detection include: 1) concentration, 2) nucleotide extraction and amplification, and 3) detection and quantification (Young et al., 2020; Haramoto et al., 2020; Balboa et al., 2020). Variety of methods have been employed to concentrate viruses like centrifugation, ultrafiltration, cell culturing, electro-negative and positive filtration (Fig. 2). From sample collection to identification there is no consensus on one standardized protocol for viral detection. Sample concentration is the most crucial step, and it is difficult to avoid false negative results. Most authors have used flocculation/precipitation as a method of virus concentration from wastewater (Bofill-Mas and Rusinol, 2020). For RNA based viruses reverse transcriptase-polymerase chain reaction (RT-PCR) assay is required while in DNA based viruses polymerase chain (PCR) reaction assay is used. SARS-CoV-2 is a RNA based virus so most studies have preferred to use this method (Lo et al., 2020; Park et al., 2020; Zhang et al., 2020a). However, some studies have used nucleocapsid staining method as well (Xiao et al., 2020).

RT-PCR results are generally reported as positive or negative and several different approaches can be used for quantification and to calculate the cycle threshold value (Ct) which is an indication or a measure of the spread of the infection. Ct values are used when specific standards are not available. The Ct values are indicators of a copy number of SARS-CoV-2 RNA in specimen through which lower cycle values correspond to higher viral copy numbers. The targeted genes for the PCR assay are the N, the E, and RdRP genes. One out of three genes detected is interpreted as a positive result; it is not necessary that all

Table 1
SARS-CoV-2 detection comparative analysis.

S. No.	Virus detection method	Sample description	Reference
1	Centrifugation, RT-qPCR	Cabin Hospital sewage Wuhan, China	Zhang et al., 2020a
2	Viral nucleocapsid staining	Feces sample of patients Guangdong, China	Xiao et al., 2020
3	RT-PCR and sequencing	Feces sample from patients traveled to Singapore from Wuhan, China	Young et al., 2020
4	RT-PCR	Feces samples of asymptomatic quarantined people in Korea	Park et al., 2020
5	RT-PCR	Nasopharyngeal swab and feces sample of 10 patients in Macau traveled from Wuhan, China	Lo et al., 2020
6	RT-qPCR	WW and river water samples from Japan	Haramoto et al., 2020
7	RT-qPCR and whole genome sequencing	Wastewater and river sampling in Italy	Rimoldi et al., 2020
8	RT-qPCR	Waste activated sludge samples in Istanbul Turkey	Kocamemi et al., 2020
9	Nested RT-PCR and real-time qPCR	Untreated wastewater of Italy	La Rosa et al., 2020
10	RT-qPCR	Primary sludge samples	Peccia et al., 2020
11	RT-qPCR	Primary and secondary wastewater treatment outlet and sludge	Balboa et al., 2020
12	RT-qPCR	Sewage samples of 7 cities and airports	Medema et al., 2020
13	RT-qPCR	Treated effluents of 11 treatment plants and 38 untreated wastewater samples	Hasan et al., 2020

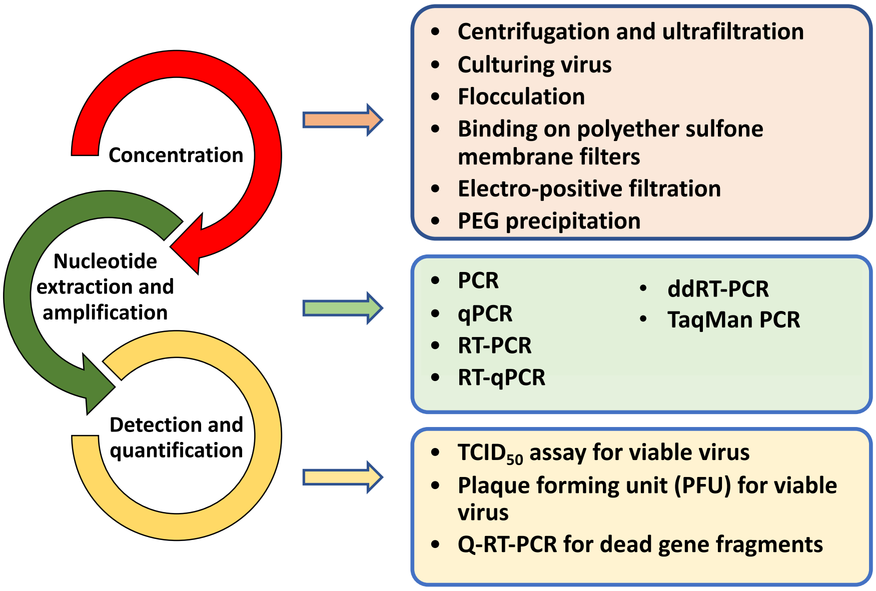

Fig. 2. Virus detection methods in wastewater.

three genes are positive in a Covid-19 positive patient. In one study; the median Ct value was 24 for all 3 genes, 36 for 2 genes and 39 for 1 gene (Drew et al., 2020). Wang et al. (2020) reported Ct $<$ 40 is positive for SARS-CoV-2 RNA and the mean Ct of the samples was 30 ($< 2.6 \times 10^4$ genome copies/mL). Kam et al. (2020) reported Ct value of $<$35 in children as positive. Virus detection can be done using TCID$_{50}$ assay, plaque forming unit (PFU) or most probable number (MPN) method. TCID$_{50}$ assay measures infectious virus titer, it quantifies the quantity of virus required to kill 50% of infected hosts. PFU measures concentration in terms of infectious dose. It is a quantity measurement of plaque forming units. Staining techniques, protein assays, transmission electron microscopy (TEM), tunable resistive pulse sensing (TRPS), flow cytometry, and enzyme-linked immunosorbent assay (ELISA) are some other quantification methods (Virus-quantification-Wikipedia, 2020).

Katayama et al. (2008) in their study used centrifugation and ultrafiltration methods for the concentration of viruses from wastewater samples, QIAamp DNA mini kit for DNA/RNA extraction, TaqMan PCR for amplification and MPN method for quantification. Bibby and Peccia (2013) used US EPA 1602 method to culture viruses for concentration, Qiagen viral RNA kit to extract RNA and PCR analysis for detection. Purnell et al. (2016) used skimmed milk flocculation to concentrate samples before nucleic acid extraction using QIAamp kit and qPCR. Costán-Longares (2008) used two different methods of concentration 1) wastewater was filtered through 0.22 μm pore-size low protein binding polyether sulfone (PES) membrane 2) adsorption on electro-positive filters was followed by isolation and propagation of viral isolates, RNA extraction and RT-PCR. Zhang et al. (2020a) detected SARS-CoV-2 using centrifugation and RT-qPCR techniques in wastewater from a Wuchang cabin hospital, built in Wuhan, China for Covid-19 patients. High virus load was found in wastewater (0.5 to 18.7×10^3 genome copies/L and disinfected using sodium hypochlorite. In recent studies; the virus concentration were reported in the range between 10^4 and 10^6 genome copies/L (Ahmed et al., 2020; Randazzo et al., 2020). Bibby and Peccia (2013) have observed the detection levels in the range of 1.9×10^3 to 5.7×10^3 genome copies/dry gram while Viau et al. (2011) have reported 1.4×10^4 to 3.2×10^4 genome copies/dry gram. Detection limits depend on the sensitivity of the PCR and the amount of sequences generated. There is a need to develop a robust detection method and standardize the protocol of virus detection. WBE of SARS-CoV-2 can be done at a faster pace if the detection method is efficient and time saving.

The mostly used nucleic acid testing (NAT) method takes up to 24 h to report the results. Since the advent of the pandemic continuous research on the development of new testing methods is on its way. One of the successful methods is imaging lungs using residual learning diagnosis detection (RLDD). Zhang et al. (2021) reported 91.33% accuracy and 91.3% precision of this method. The most attractive thing of this technology is assessment time. This technique can assess a batch of 150 samples in 4.7 s. Some other alternate methods of virus detection includes immunoassays that are based on pathogen-specific antigens or antibodies detection that are designed on lateral flow immunoassay strip (LFIAs) and it comprises of SARS-CoV-2 antigen coupled with nanoparticles. Chest computerized tomography scan, Mass spectrometry-based targeted proteomics, virus neutralization assays (VNA) (Sharma et al., 2021).

5. Virus surveillance in wastewater and desirable future measures

Asymptomatic people are huge source of shedding viruses in municipal wastewater and other sources includes public places like airports, and patients waste from hospitals. SARS-CoV-2 will persist in pipeline network and will become secondary source of infection if goes untreated (Zhang et al., 2020a; Zhang et al., 2020b). Therefore, it is important to take following measures: 1) usage and proper disposal of personal protective equipment (PPE) during patient and waste handling, 2) disinfect hospital wastewater before entering to drainage network, 3) monitoring of SARS-CoV-2 in wastewater treatment plants, 4) decentralize treatment of wastewater, and 5) monitoring of residual disinfectants in wastewater to protect from future ecological losses. Zaneti et al. (2020) published first article on quantitative risk assessment of sewage plant workers. Three scenarios were designed (moderate, aggressive and extreme) in terms of % infected population and the viral load ranges from 1.03×10^2 to 1.31×10^4 genome copies/mL. Only moderate level is below the WHO benchmark of tolerable limit. It affirms that wastewater is a transmission pathway of SARS-CoV-2. Daugthon (2020) suggested an urgent need to develop WBE methods for SARS-CoV-2 in wastewater to protect public health via early detection within a community. It is necessary to develop scale of infection spread assessment, and invention of sensors for rapid WBE and sharing of data between states and countries is required along with guidelines for poor countries who do not access to modern technology. A possible route of fecal oral transmission, human to pets and wildlife transmission also need assessment (Franklin and Bevins, 2020; Heller et al., 2020). Table 2 summarizes SARS-CoV-2 concentrations reported in wastewater during surveillance in different parts of the world.

Along with WBE standardize testing and optimize wastewater treatment process needs attention as well. Zhang et al. (2020a) suggested sodium hypochlorite (800 g/m^3) is effective in treating hospital wastewater at source however, residual chlorine decline in the piping network and slow release of SARS-Cov-2 is possible. High organic matter and suspended solids reduce free chlorine concentration in water and residual by products of chlorination are harmful for the environment. It is necessary to maximize the remove solids, OM and use alternate methods of disinfection like UV. Algal wastewater treatment systems, membrane bioreactor treatment, and ultrafiltration using membranes (Ottoson et al., 2006; O'Brien et al., 2017; Delanka-Pedige et al., 2020) are some of the methods that have proved virus treatment however, specific treatment needs to be optimized for SARS-CoV-2 and its regular monitoring should be included in the routine testing of wastewater parameters. Results of regular monitoring should be publicly available on municipality, state EPA or WWTP websites. Overflows and leakages during wastewater transmission to WWTP should be monitored. Testing should be done to recreational waters and water treatment sources. Solid waste management and biosolids management facilities also needs testing and standard protocol for management and treatment of SARS-CoV-2.

Table 2
Surveillance of SARS-CoV-2 in wastewater samples.

S. No.	Study Area	Type of sample	SARS-CoV-2 concentration	Reference
1	Temporary Cabin hospital Wuhan, China	WW from hospital in a decentralize treatment system	0.5×10^3–18.7×10^3 genome copies/L	Zhang et al., 2020a
2	Ahmedabad, Gujrat India	Untreated wastewater	5.6×10–3.5×10^2 genome copies/L	Kumar et al., 2020
3	Quito, Ecuador	River water contaminated with sewage	N1 2.9×10^5–3.19×10^6 genome copies /L N2 2.07×10^5–2.22×10^6 genome copies /L	Guerrero-Latorre et al., 2020
4	Murica, Spain	6 WWTP	$5.4 \pm 0.2 \log_{10}$ GC/L, 2.5×10^2 copies/mL	Randazzo et al., 2020
5	Paris, France	3 WWTP	50-3×10^3 equivalent genome copies /mL	Wurtzer et al., 2020
6	Queensland, Australia	Untreated wastewater at pumping station and WWTP	1.9–12 genome copies/mL	Ahmed et al., 2020
7	Porto Alegre, Brazil	WWTP	1.03×10^2 - 1.31×10^4	Zaneti et al., 2020
8	Istanbul, Turkey	Primary and Waste activated sludge samples	1.17×10^4–4.02×10^4 genome copies /L	Kocamemi et al., 2020
9	North Rhine-Westphalia, Germany	Untreated sewage samples at influent and treated water after ozonation	Influent: Solids phase 25 genome copies /mL, aqueous phase 1.8 genome copies /mL Effluent: solid phase 13 gene equivalent/mL aqueous phase 8.8 gene equivalent/mL	Westhaus et al., 2021
10	Yamanashi Prefecture, Japan	Untreated wastewater from influent and secondary treated wastewater before chlorination	Influent 4.0×10^4–8.2×10^4 genome copies /L, Secondary treated wastewater 1.2×10^2–2.5×10^3 genome copies/L	Haramoto et al., 2020

6. Viral resurgence pathways in low sanitation countries

Low sanitation countries are draining their untreated wastewater in urban streams and it ends up in rivers. The untreated sewage has been used for irrigation of vegetables, and other agricultural crops, posing an environmental and public health risk. Until 1990s research studies promoted use of wastewater for irrigation purposes but recent studies suggested that use of sewage water can alter the soil properties, microbiota and biomass of crops (Jaramillo and Restrepo, 2017). Guerrero-Latorre et al. (2020) have studied SARS-CoV-2 presence in urban streams and river water of Quito, Ecuador. Presence of pathogens such as SARS-CoV-2 in the river from untreated sewage is very high and the same situation is expected in other low sanitation countries. Contreras et al. (2017) reported diarrheal disease in children is directly linked with the use of untreated wastewater for irrigation in Mexico City and it has been decreased with changing course of water use over 25 years. Agricultural workers, consumers and residents close to areas of irrigation are people at high risk of exposure to microbial outbreaks. Untreated river and stream water has been used for food processing in low sanitation countries and it can be a source of virus spread. Viability of SARS-CoV-2 virus on inanimate surfaces such as cardboard, plastic, and stainless steel 4, 24 and 72 h (VanDoremalen et al., 2020) respectively suggests it can contaminate the food, fruit, vegetables, meat, dairy and other food products although there are no foodborne and waterborne cases of SARS-CoV-2 so far. On various surfaces in a community settings such as food table, toilet door knob, light switch, hallway bench etc. SARS-CoV-2 RNA was detected and asymptomatic food handlers, janitors and other service staff could be the source of surface contaminations (Mouchtouri et al., 2020). Cold storage food chain has also been reported a source of contamination in China as SARS-CoV-2 remains highly stable at 4 °C and freezing conditions (−10 to −80 °C) on frozen meat of fish and poultry (Han et al., 2020a, 2020b).

7. Future perspective and recommendations

In some situations, wastewater can spread SARS-CoV-2 through air droplets and fomites (Gormley et al., 2020). China Citywater (2020) claimed that hospital wastewater containing viral particles might contaminate drainage systems. Existing wastewater treatment techniques are helpful in lowering the virus load in treated wastewaters, yet innovative and more efficient treatment system must be developed. Conventional wastewater treatment systems consist of physical, biological, and chemical processes, and Okoh et al. (2010) reported physical processes can remove 90–99% of the viral load. Ultra-filtration is the most effective physical treatment. Membrane bioreactors have also proven their worth in treating viruses from wastewaters. Specific type of membranes could be developed with higher efficiencies of viral removal. Additionally, investigation on the efficiency of viral treatment in facultative ponds, constructed wetlands, and trickling filters is needed. These techniques are easy to manage in low sanitation countries, and further innovative research should focus on advanced treatment methods like anaerobic treatment, microbial fuel cells, and microbial electrolysis cells. Algal bioreactor can effectively lower viral loads, as reported by Delanka-Pedige et al. (2020). Application of biotechnological tools to develop and identify antagonistic microorganisms that can kill viruses in wastewater can enhance treatment efficiency in existing conventional treatment plants, the development of hybrid treatment systems can increase the efficiency of viral treatment in wastewater, and the use of robust disinfection techniques can remove viruses as well. Upgradation of existing WWTPs to include tertiary treatment will increase the cost of treatment. However, one time investment in clean technologies can help saving the long term cost.

Use of modern technology like biosensors should be investigated to rapidly detect viruses in wastewater. Biosensor-based techniques to detect microorganisms in wastewater is an emergent field of research (Ramírez-Castillo et al., 2015; Yu et al., 2017). It can provide a real time identification of microbial contaminant whereas traditional methods can take days. Use of nano-biosensors in wastewater monitoring has higher sensitivity and measurement reliability (Ansari, 2017). Viral protein detecting biosensors, electrochemical, or optical biosensors could help identify viruses in wastewater samples. Newly developed radiological techniques and immunoassays should be used on a commercial scale to reduce testing time.

Application of artificial intelligence (AI) in wastewater treatment plants to improve treatment efficiency is in its initial stage. It can predict, evaluate, and diagnose wastewater treatment operations. Zhao et al. (2020) applied AI in wastewater treatment plant in four areas: 1) wastewater treatment technology; 2) economy; 3) management; and 4) reuse. The results suggested that application of AI could reduce operational costs up to 30%, produce higher accuracy, and lower errors. The combination of AI and biosensors can predict the presence of viruses and help plan treatment accordingly.

Treatment of hospital wastewater at the source of production, before entering drainage systems, could be an option for the prevention of viral transmission. Pre-treatment of hospital wastewater by inactivation techniques and sludge treatment before biosolids disposal can increase the cost of treatment but it is nothing as compared to human life-saving costs. Proper disposal of hospital waste and personal protective equipment (PPE) can control the sources of viral dissemination. In low-sanitation countries, chlorination of wastewater before releasing into rivers is arguably the easiest possible method. Implications on health and ecology can be prevented with safety measures. Decentralized WWTPs should be promoted in new housing areas. The EPA should make guidelines for the disinfection of wastewater in rural communities, where people use individual septic tanks or above-ground mounds of effluent disposal and sprinkle water in farms. Wastewater treatment can prevent its route to recreational water and groundwater. Water reuse practices involve a higher level of human exposure therefore we should increase the use of advanced water treatment capacity of existing water treatment plants and monitor the reuse applications for possible exposure and transmission route. Best practices should be implemented to protect sanitation worker's health to minimize exposure.

8. Conclusions

This review describes methods of viral detection, treatment options, and inactivation in wastewater reported in recent literature, with a focus on the recent SARS-CoV-2 outbreak. Efficiency of conventional and advanced wastewater treatment methods have been summarized. Current methods are helping to reduce viral loads, but factors favoring survival and persistence need further investigation. The release of untreated viruses in recreational waters or application on land can act as secondary source of viral transmission. Specifically, low sanitation countries have greater chances of viral prevalence in the ecosystems. Therefore, biosolids treatment and upgradation of existing WWTPs to include tertiary treatment is necessary.

CRediT authorship contribution statement

Beenish Saba: Conceptualization, original draft, review & editing. **Ann Christy:** Supervision, draft review. **Birhe V. Kejjelrup:** Supervision, draft review, **Shadi W. Hassan**: draft review.

Declaration of competing interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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