

# The role of wastewater treatment plants as tools for SARS-CoV-2 early detection and removal

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## ABSTRACT

The world is facing the third coronavirus caused pandemic in less than twenty years. The SARS-CoV-2 virus not only affects the human respiratory system, but also the gastrointestinal tract. The virus has been found in human feces, in sewage and in wastewater treatment plants. It has the potential to become a zoonotic disease, as it is now proven that several mammalian species become infected. Since it has been shown that the virus can be detected in sewage even before the onset of symptoms in the local population, Wastewater Based Epidemiology should be developed not only to localize infection clusters of the primary wave but also to detect a potential second, or subsequent, wave. To prevent a zoonotic, virus removal techniques from wastewater need to be implemented to prevent the virus dissemination into the environment. In that context, this review presents recent improvements in all the fields of wastewater treatment from treatment ponds to the use of algae or nanomaterials with a particular emphasis on membrane-based techniques.

## 1. Introduction

SARS-CoV-2 is the third coronavirus to spread worldwide in the last two decades [1]. As of July 9<sup>th</sup> 2020, the virus has infected more than 12 million people with the COVID-19 disease and claimed the lives of 552,000 people. The Severe Acute Respiratory Syndrome (SARS), as its given name indicates, primarily affects the respiratory system of the patients. However, COVID-19 has a broader range of symptoms, with digestive tract symptoms widely spread amongst patients. Moreover, a substantial portion of infected people are asymptomatic or have mild symptoms but still can transmit the virus. Lastly, SARS-CoV-2 is not only supposed to have been transmitted to humans through animal carriers, but humans can also transmit the disease to other animals.

In this review, the literature concerning the presence of the disease in the digestive tract of patients and its dissemination in the wastewater network will be detailed. The present literature concerning which animals have been identified as potential carriers of SARS-CoV-2 will then be summarized.

To limit the propagation of CoVid-19 and to avoid the possibility of a pan-zoonotic disease, wastewater based epidemiology that allows rapid detection of the presence of the disease in an area and the tools available

to remove viruses from the wastewater at the wastewater treatment plant to reduce the risk of spreading the disease further within the environment, either by discharge into water systems or by the production of reclaimed water for irrigation or human consumption will be presented.

## 2. SARS-CoV-2 (2019-nCoV) information

SARS-CoV-2 is an enveloped single-stranded RNA virus which belongs to the *Coronaviridae* family. This family is subdivided into four subfamilies ranging from alpha to delta. Subfamilies alpha and beta can affect humans. SARS-CoV-2 is from the subfamily *Coronavirinae* genera  $\beta$  [2] sub-genus Sarbecovirus [3]. Since 1960, there have been seven coronaviruses from these two subfamilies that have been reported to have affected humans [4,5]: 229E, OC43, SARS-CoV, NL63, MERS, HKU1 and SARS-CoV-2. 229E, OC43, NL63, HKU1 are involved in 15 % of the common colds [5] whereas SARS-CoV, MERS and SARS-CoV-2 viruses are more virulent and they cause a severe acute respiratory syndrome (SARS). SARS-CoV outbreak was in 2002–2003 and the Middle East Respiratory Syndrome coronavirus (MERS-CoV) broke out in the Arabian Peninsula in 2012 [1]. Bar-on et al. [6] provide regularly

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updated data in which can be found the SARS-CoV-2 size, which is around 100 nm. For membrane-based treatment processes, size is important as it determines the maximum pore size of the membrane which needs to be selected to remove the viral particles. Fig. 1 provides size and shape comparisons of several viruses. In this figure, virus sizes range from 20 nm to 970 nm. With 100 nm, SARS-CoV-2 is slightly smaller than SARS-CoV but is bigger than most common viruses.

### 3. Spread of SARS-CoV-2 in the environment

The two main ways which were initially reported for SARS-CoV-2 transmission are through direct contact and through aerosols produced by contaminated people when sneezing or coughing. SARS-CoV-2 is able to survive on surfaces, with survival duration highly dependent on surface type [8–10], although it has not as yet been demonstrated as a route for transmission.

However, SARS-CoV-2 RNA has also been found in human feces [11–21] of at least 39 % of tested patients [22] and therefore the question of the possibility of fecal-oral transmission is raised [15,17].

Zang et al. [23] studied the infection of human small intestinal enterocytes and reported that two mucosa-specific serine proteases promoted virus entry in enterocytes. Mart et al. [24] also demonstrated that enterocytes are easily infected and can actually be used as experimental models. But regarding the possibility of fecal oral transmission, it was indicated that the SARS-CoV-2 was inactivated by simulated human colonic fluid [23]. These data are in vitro data and it is also reported that live SARS-CoV-2 virus was found in patient's stool samples [25,26].

Detection of SARS-CoV-2 virus was obtained in both nasopharyngeal and rectal swabs in a paediatric study of 10 children [27]. Moreover, from the day of admission, the rectal swabs returned positive for 27 days, whereas no virus was detected in any nasopharyngeal swabs after 15 days [19,27]. However, virus replication tests realized on fecal swabs returned negative. Jiang et al. [28] even detected the virus in the stools of patients for as many as 42 days.

Numerous teams have indicated that COVID-19 could be transmissible by the fecal-oral route [11–17,19,25–31], but there is no demonstrated case at this point. However, caution should be taken to limit the possibilities of virus shedding in the environment for both human and animals. He et al. [32] provided data about when and the duration that infected people can shed virus and for how long the RNA

virus can be detected [33]. Those data were compiled by ViralZone [34] and are presented in Fig. 2.

Since the virus is present in feces, is it found in municipal wastewater and this raises the possibility of spread to the wider environment from insufficiently treated effluent. Several articles have been published indicating detection of SARS-CoV-2 [35–40] in sewage.

Wurtzer et al. [36] were able to correlate the results of the quantitative monitoring of SARS-CoV-2 genome in Paris sewage to the number of people infected. They even suggested that sewage water samples should be stored in a databank to allow them to be investigated retrospectively when an outbreak is starting. The importance of this archiving was equally outlined by Dolfing [41].

Medema et al. [39] report a study in the Netherlands where they started looking for the presence of COVID-19 RNA in 6 locations on February 6<sup>th</sup> 2020. The detection started on March 4/5, six days before the first cases were reported. Similarly, Randazzo et al. [42] in Spain and Wu et al. [43] in the USA detected COVID-19 RNA in wastewater, even in low prevalence area in Spain. La Rosa et al. [44] reported the detection of COVID-19 in untreated wastewater in Italy.

This raises the question of the presence of SARS-CoV-2 in the aquatic environment. Since COVID19 is a pandemic spread in 215 countries, it covers countries with wastewater treatment plants and other developing countries where wastewater is directly sent to the aquatic environment untreated. Thus, the presence of viable SARS-CoV-2 virus or RNA in aquatic environment is reported by several authors [45–52].

### 4. SARS-CoV-2 zoonosis

COVID19 disease was first detected in the city of Wuhan in the Hubei province of China in December 2019. The corona virus that causes the disease is now named SARS-CoV-2. Since it shares 96 % whole genome identity to a bat coronavirus: BatCoV RaTG13 [53], the bat *Rhinolophus affinis* from Yunnan province is suspected to be the original mammal hosting the precursor of SARS-CoV-2. However, the two provinces are distant from each other and the contacts between bats and humans are limited. It is possible that an intermediate animal host is involved in the transmission from animals to humans. The presence of pangolins in the putative starting point of the pandemic, which is the seafood market in Wuhan China, and the 91 % nucleotide identity of the Sars-CoV-2 with a pangolin CoV [6,53] makes it a conjectured intermediate host [54],

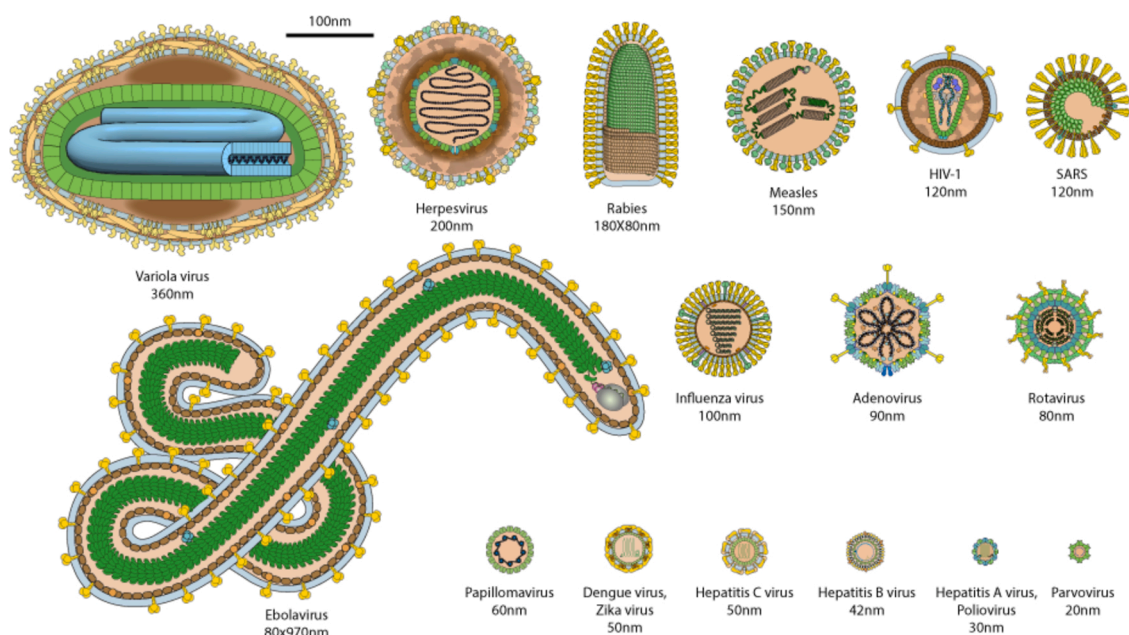


Fig. 1. Human virus relative size from <https://viralzone.expasy.org/5216> [7].

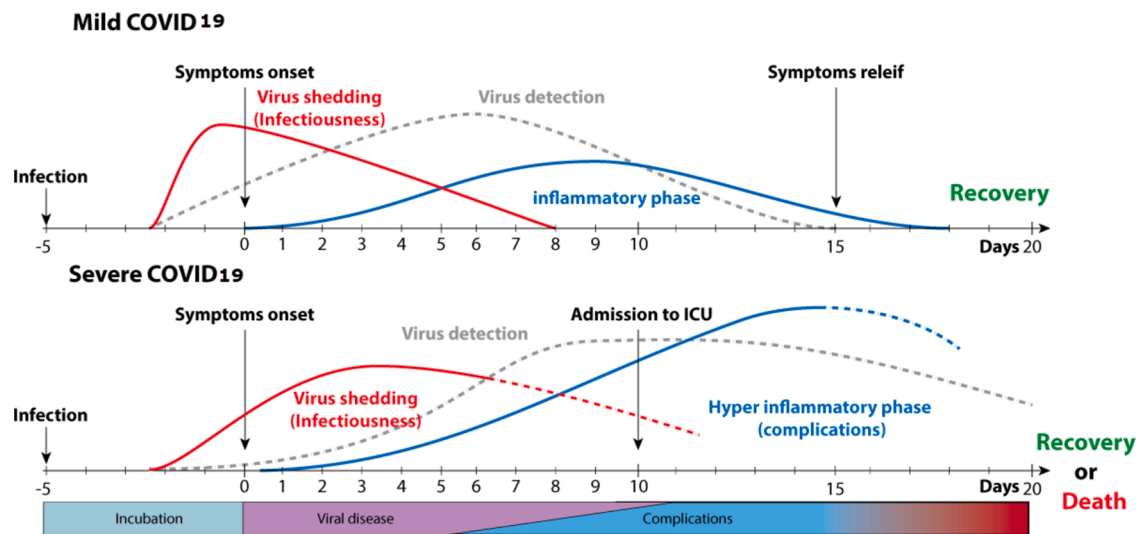


Fig. 2. Timeline for SARS-CoV-2 shedding and detection <https://viralzone.expasy.org/9116> [34].

although this is far from certain and efforts are ongoing to find other candidate intermediate hosts.

Apart from the search for the intermediate host, studies have been made to identify animal species that could have been infected. A Chinese study examined antibody levels in pigs, sheep, horses, chicken and ducks [55] and the results were negative.

However, it is reported that diseased humans have transmitted the virus naturally to ferrets, cats, dogs, tigers, lion and minks [3,56–59]. Apart from minks farms, there is no evidence so far that livestock for human consumption such as cows, sheep and pigs have been infected in farms [60]. This last point is rather important if it is considered that in the case of MERS-CoV, which belongs to *Coronaviridae* beta subfamily like SARS-CoV-2 but not to the subgenus Sarbecovirus, camels [61,62] were suspected to be possible reservoirs or intermediate hosts for successive outbreaks. Even raw milk and improperly cooked meat were discussed as potential sources of infection.

Apart from the natural transmission of SARS-CoV-2 virus from human to animals, studies have reported of experimental inoculation of the virus to animals and study the possibility of transmission from animals and the transmission route by putting in the same cage infected and non-infected animals and having other animals in another cage close to the first [63–66]. This has demonstrated that ferrets and cats are highly susceptible to the virus, whilst dogs are less susceptible and livestock (pigs, sheep and ducks) studied were not found to be susceptible. It is to be noted that those tests were done on a limited number of animals, so how representative they are of the species as a whole is not entirely known.

Transmission back to humans was discussed by Franklin et al. [67] who raised the possibility of wild hosts in North America as a potential source of perpetuation of the pathogen in the environment. Damas et al. [68] studied in-silico the probabilities of a wide range of animals to get infected and ranked them in five levels from very low to very high based on the conservation of genes for 25 amino acids important in the binding of the virus to a putatively important receptor, ACE2. The ranking used the fact that the main receptor of SARS-CoV-2 is the angiotensin I converting enzyme 2 (ACE2). Their study on 410 vertebrates, including 252 mammals could help identify potential animals that could be viral host. From this they judged that only mammals fell into the categories of medium or higher risk. In the meantime, in the Nederland on mink farms where minks had been found to be contaminated, sequencing was done on samples taken from diseased farmers and, while the topic is still under investigation, the transmission from minks to human seems to have occurred [56].

Gollakner et al. [69] underlined that although the data gathered so

far does not allow definite conclusion of the possibility of a zoonotic aspect for COVID-19, allowance should be made for the possibility. Since there is shedding of the virus to the environment through sewage, the question has been raised about the possibility of aquatic mammals to become contaminated [70].

## 5. Wastewater based epidemiology

From the previous sections it was shown that SARS-CoV-2 is found live in human feces and that it can be transmitted to animals which can also excrete it. Although the fecal oral transmission has still not been demonstrated, precaution measures should be taken to minimize this possibility. Fig. 3 summarizes the pathways that excreta from both human and animals can follow to potentially transmit the virus back to humans [71]. Sewage and then Wastewater Treatment Plant (WTT) are at the center of this potential transmission route and WTT can play an important role in detection and control of SARS-CoV-2 spread. For the detection part, Wastewater Based Epidemiology (WBE) could be used.

WBE is a relatively new tool whose idea comes from the fact that relatively stable environmental contaminants that are released in the sewage system can be sampled and quantified. It potentially provides real time information on wastewater contaminants [72] and was first used in 2001 to evaluate illicit drug usage [73]. More recently, WBE has been proposed for the early detection of viral outbreaks [74–82] as well as for the persistence of viral indicators in the aquatic environment [75, 83]. The methods used are high throughput RNA sequencing (RNA-Seq) more commonly than PCR (qRT-PCR) [75,84]. In 2019, O'Brien and Xagorarakis [74,85] underlined the importance of wastewater data collection to understand spatial and temporal evolution of viral diseases.

In the case of SARS-CoV-2, considering that the virus was found both in stool samples and wastewater as previously described, several authors have proposed to use WBE as a tool to monitor the pandemic [41,76–80, 86–100]. Thus, Medema et al. [38] reported that sewage surveillance could be a sensitive tool to monitor SARS-CoV-2 circulation in the population since it could be detected prior to symptoms being reported in the local population. Moreover, monitoring of international airport sewage would allow very early detection of the entrance of the virus into a country.

Wurtzer et al. [36] even suggested that wastewater sample storage would allow detection of an outbreak retroactively to provide historic information of the spread of the virus. In the present SARS-CoV-2 outbreak, it could have allowed the localization of the first patients and define which cities should be on lockdown instead of putting the lockdown on a whole country. For example, until now it was believed

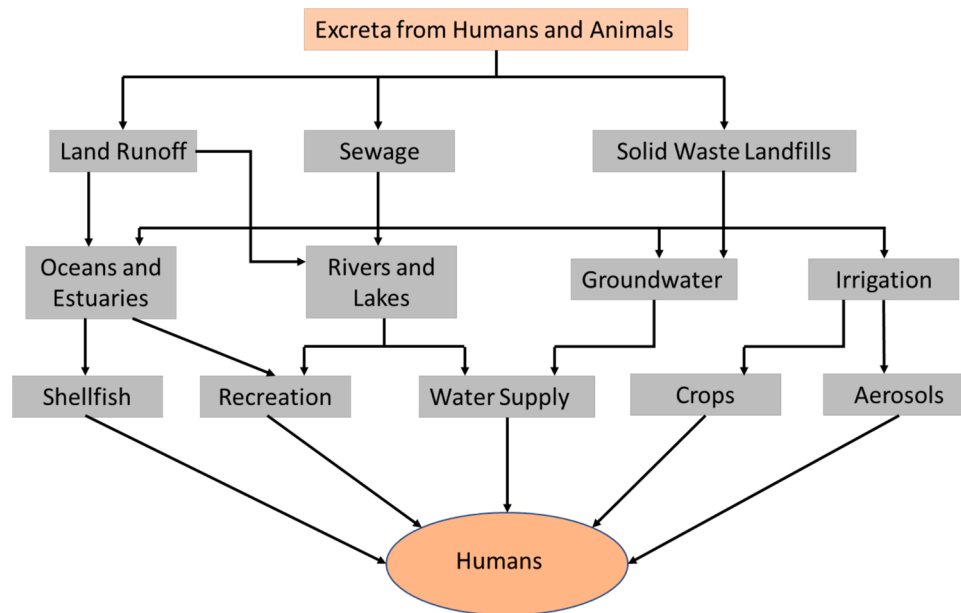


Fig. 3. Transmission routes for pathogens in human and animals excreta. Adapted with permission from ref. [71].

that the SARS-CoV-2 outbreak in France started late January 2020 but SARS-CoV-2 was detected retrospectively by RT-PCR in samples from a patient admitted in intensive care in late December 2019 [101].

Mao et al. [102] proposed a small, portable paper-based device for the detection of SARS-CoV-2 and Hata and Honda [91] provided information on the potential sensitivity of wastewater monitoring for SARS-CoV-2 which is one diseased person in 100 000.

## 6. Wastewater treatment for virus removal

The WHO [103] underlined in its interim guidance of April 23<sup>rd</sup> 2020 the importance of safely managing wastewater. Limiting the dissemination of the SARS-CoV-2 into the environment is of great importance considering that several animals have already been identified as potential hosts and that reclaimed water could be used in irrigation. Gerba et al. [104] reported the virus removal reduction factor that wastewater plants need to provide depending on the reclaimed water use. For irrigation of edible crops, they indicate a 6 log<sub>10</sub> removal is sufficient, whereas a 12 log<sub>10</sub> removal would be needed to make the water potable. The same removal targets have been reported by Ahmed et al. [105] in their 2020 article on recycled water safety.

Wastewater treatment is a wide field and its full description is not the target of this review. This section will focus on the literature containing techniques used for virus removal. After presenting the model viruses that are used to evaluate the efficiency of wastewater virus removal treatment, recently developed methods will be presented.

### 6.1. Model virus

SARS-CoV-2 is a potentially lethal virus to humans and some precautions need to be taken for scientific testing to be made. Thus, the Center for disease Control and Prevention (CDC) provides guidelines for Biosafety and COVID-19 [106]. For environment specimen testing, the CDC defines that 'Procedures that concentrate viruses, such as precipitation or membrane filtration, can be performed in a BSL-2 laboratory with unidirectional airflow and BSL-3 precautions'. As a result, in general, virus surrogates are used instead of dangerous pathogens. The surrogate to be used depends on the experiment type to be handled. For example, to compare the virus concentration methods for wastewater testing, murine hepatitis virus (MHV) has been used as a SARS-CoV-2 surrogate [84]. Other animal coronaviruses such as the transmissible

gastroenteritis virus (TGEV) and the feline infectious peritonitis virus (FIPV) have also been used for testing the persistence and disinfection of human coronaviruses in water and wastewater [107]. For safety reasons some laboratories are using the enveloped bacteriophage  $\phi$ 6 [107–109], whose size is 85 nm [110], or the non-enveloped *Escherichia* virus MS2 [109,111–122], whose size is 26 nm [123,124].

However, Shirasaki et al. [125] studied different virus models and they concluded that the Pepper mild mottle virus (PMMoV), which is a plant virus, appears to be a suitable surrogate for human enteric virus whereas MS2 and  $\phi$ X174 are not. PMMoV has also been validated together with AiV-1 and TMV as surrogates of human enteric viruses for testing wastewater virus reduction [126].

### 6.2. Wastewater treatment pond systems

Wastewater treatment ponds are very common worldwide as they are the technically simplest way to handle wastewater. Verbyla and Mihelcic [120] reviewed 71 different wastewater treatment pond systems for virus removal and concluded that one log<sub>10</sub> reduction of virus content in water was achieved between 14.5–20.9 days. Considering the World Health Organization guidelines for wastewater reuse for irrigation, a pathogen reduction of 6–7 log<sub>10</sub> units is required [127]. This target can be reached only by the combination of several methods. Ponds are used as primary treatment and there can be up to three treatment steps.

Virus removal in pond treatment systems is not very well understood and depends on the particular characteristics of the pond, including chemical composition and optical properties, but the process seems to be a combination of sunlight-mediated mechanisms and interactions of virus particles with other particulates including other microorganisms and sedimentation [120].

### 6.3. UV inactivation and chlorine treatment

Qiu et al. [128] reported the inactivation of human infectious virus at two wastewater plants. They measured the reduction using qPCR on different virus types, the mean log<sub>10</sub> reduction they obtained was 1.2 and 1.8. Simon et al. [129] reported the use of chlorine and ultraviolet light disinfection against different viruses. They obtained up to 2.5–3 log<sub>10</sub> reduction (LR) on coliphage and *E. coli*, otherwise the LRs were in the range of 0.3 to 1.3. They observed that results are better on



sand-filtered samples. UV inactivation and treatment with hypochlorite generally renders viruses inactive by damaging replicative machinery through oxidation processes [130].

#### 6.4. Ozone treatment

Ozone is highly effective for the inactivation of many viruses. A statement by the International Ozone Association (IOA) indicated that it was not aware of any research and testing that had been conducted specifically on the SARS-CoV-2 coronavirus. Peer Reviewed Research has not yet been completed and therefore definitive conclusions cannot be made regarding ozone inactivation of SARS-CoV-2 (IOA Statement on COVID-19, 2020 [131]).

The US EPA has published disinfection contact time (CT) values for virus inactivation by ozone in the surface water treatment rule (SWTR) (US EPA 1989 [132]). To assess the disinfection efficiency in water treatment, the CT-concept is applied. C represents the concentration of disinfectant (ozone) and T the contact time; CT is the product of the disinfectant concentration and contact time. Sigmon et al. [133] calculated CT values for specified log inactivation levels of a number of viruses and surrogates in wastewater at pH 7.6 and 16 °C, with log reduction values (LRV) for all viruses ranging from 0.56 for an LRV of 1–1.32 for an LRV of 1.32 [133]. These data indicated that all organisms were generally inactivated to 4-log at a CT of no more than 1.0 mg min/L.

Enteric viruses do not get proper inactivation in wastewater effluent discharged to the environment [134]. As such, effluent discharged from sewage treatment plants will have an elevated number of viruses [135]. Ozone has been used as a disinfectant instead of chlorine for the disinfection of sewage effluents used for irrigation of crops, and for discharge to surface water, due to the issue of high chlorine demand and problematic disinfectant byproducts [136]. A study by Perez-Rey et al. [137], reported that selected microorganisms including four viruses, three non-sporulated bacteria, two sporulated bacteria and one fungus, were employed in vaccine preparation for animals. Viruses were the most readily-inactivated microorganisms and sporulated bacteria the most resistant strains. The inactivation followed a second order kinetic law, depending on both ozone and microorganism concentration. The conclusion of that study was total inactivation of microorganisms in wastewater is possible by ozone application. A study by Aydogan et al. [138], indicated the effectiveness of ozone gas to inactivate *Bacillus subtilis* spores, which share the same physiological characteristics as *Bacillus anthracis* spores that cause the anthrax disease. Increased humidity during the ozone application increased the rate of inactivation.

The City of Montreal, Québec, Canada, investigated three disinfection processes (Ozone, UV, and Performic Acid) to improve wastewater treatment to reduce pharmaceuticals and endocrine chemicals in one of the primary-treated effluents (high flow system) [139]. The City of Montreal municipal wastewater has the target criteria for coliforms of 9000 UFC/100 mL that needs to be met. Ozone treatment was found to be the most efficient of the three disinfection processes investigated in removing selected pharmaceutical products from lightly treated effluents [139].

#### 6.5. Sand filters for virus removal

Sand filters are commonly used in wastewater treatment, but they usually have less than one-unit  $\log_{10}$  virus removal, they are used more for particle removal. However, Samineni et al. [112] developed functionalized sand filters using a water extract of *Moringa Oleifera* seeds. Then they tested the efficiency of the obtained sand using MS2 bacteriophage virus, obtaining an impressive 7  $\log_{10}$  reduction in virus content. The mechanism implies that MS2 has affinity for some components from the seeds and binds to it. It also implies that the functionalized sand will progressively get saturated and will eventually have to be replaced or regenerated in some way.

#### 6.6. Algal systems for virus removal

Delanka-Pedige et al. [140–142] recently published three articles dealing with the use of algae for removing viruses in wastewater. Results obtained are in the same range as for activated sludge with log removal values in the range of 1–3 depending on the virus type. They seem, however, to reduce the number of species more efficiently and thus reduce the need for chlorination. Mechanisms for viral removal in algae based wastewater treatment systems include sedimentation after viral particles have attached to algal biomass, increased temperatures denaturing algal nucleic acids and proteins and sunlight mediated degradation [140].

#### 6.7. Membranes use in wastewater treatment for virus removal

Water filtration membranes are categorized by their pore size and their distributions in four categories: Reverse Osmosis, Nanofiltration, Ultrafiltration and Microfiltration. Fig. 4 gives a representation of those pore sizes together with the main contaminants, including viruses, that can be found in water.

Considering that SARS-CoV-2 size is 100  $\mu\text{m}$ , membranes such as Reverse Osmosis, Nanofiltration and Ultrafiltration should be able to remove it. Goswami et al. [143] reviewed the use of polymeric and ceramic membranes for virus removal from water. The reported removal efficiencies were highly variable since the range reported was 0.2–7. This section will outline very recent literature showing the potential use of those membranes for SARS-CoV-2 before considering microfiltration and membrane bioreactor technology.

##### 6.7.1. Reverse osmosis

Włodarczyk and Kwarciak-Kozłowska [71] recently reviewed the treatment of waterborne pathogens by reverse osmosis. They provide complete description of the type of pathogens that are waterborne which are divided in three different groups: protozoans (5–100  $\mu\text{m}$ ), bacteria (0.5–1.0  $\mu\text{m}$ ) and viruses (0.01–0.1  $\mu\text{m}$ ); as well as requirements for Reverse Osmosis membrane materials. They indicated that Reverse Osmosis is seldom used to remove pathogens from water even if it is one of the techniques reported by the EPA with indicative log removals above 6. This is because reverse osmosis is typically coupled with a pre-treatment system, often ultrafiltration, to reduce foulants which may interfere with the reverse osmosis process. However, RO can be used in combination with such a suitable a pretreatment, to remove particulate matter, and post treatment, to complete the removal of any remaining contaminants [145].

Prado et al. [146] reported that Membrane Bioreactor/Reverse Osmosis systems were providing virus removal efficiencies in the range of 2.3–2.9  $\log_{10}$  which is rather lower than what was reported by Vickers et al. [147] who used reverse osmosis for the removal of MS2-coliphage. They obtained a nominal 5  $\log_{10}$  removal without the MBR. Considering that MS2 is 27  $\mu\text{m}$  in size, which is roughly 60 times smaller than SARS-CoV-2, the removal efficiency on the latter would be expected to be higher. The same authors [148] also reported the use of sand-anthracite filters and a membrane bioreactor/reverse osmosis system for the removal of noroviruses, which are non-enveloped capsids of about 38–40 nm in diameter [149], in raw sewage. The efficiency of the system led to a virus concentration below the detection level, which would mean, considering the initial virus concentration and the detection limit of the analysis, a  $\log_{10}$  removal higher than 6 or 7.

##### 6.7.2. Nanofiltration

Pore sizes of nanofiltration membranes are generally less than 10 nm which is smaller than any virus presented in Fig. 1 [150]. Although the literature on nanofiltration removal of viruses is not extensive, several studies have been published which generate log removal rates for various viruses at between 3 and 8 [151–155].

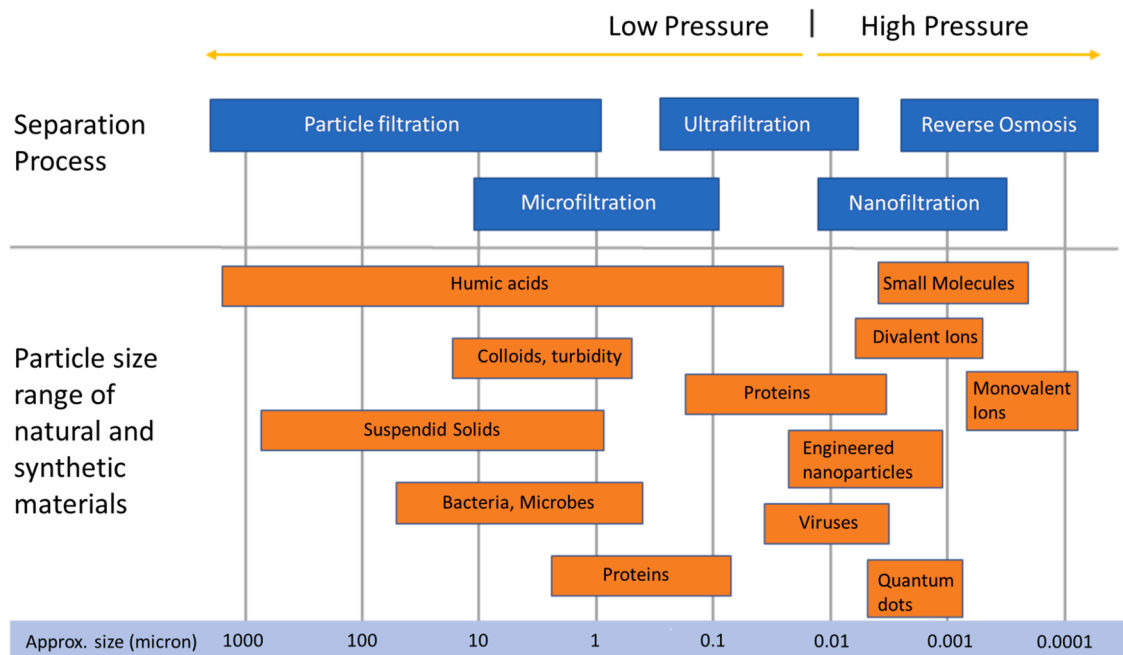


Fig. 4. Comparison of water filtration membranes pore sizes with water contaminant sizes, reproduced from open source [144].

#### 6.7.3. Ultrafiltration

As ultrafiltration is often used as a pre-treatment step prior to RO treatment, its efficacy in removal of viruses is more widely reported in the scientific literature than for other membrane-based technologies. Recently Al Aani et al. [156] reviewed the use of Ultrafiltration Membranes for wastewater and water process engineering and report their use for bacteria and virus removal.

Lee et al. [117] used a combination of coagulation and ultrafiltration (UF) on a pilot scale for wastewater reclamation. They improved removal of MS2 bacteriophage by optimizing the pH of the secondary effluent to a range of 5–6 depending on the effluent. Once improved, the virus removal factor was in the 6.8–7.5  $\log_{10}$  range. Additionally, to obtain a more stable transmembrane pressure, an additional sedimentation step was required. The ideal combination was a hybrid coagulation-sedimentation-UF system.

Other researchers [157] used a polyethersulfone Ultrafiltration membrane with average membrane pore sizes of 67 nm for the removal of the bacteriophage PP7. They studied the effect of pH in the range of 5–8 and modelled virus and membrane electrostatic interaction forces. The  $\log_{10}$  removal range obtained was in the 1.5–2.8 range depending on ionic strength. Divalent cations present in feed solutions were found to have a negative impact on the viral removal effectiveness compared with monovalent cations due to their modification of electrostatic interactions.

Grafted zwitterionic polymer hydrogels were used by Lu et al. [158] for the modification of a 150 kDa polyethersulfone membrane. They studied the membrane efficiency on MS2 and HAdV-2 human viruses. The grafting reduced water permeability but provided a  $\log_{10}$  removal in the 3–4 range.

A pore interconnectivity method of evaluation of asymmetric ultrafiltration membranes has been developed commercially specifically for virus removal [159]. Gold nanoparticles of different sizes were used and their capture by the membranes was visualized using SEM. It was found that membranes shown by this method to have poor pore interconnectivity were also membranes which had been previously shown to have significant loss of viral retention after process recovery, whereas membranes shown to have better interconnectivity were more robust in terms of virus retention over a range of operating conditions.

#### 6.7.4. Microfiltration

Since microfiltration membrane average pore sizes are larger than 100 nm, they are more suited to the removal of protozoa and bacteria rather than the smaller viruses [160]. However, this only considers separation by sieving mechanisms. Sinclair et al. [161] modified a microfiltration membranes using a cationic polymer. They reached a 3  $\log_{10}$  MS2 reduction with the resulting membrane with only 22 % reduction in flux. They proposed such membranes for low pressure point of use water filtration, due to the high-water permeability of such membranes.

#### 6.7.5. Membrane bioreactor

O'Brien and Xagorarakis reported that membrane bioreactors, under the correct conditions are capable of a 7 $\log_{10}$  reduction in virus concentration [162]. From a comparison of a number of studies it was found that the presence of a biofilm on the membrane was important in virus removal efficiency, where a biofilm, whilst reducing water flux also restricted viral penetration of the membrane. It was concluded that there must be a trade-off between maintaining a biofilm to keep viral removal efficiency high with backwashing of the membrane to remove biofilm to maintain acceptable water fluxes. They also concluded that model viruses do not necessarily show the same removal efficiency as viruses which are pathogenic to humans, with data obtained using harmless bacteriophages likely to lead to overestimation of viral removal efficiencies for pathogenic viruses.

Miura et al. [163] investigated the removal of three virus families in a full scale membrane bioreactor plant. The best results were obtained at pH4 with a  $\log_{10}$  reduction range of 2.3–4.5

#### 6.7.6. Ceramic membranes

Im et al. [122,164] first compared ozonation and coagulation as pretreatments for virus removal with a ceramic membrane. They then used a combination of ozonation, coagulation and ceramic membrane filtration for water reclamation. They obtained on a pilot plant a 12  $\log_{10}$  removal factor of MS2.

The use of hydrophobic ceramic capillary membranes for virus removal was reported by Bartels et al. [165]. They functionalized ceramic membranes with hexyl or octyl triethoxysilanes in order to increase hydrophobic surface properties. Without functionalization, they

obtained log<sub>10</sub> removal in the range of 0.3–3.4 for the bacteriophages MS2 and PhiX174, whereas after functionalization the log<sub>10</sub> removal was around 9. This increase in removal efficiency was due to favorable virus- treated membrane surface interactions, primarily due to hydrophobic interactions, which lead to adsorption of viruses to the membrane material. Presumably, such membranes would potentially be subjected to a sufficiently high saturation of viral concentration, thus necessitating cleaning.

In-line coagulation with ceramic membrane filtration for the removal of the bacteriophage Q $\beta$  was investigated by Wattanachira et al. [166]. The coagulant they used is polyaluminum chloride. They underlined that they had to adjust coagulant dosage with virus concentration. Overall, they obtained 6.7 log<sub>10</sub> virus removal under optimal conditions.

#### 6.7.7. Nanomaterials

The use of nanomaterials to remove and de-activate viruses in wastewater is a broad field and includes the use of fullerenes, photocatalysts and membranes incorporating nanomaterials, such as CNTs, TiO<sub>2</sub> and ZVIs [167–169]. For instance, Kim et al. [170] reported that Silver Multiwall nanotubes (Ag-MWCNT) used in a nanocomposite filter system reached a 100 % removal for several different viruses [170]. Domaga et al. [113] studied Cu<sub>2</sub>O/MWCNTs filters for MS2 virus removal from water. One of their three samples reached a 7 log<sub>10</sub> reduction in MS2 at pH5. Nemeth et al. [171] used a Cu<sub>2</sub>O-coated MWCNT membrane and obtained a 4 log<sub>10</sub> MS2 removal in a pH range of 5–9. Kuo et al. [115] obtained a 7 log<sub>10</sub> reduction on three viruses, Q $\beta$  bacteriophage, MS2 bacteriophage, and Aichi virus, with smectic liquid-crystalline ionic membranes.

#### 6.8. Integrity testing techniques and imperfection fixing

For the Reverse Osmosis, Nano-Filtration and Ultra-Filtration membranes to efficiently remove viruses, their integrity has to be optimal. For instance, membranes may contain defect obtained during manufacture or may suffer mechanical damage during use from high hydraulic operating pressures or scouring by particulates in the feed water. A number of reviews are found in the literature for techniques to assess the integrity and structure property relationships of separation membranes including by Wang et al. [172], Ostarcevic et al. [144] and Pype et al. [173]. High-pressure membranes are only credited at 2 log<sub>10</sub> virus removal by regulatory agencies, whereas in the sections above articles were presented where it reached as high as 7 log<sub>10</sub>. This difference is due to a lack of sufficiently sensitive detection technologies available industrially [174].

Frenkel and Cohen [175] proposed new techniques for real-time monitoring of reverse osmosis membrane integrity for virus removal. They proposed a pulsed marker membrane integrity monitoring system. The potentials and limitations of this type of fluorescent-based technique was then investigated by Yoon [174], who managed to obtain a sensitivity level at the 4 log<sub>10</sub> level.

Then, Niewersch et al. [176] proposed a solution-diffusion-imperfection model with a decision tree for diagnosis to help monitor the integrity of membranes, which was capable of identifying the types of imperfections present in a particular membrane under study.

For ultrafiltration, Khannstover et al. [177] published a comparison of different methods that can be used to assess integrity and separation efficiency. Nanoparticle counting was found to be the most sensitive method overall in terms of lowest LRV values reported.

As a solution to imperfections in nanoscale membranes, Suzuki et al. [178] proposed to use a small volume of aqueous polyvinyl alcohol to selectively plug imperfections. These plugs were then stabilized through exposures to citric acid, sodium hydroxide, and ethylenediaminetetraacetic acid.

## 7. Conclusions

SARS-CoV-2 is a virus that emerged at the end of 2019 in China and generated a pandemic of COVID-19 within a few months. Initially thought to affect only the respiratory system, it was soon proven to be also gastrointestinal. Then, the environmental implication is that SARS-CoV-2 is shed into the sewage system and thus reaches Wastewater Treatment Plants when existing or more globally the aquatic environment.

SARS-CoV-2 has also been proven to be transmitted from humans to animals, which can potentially be a virus reservoir. Considering the possibility of SARS-CoV-2 being a zoonotic, it becomes even more important to limit SARS-CoV-2 spread into the environment. Wastewater Treatment Plants could be a solution for the early detection of the disease in an area through the development of a Wastewater Based Epidemiology plan. It could also be used as a decision helper for lockdown application or removal.

After reporting some of the virus models that can be used to characterize virus removal efficiency, a wide range of recently developed wastewater treatments were presented. This include some classical and advanced technologies treatments (Ozone, UV, etc.) and numerous membrane-based systems. As a general rule, it is the combination of different techniques in primary, secondary and tertiary treatments that would allow enough virus removal to limit the possibility of environmental contamination.

The SARS-CoV-2 pandemic should develop a global awareness that solutions have to be found for improving the wastewater treatment even in developing countries, due to the possibility of its spread through the aquatic environment from inadequately treated waste water. SARS-CoV-2 transmission through the fecal oral route is not predominant, but considering that it is the third new coronavirus to evolve to a pandemic in less than twenty years, preparations should be made for the possibility of a virus pandemic more readily transmissible through this route. Considering the economic costs of the present pandemic, greater efforts to improve wastewater treatment specifically with the removal or inactivation of viral contaminants on a global scale should be an urgent priority.

## Declaration of Competing Interest

The authors of this manuscript declare that there is no conflict of interest.

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