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# Novel wastewater surveillance strategy for early detection of coronavirus disease 2019 hotspots

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**Abstract**

The novel coronavirus disease 2019, a pandemic of global concern, caused by the novel severe acute respiratory syndrome coronavirus 2 has severely revealed the need for public monitoring and efficient screening techniques. Despite the various advancements made in the medical and research field, containment of this virus has proven to be difficult on several levels. As such, it is a necessary requirement to identify possible hotspots in the early stages of any disease. Based on previous studies carried out on coronaviruses, there is a high likelihood that severe acute respiratory syndrome coronavirus 2 may also survive in wastewater. Hence, we propose the use of nanofiber filters as a wastewater pretreatment routine and upgradation of existing wastewater evaluation and treatment systems to serve as a beneficial surveillance tool.

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COVID-19, Wastewater treatment, Nanofiber, Monitoring tool, Biofilm, Surveillance.

## Coronavirus in wastewater

Coronaviruses, named as such due to their crown-like spikes, are enveloped single-stranded, positive-sense RNA genomes that encompass a genome size of 26–32 kb in length belonging to the family *Coronaviridae* [1]. The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first reported in December 2019, is identified as a zoonotic virus, similar to its counterparts, severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus [2]. SARS-CoV-2 is the underlying cause of the global pandemic, novel coronavirus disease 2019 (COVID-19). The spread of infection of this pandemic has reached a whopping 41,79,479 reported cases, with 2,87,525 deaths as per the World Health Organization on May 14, 2020. The infection has spread to around 215 countries and territories with the highest number of cases reported in the USA, followed by Spain, Russia and the United Kingdom.

Although coronavirus infection is primarily believed to occur by inhaling or coming in contact with aerosols [3], viral RNA has also been detected in the stools of infected individuals with severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) [4] infection. There are reports of the presence of SARS-CoV-2 virus in the stool of infected individuals in the Netherlands, USA, France and Australia [5–9]. Another study by Xu et al [10] showed potential evidence of the faecal–oral transmission of severe acute respiratory syndrome coronavirus, and hence concerns have been raised regarding the spread of this virus through the water system. Water treatment systems play a significant role in public health protection. Treated wastewater has a wide range of uses from drinking to recreational purposes such as a spa or an open pool for irrigation and food production [11]. With the potential for being part of the environmental transmission, a significant concern regarding the safety of drinking water, wastewater sanitation and the sewages was raised. In addition, the role of biofilms has also been questioned because it is possible for slimy bacteria that form a thin coat over the pipelines to aid in the spread of the virus [12]. This article discusses the current water management system and the benefits of installing a monitoring tool in wastewater treatment facilities.

## Current wastewater treatment systems

Impure water serves as a favourable habitat for the growth of a range of microbiota, including bacteria such as *Escherichia coli*, faecal streptococci and *Clostridium perfringens*, pathogenic protozoans such as *Cryptosporidium* and *Giardia* and an array of viruses and microalgae [13]. These pathogens, when left unchecked, cause havoc in the ecosystem.

The present wastewater treatment systems include three grades of water processing. The first grade, known as primary, separates organic and inorganic solids, the secondary grade removes suspended, dispersed and dissolved solids and finally, the tertiary grade treatment specializes in improving the water quality by using biological, physical or chemical approaches. Commonly exercised treatment systems include coagulation, flocculation, precipitation, ion-exchange, adsorption and membrane filtration techniques such as ultrafiltration, reverse osmosis and nanofiltration (Table 1) [14,15]. Among the aforementioned techniques, chemical and autoflocculation treatments have proved as reliable methods for microorganism elimination.

Water treatment processes that use membrane bioreactors and beneficial microorganisms are believed to be capable of effectively removing viruses and bacteria in both drinking water and treated wastewater. But the same is not valid for untreated wastewater [16]. A study using SARS and poliovirus showed that the survival of the viruses decreased drastically when factors such as temperature, organic matter, light exposure and aerobic microorganisms were unfavourable for the viruses under study. The use of detergents, solvents and protozoa is also capable of inactivating viruses [17]. For the virus to infect a person through water, it is necessary to survive through the treatment process and retain its infectivity before coming in contact with a host, which is very unlikely [18]. Studies have shown that the rate of inactivation of SARS is faster than that of poliovirus, which indicates that enveloped viruses are unstable, and the

**Table 1**

**Water treatment methods. The table depicts the various possible techniques available for use in the wastewater treatment process and their impact on microorganisms. BOD, Biological Oxygen Demand.**

| Treatments | Action | Pathogen level |
|---|---|---|
| **Pretreatment stage** | | |
| Nanofibre membrane | Capture of high-risk microorganisms for screening | High |
| **Primary stage** | | |
| Sedimentation | Suspended solids removed | High |
| **Secondary stage** | | |
| Aeration | BOD reduction<br>Pathogen removal | Medium |
| Filtration | Nutrient management | Medium |
| **Tertiary stage** | | |
| Coagulation | Pathogen removal | Low |
| Flocculation | Pathogen removal | Low |
| Precipitation | Pathogen removal | Low |
| Ion exchange | Pathogen removal | Low |
| Adsorption | Pathogen removal | Low |
| Membrane filtration | Pathogen removal | Low |
| Electrochemical | Pathogen removal | Low |
| Chlorination | Pathogen removal | Low |
| Ozonation | Pathogen removal | Low |
| Ultraviolet radiation | Pathogen removal | Low |

mode of transmission through wastewater is unlikely [17].

## Influence of wastewater effluent

The method of treatment is chiefly determined by the range of effluents contaminating the water. Wastewater contaminants comprise a range of substances discharged from industrial environments. Eliminating these contaminants has proven to be challenging, and researchers have proposed various alternatives to traditional treatment methods. A few suggestions for upgrading the purification process are pretreatment by nanofiber filtration, electrocoagulation, photocatalysis and the use of specific plant species such as *Eucalyptus camaldulensis* and *Arundo donax*. Pretreatment methods such as wet oxidation, microwaves and ultrasonic waves can also be implemented before the effluent enters the treatment routine. Other proposed treatments include the use of vertical membrane bioreactors comprising anoxic and oxic zones, as well as the horizontal and vertical flow used in wetlands and stabilization ponds [19–21].

The effluents also contain several microorganisms affecting the water source. The efficiency of the water plant in removing some related undesirable components should be assessed by various analyses [21]. An effective technique for analysis of microfauna in wastewater is the use of polymeric alginate beads to immobilize microalgae such as *Chlorella sorokiniana* and symbiotic bacteria such as *Azospirillum brasilense*. The surfaces of these submerged beads on examination by fluorescence in situ hybridization and scanning electron microscopy indicated the possibility of biofilm formation and growth [22].

## Persistence of water-borne microorganisms in biofilms

Human T-cell leukaemia virus type 1 serves as an ideal model for the study of viral biofilm formation and interaction with the surrounding bacterial environment. These viral communities display characteristics similar to a bacterial biofilm and exhibit a rare propagation mechanism by forming a carbohydrate-rich scaffold that aids in an attachment to the T-cell surface and encapsulation of the virion. The infected cells within this matrix provide a compatible surface for increased viral adhesion, cohesiveness and dispersion. This process proves that specific viruses have a certain range of control over the extracellular matrix through biofilm formation [23]. Pais-Correia et al. [24] further explained that various virus-induced components such as collagen and agrin govern the formation of a biofilm and other linker proteins, including tetherin and galectin-3, and facilitate cell-to-cell interactions. A striking difference in the propagation was observed in both microorganisms isolated from free-flowing, as well as stagnant wastewater and those thriving in a biofilm. Research indicates that the virus persists longer in the biofilm than in wastewater [24,25].

## SARS-CoV-2 in wastewater

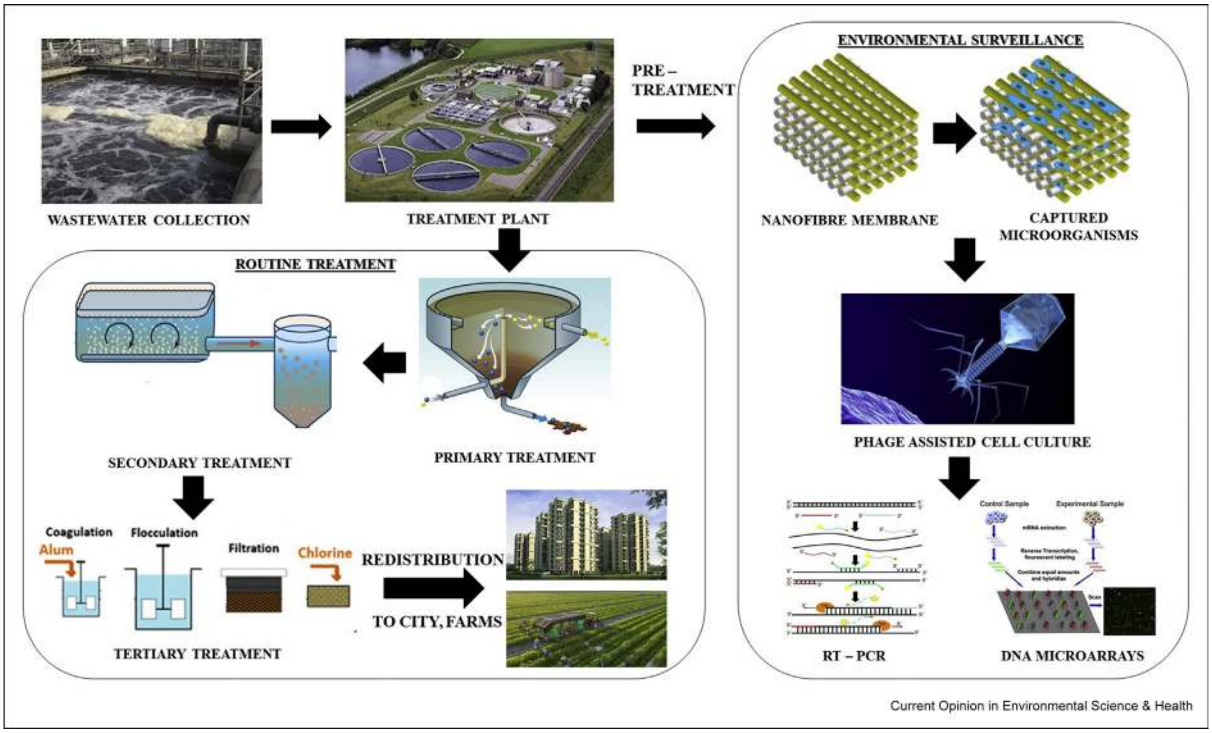
Studies on previous coronaviruses, namely SARS and MERS, have shown that these single-stranded RNAs are less resistant and more fragile to water treatment processes than other kinds of viruses. La Rosa et al. [27] discuss about the presence of coronaviruses in the water environment and their abundance in the influent [26]. Because very few studies are carried out on the novel SARS-CoV-2, no conclusive results can be obtained regarding the effectiveness of these treatment processes [28,29]. To date, due to the lack of evidence, the World Health Organization has deemed it necessary to use only the usual preventive protection equipment for individuals working in wastewater sanitation facilities.

To curtail the quick spread of COVID-19 and to estimate the affected populations, wastewater samples (treated and untreated) from a few regions in the Netherlands were taken after the first case of infectious disease was reported [30]. The virus can be grown with the help of bacteriophages in an appropriate cell culture media for propagation. The RNA isolated from them was then tested for SARS-CoV-2 activity using a real-time Reverse transcription-polymerase chain reaction (RT–PCR) assay [31,32]. The untreated samples tested positive, whereas the treated samples showed the presence of viral RNA, although it is unclear if the virus retained their infectious properties or faecal–oral transmission after the routine treatment [30].

## Environmental surveillance

Analysis of wastewater treatment systems serves as an invaluable source for the study of bacterial and viral community interactions (Figure 1). Because it is nearly impossible to test every individual during a community spread of the COVID-19 pandemic, it is necessary to locate the hotspots of the disease and begin isolation and treatment from there. Environmental surveillance is a tool used to monitor the extent and duration of the spread of the virus in specific populations. It can give a measure of contaminants and also warn us of possible threats emerging in that particular confinement [33]. Monitoring tools have already been successfully implemented for viruses such as poliovirus and Aichi virus [30,31], which have aided in strategies that resulted in their elimination. The efficiency of a monitoring tool depends on various factors such as geographical location, general sanitary, climatic conditions, sampling methods such as trap sampling, precipitation methods, charge-based filters and detection methods [34]. A significant shortcoming of wastewater screening techniques is the inability to recognize individuals from the affected area [35,36].

**Figure 1**




**An illustration of an upgraded wastewater treatment facility**. The figure shows the entire upgraded wastewater treatment routine along with the pretreatment process necessary for environmental surveillance.

This technique is in line with the wastewater-based epidemiology (WBE) concept. WBE is a reliable surveillance model for identifying global hotspots of COVID-19 [37–40]. It was widely used for monitoring polio and hepatitis A [36]. It will be easier to survey regions for viral infections, especially in the asymptomatic cases of COVID-19, comprehensively and in real time [9,41]. Another advantage of wastewater monitoring is the possibility to detect variations of viral strains through phylogenetic studies [9]. Several studies have used WBE for the detection of SARS-CoV-2 with no significant outcomes [30,38,42,43]. There are approximately 105,600 wastewater treatment facilities globally. By using these facilities for surveillance, it is possible to monitor the health profile of billions of individuals [36,44,45].

Although there is no concrete evidence of COVID-19 transmission through water systems, it is essential to study the survival rate of SARS-CoV-2 in surface wastewater and sewage water as a precaution. Because there are several cases of asymptomatic infections of COVID-19, it will be useful to identify the populations with these carriers to prevent further spread of this virus. The study by Gundy et al. [17] showed that SARS survives in wastewater for a limited time. The number of viruses present in the water is proportional to the number of infected individuals in the area. By constantly monitoring the levels of these viruses, it is possible to determine the spread of the COVID-19 infections. This tool will serve as an efficient and sensitive monitoring tool to measure virus levels in hotspot populations and provide early warning signs before a potential epidemic in the future. A major drawback of this tool is the lack of detection of enveloped viruses during the treatment process, the need for special laboratory skills and sustainable financial resources. It will be invaluable to develop direct detection methods such as enzyme-linked immunosorbent assay (ELISAs), RT–PCR, digital RT–PCR, multiplex PCR, cDNA microarrays, isothermal nucleic acid amplification-based methods or the newly discovered paper-based device for coronaviruses in these wastewater treatment facilities [46–48]. Another aspect to be noted is sensitivity. The tools should be sensitive enough to capture even the smallest amount of viral infection during the initial stages to prevent the spread of viral particles.

## Nanofiber as a monitoring tool

One of the most effective monitoring tools to date is the use of electrospun nanofiber membranes to screen disease-causing pathogens. These nanofibers are effective in isolating pathogens using a polyurethane nanofiber material (147 nm), which is active in filtering intestinal enterococci, *E. coli* and coliform bacteria [49]. Characterization of the nanofiber membranes could be achieved by Fourier transform infrared spectrometers with attenuated total reflectance, scanning electron microscopy, surface area analysis and contact angle measurements. The bioengineering of these nanofibers with specific binding sites increases the specificity towards its target, in this case, SARS-CoV-2 [49]. This pretreatment technique has proven effective in filtration. These flat sheet membranes are electrospun with a range of functionalizing agents such as nanosilver, bronopol and WSCP (Poly[(dimethylimino)(2-hydroxy-1,3-propanedily) chloride] based on the required pore size and colony-forming units of the microorganism. Although there is a high risk of losing functionality during leaching, the microorganism removal capacity of these nanofibers was shown to be preserved [50]. Hence, we propose that a custom-made, removable, electrospun nanofiber membrane with a positive charge could be effective in attracting the genetic material of a virus, making it a potential monitoring tool. This membrane, when lysed using standard protocols [50], would give a higher yield of the genetic material when compared with isolation from a crude sample.

## Conclusion

As the current water treatment routine has not been confirmed to remove SARS-CoV-2, it is necessary to take extra steps to ensure the complete removal of these viruses from water. Providing additional treatments to the wastewater before it enters the treatment routine, thus upgrading the existing process, is a well-conceived notion. Home-made treatments such as boiling and large-scale processes such as ultrafiltration, inactivation by ultraviolet irradiation and chlorination are promising tactics that can be used to upgrade the treatment, especially in pandemic hotspots. An investigation of the spread of viruses through biofilms also needs to be better prepared for future situations. Once the countermeasures of social distancing are uplifted and people return to their routine lifestyle, there is a high probability of the reemergence of this virus. Hence, facilities such as a monitoring tool for increased scrutiny of wastewater treatment industries must be enforced to minimize public health risks. An efficient monitoring tool can serve as an alarm when there is an increase in virus particles above the threshold levels, thus allowing us to act faster and contain the infection before it spreads at an alarming rate.

## Author contributions

B.V. and A.V. contributed to conceptualization and study design; A.V. and H.G. contributed to investigation, resources and original manuscript writing; S.S.S.R., V.G., M.A., A.N., P.S. and P.K.S.M.R. contributed to manuscript review and editing and V.G. and B.V. contributed to the final approval of the study.

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## Conflict of interest statement

Nothing declared.

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