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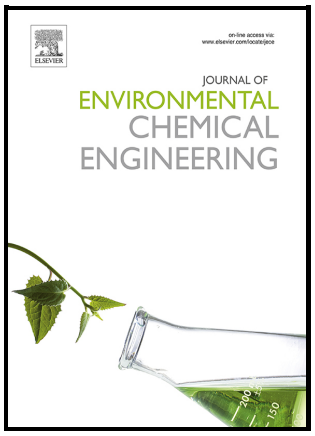

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Recent Advances in the Biosensors Application for the Detection of Bacteria and Viruses in Wastewater

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Abstract

The presence of disease-causing pathogens in wastewater can provide an excellent diagnostic tool for infectious diseases. Biosensors are far superior to conventional methods used for regular infection screening and surveillance testing. They are rapid, sensitive, inexpensive portable and carry no risk of exposure in their detection schemes. In this context, this review summarizes the most recently developed biosensors for the detection of bacteria and viruses in wastewater. The review also provides information on the new detection methods aimed at screening for SARS-

CoV-2, which has now caused more than 4 million deaths. In addition, the review highlights the potential behind on-line and real-time detection of pathogens in wastewater pipelines. Most of the biosensors reported were not targeted to wastewater samples due to the complexity of the matrix. However, this review highlights on the performance factors of recently developed biosensors and discusses the importance of nanotechnology in amplifying the output signals, which in turn increases the accuracy and reliability of biosensors. Current research on the applicability of biosensors in wastewater promises a dramatic change to the conventional approach in the field of medical screening.

Keywords: Wastewater-based epidemiology; Biosensing; On-site; Real-time; Nanotechnology.

1. Introduction

Water scarcity has continued to grow into a major challenge over the past several decades as a result of the increasing demand caused by population growth and industrial development. While many areas around the world suffer due to insufficient freshwater bodies and are relying on alternative water resources such as desalination, the quality of freshwater is deteriorating [1]. In addition to population growth, habitat encroachment, international travel, and globalization have led to the emergence of new pathogens that could pose a threat to general health alarming [2]. Water pollution has led to an increase in contaminants such as heavy metals, organic material, and microorganisms in water. Monitoring and detection protocols are necessary to select appropriate treatment processes before water is discharged into the environment or re-utilized [1]. Furthermore, they are also a crucial part of wastewater-based epidemiology (WBE) and are used to provide data on a community level. WBE is a relatively new approach that measures the presence and quantity of pollutants and biomarkers in wastewater and is in constant need of development and research due to the deterioration of water quality. Conventional detection methods, on the other hand, generally identify pathogens based on specific constituents and are often used to provide data at the individual level. Despite the many modifications introduced to conventional methods over the years, each belongs to one of the three categories, quantitative polymerase chain reaction (qPCR), culture-based methods, and immunology-based methods. These conventional analytical tools are known to have high sensitivity, selectivity, and stability;

however, their high cost and laboratory requirements could limit their broad applications, especially in jurisdictions with limited resources [3].

Infection control could greatly benefit from the rapid detection of pathogens in wastewater. Pathogen-causing infectious diseases spread through different routes, making newly emerging pathogens, such as the SARS-CoV-2 virus, difficult to control. Recent studies have demonstrated that the virus could be detected by qPCR in the stool of infected individuals [4]. This makes WBE a viable method to track the COVID-19 pandemic. Currently, qPCR continues to be an efficient method for COVID-19 testing, although it has the risk of exposing individuals conducting the tests to the virus. While control measures such as social distancing and isolation would probably suppress the current pandemic, the outbreak of this disease has already exceeded SARS and is expected to return in several waves of infections. The most effective way to detect such infectious diseases is by mass testing and ensuring proper isolation and treatment. The integration of biosensors in wastewater systems could provide mass testing and ensure proper isolation and treatment, to a much higher degree than conventional detection methods.

Wastewater treatment plants are often investigated for their performance in the elimination of pathogens. This is usually done by monitoring biological constituents in effluent streams using conventional detection methods. The introduction of biosensors proved to offer several advantages as compared to conventional methods because they are rapid, easy to use, and portable devices. The installation of pathogenic biosensors in wastewater pipelines could provide real-time data and online detection of pathogens. In turn, early warnings of outbreaks of infectious disease outbreaks can be obtained to protect the population from future threats to public health. Simultaneously, the use of biosensors within wastewater treatment plants could automate the modification of certain control parameters. For example, if a high concentration of a certain pathogen is detected in an effluent stream, the process could be designed to automatically adjust the dose of disinfectant used. In addition to that, miniaturization technologies could be applied in biosensor designs. Hence, biosensors can be designed in a cost-effective way, facilitating their commercialization and real-life applications.

This review presents recent research publications on recently developed biosensors for the detection of bacteria and viruses in wastewater. A thorough search process was conducted to identify recently published research articles. The process used two search engines, Google Scholar and Science Direct. The search terms used were (sensor OR aptasensor OR biosensor OR immunosensor) AND (bacteria OR virus OR RNA OR DNA OR antibody OR antigen) AND (detection OR identification OR recognition) AND (electrochemical OR optical OR thermal OR fluorescence). Appropriate adjustments were made to suit different search engines. In total, approximately 45 articles were identified and reviewed. For consistency, all the values reported in this review were converted to equivalent units.

2. Biosensors for the detection of pathogens

Biosensors are defined as chemical sensors that use biochemical reactions as a recognition element. They are often made up of two main components, the biorecognition element and the transducer. The biorecognition element is the biological receptor, which could be antibodies, enzymes, microorganisms, genetic material (DNA, RNA), or cells, while the transducer detects changes in sensor response (optical, thermal, or electrochemical) after binding of biological elements to receptors and converts them to an electrical signal [5]. Biosensors were first introduced by Clark and Lyons for the measurement of glucose levels in 1962 [6]. They have then gained the interest of researchers and have been developed to become fast, sensitive, low-cost, and portable analytical devices. As a result, they have been proven to successfully quantify the concentrations of certain drugs, biomolecules, and microorganisms in wastewater [7]–[9].

Biosensors have previously been developed for the detection of biomarkers in wastewater, such as inorganic ions, organic pollutants, pharmaceuticals, and pathogens. Inorganic ions are often present in extremely low concentrations in wastewater, which is why further studies and verification are required before inorganic sensors become more commonly used. On the other hand, biosensors for organic pollutants and pharmaceuticals have been widely explored with sensitivities that are more appropriate than those of inorganic biosensors [3]. Presently, there is a growing number of studies on the development of biosensors for the detection of pathogens [3], [10]. At the same time, research on biosensors for the detection of pathogens in wastewater is still currently not mature enough, suggesting the need for further

research. A vital element in these biosensors is the biological receptor. Whether target molecules are human nucleic acids, peptides, proteins, or markers of antimicrobial resistance, the biological receptor is a critical component that determines the selectivity and limit of detection (LOD) of a biosensor [3]. The biological element could be an antibody, enzyme, cell, microorganism, or nucleic acid aptamers. In general, optimizing a biosensor requires the selection of a biological element which interacts with the target analyte from a given sample, whilst providing rapid and reliable output. Based on research, it is found that nucleic acid aptamers exhibit the highest affinity towards target molecules, despite their cost and detection time. However, antibodies remain the gold-standard biological elements due to their high selectivity, affinity, and regeneration for various pathogens [11]. The most reported biological receptors are aptamers, antibodies, enzymes, and microorganisms. **Figure 1** illustrates the working principle of biosensors.

It is important to consider the accuracy of the biosensor detection of pathogens in wastewater because of the presence of a complex wastewater matrix and, therefore, it is of significance to optimize the fabrication parameters to enhance the biosensor response. Nanomaterials are often used to enhance the sensitivity of biosensors, especially when the applicability of biosensors is extended to real samples. When nanomaterials are introduced into the field of biosensors, it is important to consider their affinity for biological receptors. As an example, carbon nanotubes (CNTs), which have been frequently used for their exceptional electrical properties in biosensors, do not show affinity for biological receptors [12]. Therefore, the incorporation of linking molecules to immobilize receptors on the surface of nanomaterial-based biosensors is crucial. As with most biosensors, once nanomaterials are functionalized with bioreceptors such as enzymes or antibodies, a biochemical reaction would occur upon binding to targeted biological molecules or proteins. Such reactions cause electrical shifts in the given medium, which is a sensing indication. The electrical properties of nanomaterials play a significant role in the strength of the generated electrical shifts. In turn, the introduction of nanomaterials into the biosensing field has paved the way for the design of more sensitive biosensors. The exploitation of such unique properties has driven nanomaterial-based biosensors to compete or even surpass conventional detection methods. The subsequent sections review recently developed biosensors for the detection of bacteria and viruses with a specific focus on the newly emerging SARS-CoV-2.

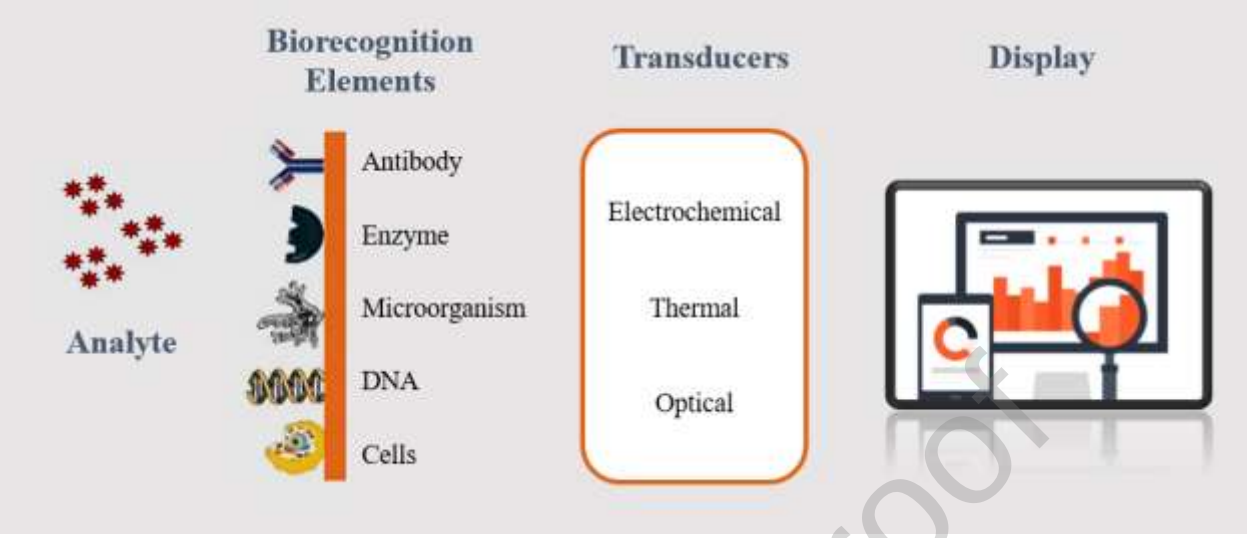

Figure 1. Illustration of the working principle of biosensors.

2.1. Bacteria biosensors

Wastewater environments contain a wide range of pathogens, with bacteria being the most dominant by mass. While most bacteria are harmless, some have been shown to cause infections such as diarrhea, dysentery, skin, and tissue infections. According to Stevik et al. (2004), the most important pathogenic bacteria are *Salmonella* sp., *Shigella* sp., *Vibrio cholerae*, *Yersinia enterocolitica, Y. pseudotuberculosis, Leptospira* sp., *Francisella tularensis, Dyspepsia coli,* enterotoxine producing *Escherichia coli* and *Pseudomonas* [13]. Therefore, several treatment and detection mechanisms have been designed and proposed to improve the efficiency of existing wastewater treatment plants (WWTPs). While qPCR, cell culture and colony counting, and immunology-based methods are reliable and accurate, they are often expensive and time-consuming. Therefore, biosensors have been proposed for the early detection of bacteria, with several developments leading to their enhanced sensitivity. A summary of all studies reporting biosensors for the detection of bacteria is presented in **Table 1**.

E. coli is a long-known dangerous foodborne disease-causing bacterial species and has been repeatedly used as a standard indicator of coliforms in water [14]. Using conventional techniques, the detection of *E. coli* serotypes is expected to take 2-3 days, delaying corrective measures. In previous years, efforts have been aimed at developing biosensors, being rapid tools,

for the detection of *E. coli* serotypes, with the resulting limits of detection in the range of 10^2 to 10^3 CFU/mL [15]–[17]. More recently, an electrochemical biosensor for the detection of *E. coli* O157 via carbon screen printed electrodes (SPEs) was developed [18]. Through the utilization of gold nanoparticles to modify the SPEs, the biosensor gained stability and effectiveness. The biorecognition elements in this bacterial sensor were anti-*E. coli* O157 antibodies, which were immobilized on the materialized surface to make the sensor specific for *E. coli* O157. The developed biosensor was tested through electrochemical impedance spectroscopy to determine its electrochemical properties in the presence of the target molecule. It was found that the device was able to detect concentrations as low as 15 CFU/mL of *E. coli* O157 in 30 minutes. Another study also reported the fabrication of an electrochemical biosensor for the detection of *E. coli* strain MG1655 in water, in the absence of a biorecognition element [14]. The biosensor detection principle was based on the reaction of *E. coli* strain MG1655 with a locally formed catalyst. Although this method could detect *E. coli* strain MG1655 in under half a second, the quantification step took around 10 minutes. The novelty behind this method relied on the biosensor's ability to detect the presence of target molecules without the requirement of a biorecognition element and the optimization of its immobilization on the sensor surface.

In addition to electrochemical biosensors, other studies have used different transduction methods in the detection of *E. coli* serotypes. For example, reduced graphene oxide (GO) was used in the fabrication of a bacterial chemiresistor biosensor [19]. In this study, M13 phage was used to modify the sensor material on the sensor and make it selective towards F-pili of *E. coli* strains such as XL1-blue. SEM, XRD, FTIR, and AFM results were used to provide morphological and spectroscopic characterizations of GO and rGO. Electrical characterization confirmed the sensitivity and precision of the developed chemiresistor biosensor with an LOD value of 45 CFU/mL which was in line with reported literature [20]. Selectivity was also confirmed by analyzing the sensor response to *Pseudomonas chlororaphis* strain. Furthermore, the detection of *E. coli* O157:H7 pathogenic bacteria was reported by Petrovszki et al. (2021) using an integrated electro-optical biosensor [21]. An integrated microsystem that consists microfluidic channels and dielectrophoretic surface electrodes along with a rib waveguide was used to create a label-free sensing platform for the detection of *E. coli* O157:H7. The principle of sensing is based on analyzing the light scattering in the presence of targeted molecules in the vicinity of the waveguide. Quantification of *E. coli* O157:H7 was also possible with a LOD of

10^2 CFU/mL, reached within 10 minutes. Compared to other research articles reviewed in this work, the *E. coli* O157:H7 biosensor developed by Petrovszki et al. (2021) showed less sensitivity, demonstrated by its LOD of 10^2 CFU/mL [21]. However, with the reported LOD value, the biosensor can detect *E. coli* O157:H7 at concentrations lower than the infection dose. A fully optical transducer system for the detection of E. coli was studied by Janik et al. (2021) [22]. The developed optical fiber device utilized a sensing mechanism based on microcavity in-line Mach-Zehnder interferometer. With that, and the use of low molecular weight peptide aptamers, detection capability has been reported. The biosensors detected *E. coli* O157:H7 at low concentrations of around 10 CFU/mL. In comparison with other reported optical-based biosensors for the detection of the same target, Janik et al. (2021) achieved the lowest LOD with their optical fiber sensor.

In addition, a study aimed at the development of a fluorescent biosensor for the detection of two common bacteria, *E. coli* O157:H7 and *Salmonella Typhimurium* [23]. The fabrication of this biosensor did not require the complex immobilization of biorecognition elements on the sensor surface. The use of a fluorescent-labeled aptasensor was sufficient to recognize targeted molecules, with the fiber nanotube and nanoporous layer utilized as transducer. With this design, the authors reported LOD values of 340 and 180 CFU/mL for *E. coli* O157:H7 and *S. Typhimurium*, respectively, with a quantification element achieved in less than 35 minutes. Sheini (2021), too, developed a fluorescent biosensor for the detection of four bacteria strains, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* [24]. Being the main cause of sepsis in children, Sheini expanded the testing scope of her reported biosensor to diagnose septicemia in children. The paper-based device was composed of hydrophilic zones and hydrophobic barriers divided into six gold and copper nanoclusters. Detection was confirmed within 0.25 minutes via fluorescence emission under UV light, with the use of a smartphone. Through the introduction of serum samples, the biosensor was found to have a limit of detection of 43, 63.5, 26, and 47 for *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa*, respectively. In contrast, Maria et al. (2021) used polyclonal anti-*Salmonella* antibodies in the fabrication of an immunosensor for the detection of *Salmonella enterica* serovar Typhimurium, a predominant causative-agent of foodborne diseases [25]. A carboxymethylated cashew gum film was deposited on a gold sensor surface and functionalized with antibodies. Electrical measurements were performed on the

developed design, yielding an LOD of 10 CFU/mL in 125 minutes. The detection was successful in contaminated whole and skim milk samples. Before the introduction of biosensors, the duration of rapid tests for the detection of the Salmonella pathogen included an incubation time of around 24 hours, causing delay in corrective action. This enrichment step was crucial to increase the bacterial count and meet the LOD of rapid tests, which was in the range of 10^3–10^5 CFU/mL for *Salmonella*. Biosensors, on the other hand, provide much higher sensitivities, with the most recent LODs being in the range of 10–10^2 CFU/mL [23], [25].

The presence of other types of bacteria was also investigated using electrochemical biosensors. Song et al. (2021) reported a biosensor design for the detection of *Helicobacter pylori* (*H. pylori*) [26]. The sensor was fabricated to detect the targeted DNA of the *H. pylori* genome. That was done by immobilizing hairpin DNA, which are specific to the targeted molecule on the sensor surface. The novel assay strategy was also based on the linear isothermal amplification reaction, which enhances the sensitivity, selectivity, and repeatability of the biosensor. The designed sensor was found to be applicable to DNA sequences from other pathogens. In addition, the electrochemical sensor reported excellent selectivity against muted DNA and other pathogens with an LOD of 1.3 pg. In another study by Cai et al. (2021), a triple-helix molecular switch was used to design an electrochemical biosensor for the detection of *Staphylococcus aureus* (*S. aureus*) [27]. 5'-biotinylated aptamers were used to bind to the target when present in analytes such as lake water, tap water, or honey samples. Conventionally, traditional culture, instrument detection, immunological detection, and molecular biological detection were used for the detection of *S. aureus.* Their use is considered problematic due to time consumption, high cost, professional operation requirement, and inaccuracy. Previously reported biosensors for the detection of *S. aureus* had LODs between 5–300 CFU/mL. The high sensitivity, specificity, and versatility of the biosensor fabricated by Cai et al. (2021) were the result of the combination of the chosen aptamer and the triple helix molecular switch [27]. An LOD of 8 CFU/mL was reported, after deoxygenating the surface with nitrogen prior to testing, to avoid any interference with the results. The resulting performance was highly comparable with that of previously reported biosensors. Wang et al. (2021) have used molecularly imprinted polymers as biorecognition elements [28]. They have reported a biosensor for the detection of *S. aureus* using molecularly imprinted polymers. A prepared bacteria-imprinted conductive poly (3-thiopheneacetic acid) film was deposited on gold electrodes. The structure and performance of

the biosensor was characterized by microscopy and electrical measurements, and the results suggested an LOD of 2 CFU/mL within a response time of 10 minutes [28]. The sensitivity of this design was the highest compared to previously reported *S. aureus* biosensors. It is also worth mentioning that this design, unlike other designs, omitted the drawbacks of using cross-linkers and organic solvents.

Moreover, Hou et al. (2021) focused on photoelectrochemical (PEC) biosensors as a newly emerging detection technique that offers several advantages such as low cost, low noise, simplicity, high sensitivity and accuracy, compared to traditional techniques [29]. A photoelectrochemical biosensor was fabricated with *Vibrio parahaemolyticus* aptamers as biorecognition elements for the detection of *Vibrio parahaemolyticus*. The sensor was fabricated using a layer-by-layer assembly method and optimized to produce the best photocurrent response. An LOD of 40 CFU/mL was documented using this design, being the lowest reported value so far. The reproducibility and sensitivity of the sensor make it a promising candidate for the detection of other pathogenic bacteria in food [29]. The differentiation between various strains of *S. aureus* was studied by Jia et al. (2021) [30]. An optical biosensor was manufactured and proven to differentiate between the *S. aureus* strain RN4220, the *S. aureus* strain N315, the *E. coli* strain DH5α, and the *E. coli* strain E32511. Each of these strains has different compositions of α-glucosidase, β-galactosidase and β-glucuronidase. Functionalized biosensors with enzymatic substrates reported a rapid distinction with LOD values in the range of 0.2 to 4.5 nM and a response time of 60 minutes.

According to Cui and Liang (2021), wastewater contamination has a huge impact on the rise of foodborne pathogens, including bacteria [31]. Therefore, the detection of bacteria in food is correlated with the root cause of water contamination. This also explains why researchers have long been interested in the development of biosensors for the detection of foodborne bacteria. *S. Typhimurium* is one of the most common foodborne pathogens, with the potential to cause several symptoms post-infection. Huang et al. (2021) developed a method for the detection of *Salmonella* in synthetic samples [32]. A rotary magnetic separation technique was operated by a stepper motor and magnetic nanoparticles (MNPs). The authors introduced *Salmonella* polyclonal antibodies to MNPs to make the biosensor specific to *Salmonella* molecules. A capillary tube was used to inject the targeted bacteria and allows its interaction with the

biosensor. In 120 minutes, the biosensor could detect as little as 10 CFU/mL of *Salmonella*, with a linear range of 10 to 10^6 CFU/mL. Jiang et al. (2021) also developed a biosensor for the detection of *Salmonella enteritis*, using molecularly imprinted polymers as recognition units instead of biological elements [33]. The reported sensor was an electrochemical one with a polyethylene terephthalate (PET) chip, driving liquid flow based on siphonage and hydrophilicity. Differential pulse voltammetry was used to interpret the detection of Salmonella. A limit of detection of 100 CFU/mL and a linear range of 3×10^2 - 3×10^7 were reported. Interfering molecules were also introduced to examine the selectivity of the biosensor. Bacteria used were E. coli, L. monocytogenes, and P. aeruginosa, and selectivity was verified by interpreting peak current values for different bacteria. The biosensor was also successfully tested on real samples to assess the effect of matrix complexity on its performance [33].

Table 1. Studies on biosensors developed for the detection of bacteria.

a: pg, b: nM, c: cells/mL.

Biosensor type	**Biorecognition element**	**Target**	**LOD (CFU/mL)**	**linear range (CFU/mL)**	**Response time (minutes)**	**Reference**
Electrochemical	Anti-*Escherichia coli* O157 antibody	*Escherichia coli* O157	15	10 - 10^6	30	[18]
Electrochemical	Hairpin DNA containing Md-Dz substrate & G-quadruplex DNAzyme (Gq-Dz),	*Helicobacter pylori* genomic DNA	1.3 [a]	2.1 - 67.2 [a]	-	[26]

Electrochemical	5'-biotinylated aptamers	*Staphylococcus aureus*	8	30 - 3×10^8	-	[27]
Electro-optical	-	*Escherichia coli* O157:H7	10^2	-	10	[21]
Photo-electrochemical	*Vibrio parahaemolyticus* aptamer	*Vibrio parahaemolyticus*	40	3.2×10^2 - 3.2×10^8	-	[29]
Optical	4-Methylumbelliferyl α-D-glucopyranoside (MUD), 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and 4-Nitrophenyl-β-D-glucuronide (PNPG) enzyme substrates	*Staphylococcus aureus* strain RN4220 *Escherichia coli* (EHEC) strain E32511 *Escherichia coli* strain DH5α	0.2 [b] 3.4 [b] 4.5 [b]	-	60	[30]
Chemiresistor	M13 phage	F-pili containing *Escherichia coli* strains	45	10^2 - 10^7	-	[19]

Electrochemical immunosensor	Polyclonal anti-*Salmonella* antibodies	*Salmonella* enterica serovar Typhimurium	10	$10 - 10^{5}$	125	[25]
Electrochemical	Probe single strand DNA	Haemophilus Influenzae	10^{-10} [b]	10^{-10} - 10^{-5} [b]	-	[37]
Electrochemical	Molecularly imprinted polymers	*Staphylococcus aureus*	2	$10 - 10^{8}$	10	[28]
Fluorescence aptasensor	Aptasensor Cy3-apt-E & Cy5.5-apt-S	*Escherichia coli* O157:H7 *Salmonella Typhimurium*	340 180	-	35	[23]
Electrochemical impedance spectroscopy	Vancomycin	*Staphylococcus aureus*	<39	-	-	[38]
Electrochemical	Anti-*Salmonella* polyclonal antibodies	*Salmonella Typhimurium*	10	$10 - 10^{6}$	120	[32]
Electrochemical	DNA	*Vibrio cholerae*	7.41×10^{-21} [b]	$10^{2} - 10^{-5}$ [b] and 10^{-5} -10^{-18} [b]	-	[34]
Immunoelectrochemical	Anti-*Escherichia coli*	*Escherichia coli* O157:H7	400 [c]	-	180	[35]

	immunoglobulin G (IgG)					
Electrochemical	Functional DNA aptamer	*Escherichia coli* O157:H7	19	$10 - 10^6$	60	[39]
Optical	Nanozyme	*Salmonella Typhimurium*	100	$10^4 - 10^6$	50	[40]
Fluorescence	-	*Staphylococcus aureus*	43	50 - 1×10^8	0.25	[24]
		Streptococcus pyogenes	63.5	70 - 1×10^8		
		Escherichia coli	26 47	30 - 1×10^8		
		Pseudomonas aeruginosa		50 - 1×10^8		
Electrochemical	Molecularly imprinted polymers	*Salmonella enteritis*	100	3×10^2 - 3×10^7	20	[33]
Optical	Peptide aptamers	*Escherichia coli* O157:H7	10	-	-	[22]
Fluorescence	DNAzyme	*Aeromonas hydrophila*	36	$0 - 10^3$	10	[36]
Colorimetric	CRISPR-Cas12a	*Salmonella*	1	$10^0 - 10^8$	-	[41]

Vibrio cholerae is another pathogen that is transmitted via food and water. Ali et al. (2021) reported an advanced DNA biosensor that could detect the target analyte in a complex sample such as poultry feces [34]. The gold nanocube and modified glassy carbon electrodes

were functionalized with a DNA carrier matrix as the biorecognition element. When the biosensor was electrically tested, an LOD of 7.41×10^{-21} nM was reported with two linear detection ranges of 10^{2}–10^{-5} and 10^{-5}–10^{-18} nM. Another study reported the development of an immunoelectrochemical biosensor for the detection of *E. coli* O157 in food [35]. The biosensor used a porous graphite felt electrode (GF) electrode that was coated with anti-*E. coli* immunoglobulin G (IgG). GF has been used in several electrochemical applications because of its good electrical conductivity, compressibility, cost, and mechanical flexibility. Most importantly, it generates a high current intensity, which leads to lower detection limits. Consequently, target detection in food samples was achieved with concentrations as low as 400 cells/mL. Moreover, Ma et al. (2021) investigated the use of DNAzymes as biorecognition elements for the detection of *Aeromonas hydrophila*, which is a highly pathogenic bacteria posing human health threats with their presence in food and the environment [36]. An in vitro selection process had been carried out to select the used DNAzyme which exhibited the highest activity. Fluorescent signals confirmed an LOD of 36 CFU/mL within a period of 10 minutes, and the stability of the biosensor was confirmed for a duration of at least six months.

2.2. Virus biosensors

Enteric waterborne viruses play a vital role in the transmission and spread of diseases. Wastewater presents a hostile environment for viruses, and hence its constant investigation for viral constituents. A main source of waterborne viruses is human fecal matter, as each infected person sheds between 10^{5} and 10^{12} viral particles per gram [4], [42], [43]. Therefore, the effectiveness of wastewater treatment plants is often investigated with respect to the elimination of viruses [44]–[46]. However, the uprising of highly infectious diseases, such as COVID-19, calls for the need to focus on rapid viral detection technologies. Therefore, the development of biosensors has gained the interest of researchers [3]. A major benefit and contribution that biosensors could provide is the on-line detection of viruses. This could enable early detection of preexisting life-threatening viruses in addition to newly emerging ones. A summary of all studies reporting biosensors for the detection of viruses is presented in **Table 2**.

Several efforts and models have already begun to study the feasibility of complete systems that utilize biosensors for the automated detection of viruses. Jain and Manocha (2021) presented a powerful technique for real-time virus monitoring and spread control [47]. This monitoring system detects changes in human body temperatures through thermal imaging systems embedded in smart wrist bands. All collected data are displayed on an application that is linked by the Global Positioning System (GPS) to the appropriate authorities. This technique also uses Internet of Things (IoT) based sanitization tanks to ensure that the spread of viruses is avoided. In some cases, such as universities, sanitization will automatically take place when the presence of viruses is detected. In other efforts to combat the ongoing transmission of infectious diseases such as COVID-19, Wang et al. (2021) have developed a method to prevent the further spread of SARS-CoV-2 [48]. In particular, the study focused on developing a numeric model based on the electromechanical response of piezoelectric fiber/epoxy matrix composites. The main objective of such a model was to optimize the biosensors. Some of the factors considered in the proposed model are the frequency, position, and size of the resonant biomarker.

Human immunodeficiency virus (HIV) has previously been declared a pandemic previously, and several efforts have been made to early diagnosis of HIV infected individuals. Compared to conventional diagnostic tests, point-of-care (PoC) devices have become a preferred option due to faster diagnostic capabilities and earlier treatment possibilities. Among these, Song et al. (2021) recently fabricated field effect transistors through rolled-up nanotechnology as microfluidic diagnostic biosensors [49]. The biorecognition element used was the HIV gp41 antigen to detect gp41 HIV antibodies. Upon introduction of HIV antibodies in serum samples, the biosensor showed an LOD of 2.5×10^{-3} nM, which holds great potential for the diagnosis of PoC. Unlike the detection of HIV antibodies, the p24 structural protein plays a greater role in the early detection of HIV in infected patients. This is due to the primary immune response that causes HIV virus to be surrounded by the p24 protein in the blood serum. The detection of p24 proteins is feasible in the early infection stages, enabling detection several days earlier than the detection of HIV antibodies. In this regard, Gogola et al. (2021) have reported the development of an aptasensor that is specific to the detection of HIV through p24 proteins [50]. In their work, graphene quantum dots were used to strengthen the amplification of an electrochemical signal and aid in the immobilization of the p24-HIV aptamer onto the device. The sensor was tested using solutions containing p24-HIV aptamers and was able to meet an LOD of 5.17×10^{-2} ng/mL.

Furthermore, it was reported that the developed aptasensor was successful in differentiating between positive and negative samples in spiked human serum [50]. Such high sensitivities presented upon testing in more complex environments are a step forward towards eventually achieving wastewater-sensitive biosensors.

Electrochemiluminescence (ECL) biosensors for the detection of viruses, namely HIV and human papilloma virus (HPV) have been reported [51]–[53]. Zhao et al. (2021) integrated spherical nucleic acid (SNA) with CRISPR/Cas12a for the detection of HIV and HPV [51]. An "on/off" signal switchable biosensor was fabricated such that a sandwich structure is formed by the connection of the target HIV DNA to loaded SNA. The sensor detected HIV and HPV at concentrations as low as 0.00003 nM and 0.00032 nM, respectively, in 120 minutes. In general, multiplex detection schemes require simplification to enable their extension to real life applications. In this case, too, the detection of ssDNA is accompanied with biosensor complexity. Thus, it is recommended that further studied be made in efforts to simplify such detection schemes. Liu et al. (2021) reported the development of a similar ECL biosensor using CRISPR/Cas for the detection of HPV-16 DNA [52]. Their biosensor was found to exhibit an LOD of 0.00048 nM. Although the biosensor developed by Zhao et al. (2021) outperforms that of Liu et al. (2021) with respect to the detection of HPV, its selectivity is high enough to promise its potential application in point of care testing.

Hepatitis B virus (HBV) is known to be associated with hepatocellular carcinoma, liver cirrhosis, and a high mortality rate. Although HBV presents a global public health concern, there are no preventative actions against its spread in addition to vaccination. Traditionally, HBV was diagnosed through long and sophisticated detection procedures such as radioimmunoassay, enzyme-linked immunosorbent assay, and chemiluminescence. Therefore, suitable PoC diagnostic tools are needed for the fast and simple detection of HBV. In agreement, Shariati et al. (2021) reported a simple, accurate, and cost-effective PoC device for HBV detection [54]. A field effect transistor (FET) was developed for the detection of HBV deoxyribonucleic acid with ultrasensitive capability. ZnO-doped MoS_2 nanowires were used to materialize the device, and the high sensitivity and low response time obtained were owed to the excellent electrical and structural properties. In addition, the developed biosensor proved its reproducibility with the ability to maintain its initial response with up to 96%. The authors also tested the sensor for its

specificity against similar DNA types and confirmed its high performance. In another study, efforts were dedicated to developing a system for the detection of HBV, which is composed of an electrochemical sensor and an easy-to-access control element [55]. By integrating the sensor into a smartphone, Teengam et al. (2021) presented a complete platform for simple and portable analytical tools. To obtain high sensitivity, the authors electropolymerized β-cyclodextrin on the surface of the sensor and incorporated gold nanoparticles in the electrodes [55]. Hepatitis B surface antigens were used to examine sensor performance reporting an LOD as low as 170 ng/mL. In their study, the authors were able to achieve good sensitivity along with real-time monitoring through a smartphone-based system operated via near field communication (NFC).

Similar to HBV, hepatitis C virus (HCV) represents one of the main causes of liver-related diseases. Conventional HCV detection tools often aim to identify antibodies that only form after 2-4 weeks of patients showing clinical symptoms. In turn, testing could sometimes not distinguish between present and previous infections. Therefore, detection of HCV has been better studied by identification of viral RNA segments. However, the usual drawbacks associated with any detection of viral RNA remain. Therefore, researchers have focused their efforts on HCV detection through surface antigens. Recently, the development of a biosensor for the detection of the HCV envelope protein E2 has been reported [56]. Several fragments of CD81 biological cell receptors were tested and their growth was optimized on the sensor surface. Due to its similarity in binding affinity to the targeted E2 protein, the cheapest receptor, which is the linear peptide, was selected for biosensor fabrication. The reported HCV biosensor has also demonstrated its high performance in a more complicated matrix containing interfering protein conalbumi. Furthermore, for a solution containing E2 proteins in phosphate buffered saline and blood plasma, the sensor could detect a concentration as low as 21 ng/mL. In another study, molecularly imprinted polymer sensors were investigated for the detection of a similar virus, the weakly fluorescent hepatitis A virus (HAV) [57]. A luminescent metal-organic framework was used to produce fluorescent output signals as an indication of the presence of HAV. Within 15 minutes, the sensor was able to detect HAV at concentrations as low as 3×10^{-3} nM in binary systems. Therefore, the sensor is highly selective, even in the presence of similar competitive viruses such as HBV. The reviewed HCV and HAV sensors were selective in the presence of interfering molecules, but further validation and testing are required to extend their applications to higher complex environments such as wastewater.

Furthermore, flu viruses are highly common as they infect 5-10% of the global population. In the past couple of years, viral isolation, serology, nucleic amplification, and lab-on-chip assays have been used and modified for Flu viruses' diagnostics. However, due to the common drawbacks of these diagnostic measures and the overwhelming concerns associated with mortality rates, there continues to be a need for rapid diagnostic tools for Flu viruses [58], [59]. Raji et al. (2021) reported the development of biosensors for the detection of Flu A and Flu B viruses [58]. This study used antibodies for Flu A and Flu B viruses and immobilized them on the surface of colorimetric biosensors. In mucus samples, the biosensor had an LOD of 0.04 ng/mL. The specificity of the sensor was also confirmed against the MERS CoV and HCoV viruses. Similarly, conventional diagnostic techniques used for the detection of respiratory syncytial virus subgroups A (RSVA) and B (RSVB) are no longer preferred for the beforementioned drawbacks. Recently, a reported toehold switch sensor was investigated for its performance as a detection tool for RSVA and RSVB [60]. In the study, target trigger RNAs of RSVA and RSVB were used as biorecognition elements in the sensor design. These RNAs bind to RSVA and RSVB when present, producing an eye-detectable colorimetric result. After optimization, the sensor had an LOD of 5.2×10^{-9} nM and 9.1×10^{-9} nM for RSVA and RSVB, respectively.

Hand, foot, and mouth disease (HFMD) outbreaks occur in livestock every few years. The disease is caused by several enteroviruses, including Enterovirus A71 (EV-A71). Several efforts have been reported to develop biosensors for the rapid diagnosis of HFMD, without thorough investigation of their performance. Udos et al. (2021) reported the development of a biosensor for the detection of Enterovirus A71 in synthetic analytes [61]. The fabrication and functionalization of the sensor was optimized, with a noted focus on eliminating false detection which may be caused by refractive-index noise. The biosensor was tested by measuring the optical spectrum and quantifying the viral concentration. It was noted that the sensor could detect a concentration of 0.343 ng/mL in 4 minutes, with a marked selectivity towards EV-A71 in the presence of other HFMD-causative viruses [61]. In addition, Liu et al. (2021) developed a biosensor for the detection of Singapore grouper iridovirus (SGIV), which is a dangerous form of iridoviruses [62]. Although lateral flow biosensors have been used for the detection of bacteria, cells, proteins, and chemical contaminants, they have never been investigated for the detection of SGIV. The authors used lateral flow biosensors and functionalized them with DNA aptamers to

target SGIV molecules [62]. With high specificity and sensitivity, the biosensor could detect SGIV at concentrations of 5×10^4 cells/mL in less than 90 minutes.

The Dengue virus (DENV), which is directly correlated with several fatal diseases with very limited treatment options, has also been studied by Lee et al. (2021) [63]. Being a fatal disease, timely diagnosis provided by biosensors is of high importance. As a diagnostic tool, the authors developed a sensitive electrochemical biosensor for the early detection of DENV. Using clustered regularly interspaced short palindromic repeats (CRISPR) RNA and Cpf1 together as biorecognition elements, the target DENV molecules were detected. The biosensor developed could detect DENV molecules at concentrations as low as 10^{-5} nM in 30 minutes. Despite the novelty and success of the reported design, the sensitivity of the sensor is relatively low compared to other similar CRISPR/Cpf1-based sensors [63]. In another study, Dengue virus non-structural protein 1 (NS1) was used as a biomarker [64]. The specific 6-mercapto-1-hexanol (MCH) aptamer was immobilized on gold electrodes and optimized to obtain a monolayer. During testing, the biosensor showed sensitivity towards the targeted NS1, even in the presence of Dengue virus e envelope protein. In spiked human serum samples, the reported LOD ranged from 0.05 to 0.025 ng/mL. It is also worth noting that bovine serum albumin was added to the tested samples to avoid nonspecific and undesirable blockage of the biosensor surface during testing. The sensor was also sensitive to NS1 in clinical range concentrations, demonstrating its potential application as a miniaturized POC device, which could also be further extended to other Dengue serotypes [64]. Although the sensor was tested for selectivity in the presence of other proteins of the same virus, no data was provided to show the sensor's capability in real-patient or environmental samples.

The detection of viral hemorrhagic septicemia (VHS), a very common infection in water and fish, has been investigated [65]. According to the authors, RT-PCR and real-time PCR are the two most common detection tools for VHS due to their rapidness and sensitivity. However, they are not cost-effective and require professional operation inside well-equipped laboratories. An electrochemical biosensor was developed by Moattari et al. (2021) through the immobilization of VHS-specific DNA probes on pencil graphite electrodes [65]. Using methylene blue, several DNA sequences were investigated on the reported sensor and high-sensitivity results were obtained. Furthermore, the genosensor showed an LOD of 0.125 nM and

a linear detection range of 10^5 to 1 nM. In their study, the authors further validated the capability of their sensor on real fish samples, which may be highly indicative of the presence of VHS in water bodies. In comparison with other reviewed papers, the VHS sensor might have good potential for extension to wastewater samples, given its successful tests on real fish samples. In addition, conventional methods for the detection of immunoglobulin G (IgG), which is an indicative antibody of several diseases, including measles, require antibodies. Because IgG antibodies are expensive and difficult to prepare, molecularly imprinted polymers are perfect substitutes. Bai et al. (2021) manufactured molecularly imprinted polymers on top of nano Au/nano Ni modified Au electrodes through metal free visible light induced atom transfer radical polymerization (MVL ATRP) [66]. During fabrication, IgGs were conjugated with fluorescein isothiocyanate as a template and photocatalyst. The biosensor could identify IgG at concentrations as low as 2.0×10^{-5} ng/mL and up to 10^3 ng/mL. The authors claim that their reported biosensors show a broader detection range and lower LOD than those previously reported [66], making it a more promising potential for wastewater applications.

Table 2. Studies on biosensors developed for the detection of viruses.

Biosensor type	Biorecognition element	Target	LOD (ng/mL)	Linear range (ng/mL)	Response time (minutes)	Reference
Electrochemical	p24 ssDNA, p24-HIV, and p24-HTLV aptamers	p24-HIV protein	5.17×10^{-2}	0.93 – 93,000	-	[50]
Electrochemical	Hepatitis B virus DNA	Hepatitis B virus DNA	10^{-7} [a]	5×10^{-4} - 5×10^{7} [a]	0.417	[54]

	oligonucleotides					
Electrochemical	Anti-HBV monoclonal antibodies	Hepatitis B surface antigen	170	10,000 – 200,000	-	[55]
Electrochemical	Recombinant LEL fragment of CD81 2 synthetic peptides imitating linear and loop like peptides of CD81	Hepatitis C virus surface antigen: envelope protein (E2)	21	-	-	[56]
Fluorescence	Molecularly imprinted polymers	Hepatitis A virus	3×10^{-3} [a]	2×10^{-2} - 2.5 [a]	15	[57]
Toehold switch sensor	Target trigger RNAs of RSVA and RSVB	Respiratory syncytial virus (RSV): subgroups A (RSVA) and B (RSVB)	5.2×10^{-9} [a] (RSVA) and 9.1×10^{-9} [a] (RSVB	-	-	[60]

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Colorimetric	Flu A and Flu B antibodies	Flu A and Flu B viruses	0.04	0.04 - 40		[58]
Lateral flow biosensor	Q2 and Q3 aptamers	Singapore grouper iridovirus	5×10^{4} [b]	-	<90	[62]
Resonance tilted fiber Bragg grating (SPR-TFBG)	Monoclonal antibody Mab	Enterovirus A71	0.343	-	4	[61]
Electrochemical	CRISPR RNA & Cpf1	Dengue virus	10^{-5} [a]	-	30	[63]
Electrochemical	IgG imprinted polymers	Immunoglobulin G	2.0×10^{-5}	10^{-4} - 10^{3}	-	[66]
Microfluidic FET	Human immunodeficiency virus gp41 antibody probes	Human immunodeficiency virus gp41 antibodies	0.0025 [a]	-	-	[49]
Fluorescence	Hepatitis C virus DNA	Highly specific pyrrolidinyl peptide nucleic acid probe	5 [c]	5 - 100 [c]	-	[67]
Fluorescence	DNA walker	H5N1 DNA	0.06 [a]	0.2 - 20 [a]	-	[68]
Electrochemical	DNA aptamer	Dengue virus	0.05 -	0.01 -	-	[64]

		(NS1 antigens)	0.025	1000		
Electrochemical	DNA probe	Viral hemorrhagic septicemia virus	0.125 [a]	10^5 - 1 [a]	-	[65]
Electrochemical	Anti-hepatitis B antibody	Hepatitis B surface antigen	0.018	0.1 - 250	8.33	[69]
	Anti-hepatitis C antibody	Hepatitis C core antigen	0.0012	0.001 - 250		
SERS-Based Biosensor	Monoclonal anti-FluA antibodies	Influenza A H1N1 virus	50 [d]	-	30	[59]
	Monoclonal anti-adenovirus antibodies	Human adenovirus (HAdV)	10 [d]			
Electrochemiluminescence	Spherical nucleic acid and CRISPR/Cas12a	Human immunodeficiency virus	0.00003 [a]	-	120	[51]
		Human papilloma virus	0.00002 [a]			
Electrochemiluminescence	CRISPR/Cas12a	Human papilloma virus subtype	0.00048 [a]	-	70	[52]
Electrochemiluminescence	Metal organic framework (ZIF-8)	Human immunodeficiency virus (HIV-1	3×10^{-7}	0.000001 - 1 [a]	-	[53]

protein)

a: nM, b: cells/mL, c: pmol.

2.3. SARS-CoV-2 biosensors

Human coronaviruses have been recognized since the 1960s. The most impactful viruses on public health are MERS-CoV, SARS-CoV, and SARS-CoV-2. For most pathogenic identifications, real-time polymerase chain reaction (RT-PCR) is used as the standard testing methodology. Given that most conventional methods, including RT-PCR, required diagnosis to be carried out in well-equipped laboratories, and that emerging pathogens carry high risks of being infectious, point-of-care and risk-free testing is gaining the interest of researchers [70]. More studies are being carried out to find fast and easy alternative detection methods with lower risks of viral transmission. According to Layqah and Eissa (2019), several biosensors have been reported for the detection of coronaviruses, some having much higher sensitivity values than standard qPCR tests [71]. For example, a label-free bio-optical sensor for RNA amplification was found to have 10-fold sensitivity of RT-PCR assays for the detection of MERS-CoV [72]. Therefore, great efforts have been made with regards to the development of SARS-CoV-2 biosensors, given that they can be used in resource limited settings [73]. In addition, SARS-CoV-2 has already been detected in feces and wastewater [74]. In fact, wastewater collection networks are already being used to collect information on the spread of infectious diseases, such as COVID-19, within communities [75]. Therefore, sensitive and selective SARS-CoV-2 biosensors can be used in wastewater applications. A summary of all studies reporting biosensors for the detection of SARS-CoV-2 is presented in **Table 3**.

Seo et al. (2020) designed a graphene-based field effect transistor for the detection of SARS-CoV-2 virus in swab specimens [76]. The FET sensor was composed of monolayer graphene, which was functionalized with SARS-CoV-2 spike antibody, being selective towards the spike proteins. 1-pyrenebutyric acid N-hydroxysuccinimide ester (PBASE), which binds to graphene via pi-pi bonding, was first used as an intermediate layer to link the antibody to the graphene monolayer. This bonding was verified through Raman and XPS analysis. Before

attaching the antibody to the FET surface, the antibody's selectivity towards the target spike proteins being used was verified through ELISA. After the fabrication of the biosensor, electrical characterization was conducted to test its detection performance upon antibody-antigen binding. Through IV characterization, the successful detection of SARS-CoV-2 spike proteins was verified. The detection was noted with concentrations as low as 100 fg/mL. Molecularly imprinted polymers were used by Raziq et al. (2021), for the first time, as biorecognition elements for the detection of SARS-CoV-2 using electrochemical biosensors. Their reported sensor was composed of a disposable Au-thin film electrode (TFE) chip and possessed high selectivity towards SARS-CoV-2 nucleoproteins when modified with SARS-CoV-2 nucleoproteins. The selectivity of the developed biosensor was demonstrated by its differentiation against similar proteins such as S1 and E2 HCV, and the performance of the developed sensor was tested against analytes prepared from commercial SARS-CoV-2 antigens. The results have shown a linear response between 2.2×10^{-8} and 3.33×10^{-4} nM, an LOD of 1.5×10^{-6} nM, and a quantification limit of 5×10^{-6} nM or 0.7-2.2 pg/mL [77]. Since samples taken from real patients with COVID-19 have been found to contain nucleoproteins at concentrations less than 10 pg/mL [78], the designed biosensor was sensitive enough for further applications. Therefore, the optimized sensor was then further investigated with clinical nasopharyngeal swab samples, and its performance was found to be promising in complex media and in buffer. Furthermore, Yakoh et al. (2021) reported a method for the detection of SARS-CoV-2 antibodies, as an alternative to lateral flow-based assays (LFAs), which have been widely used to complement RT-PCR tests, specifically after the second week of infection [79]. The difference in this detection method, compared to conventional serological assays, is the unnecessity to use antibodies. An electrochemical biosensor was developed as a label-free, paper-based platform capable of detecting SARS-CoV-2 without the need to immobilize antibodies on the surface. The biosensor proved its performance in the SARS-CoV-2 presence of the spike protein of SARS-CoV-2. In 30 minutes, the biosensor reported an LOD of 1 ng/mL and linear range of 1-1000 ng/mL. Despite the sensor's ability to detect antibodies in clinical sera, the current performance of the reported biosensor did not suffice for the detection level in nasopharyngeal swab specimens. However, it should be noted that the biosensor achieved a sensitivity that is 3 times higher than the most recently developed colorimetric LFA for SARS-CoV-2 antibody detection.

Furthermore, Peng et al. (2021) have proposed a SARS-CoV-2 detection method that uses a catalytic assembly circuit and DNA polymerization in the presence of targeted RNA [80]. The biorecognition elements used were hairpin structures and were immobilized on the surface for specificity. The sensor has shown its capability in SARS-CoV-2 RNA detection at concentrations as low as 2.6×10^{-6} nM, along with linear responses in concentration ranges between 10^{-5} and 1 nM [80]. In a similar study targeting SARS-CoV-2 RNA, Zhao et al. (2021) reported the development of a biosensor that does not require any pretreatment steps, such as nucleic acid amplification and reverse transcription, which conventional methods often require [81]. A portable electrochemical biosensor was designed to detect SARS-CoV-2 RNA at high sensitivity using a supersandwich-type recognition strategy and calixarene-graphene oxide, which were designed to enrich toluidine blue. While calixarene shows excellent recognition and enrichment properties of TB, modification with Au nanoparticles serves to increase biosensor sensitivity. For the first time, a biosensor for the detection of SARS-CoV-2 was equipped with a smartphone to improve point-of-care testing by reporting detection signals. In their study, Zhao et al. (2021) collected 88 RNA extracts from positive and recovering patients to confirm the effectiveness of the biosensor. The results showed that the developed biosensor produced higher detectable ratios than RT-PCR, with a LOD of 200 copies/mL [81]. In comparison, Broughton et al. (2020) developed DETECTR, a CRISPS-Cas12-based lateral flow assay, for the detection of SARS-CoV-2 RNA extracts with an LOD of 10,000 copies/mL [82], which is much higher than that of Zhao et al. (2021). However, the rapid detection provided by DETECTR, in comparison with conventional methods, is significant.

Several studied investigated optical biosensors for the detection of SARS-CoV-2 [83]–[85]. Cennamo et al. (2021) reported an optical biosensor for the detection of SARS-CoV-2 using DNA sequences [83]. Specific DNA sequences were immobilized on the sensor surface to trigger protein bonding in the presence of the S1 protein, the receptor binding domain. A D-shaped plastic optical fiber was modified with a gold nanofilm and a short poly (ethylene glycol) (PEG) interface that bonds to the biorecognition element. The specificity and sensitivity of the sensor were demonstrated by testing against different interferences, namely BSA, AH1N1 hemagglutinin protein and MERS spike protein. As a result, an LOD of 37 nM was obtained, which is highly comparable to similar reported optical sensor performances in the literature [83]. In general, the sensitivities of optical sensors were significantly lower than those of

electrochemical sensors [86]–[88]. However, the sensor was only tested on synthetic samples containing the S1 protein. According to the authors, preliminary tests in human serum have given potential to further implementation in point-of-care facilities. Another research publication has reported the development of an optical biosensor for SARS-CoV-2 detection. Huang et al. (2021) have designed a one-step detection and quantification method for SARS-CoV-2 [85]. In their study, a specific nanoplasmonic resonance biosensor was manufactured by immobilizing SARS-CoV-2 monoclonal antibodies on the chip surface. A generic microplate reader was used to detect particles from the SARS-CoV-2 virus. Through direct optical measurement of SARS-CoV-2 particles, the sensor was able to detect concentrations as low as 370 vp/mL in a time frame of 15 minutes. The specificity of the sensor was assessed in the presence of SARS, MERS, and VSV pseudovirus. The results show a remarkable difference between the SARS-CoV-2 particles and the rest, demonstrating high specificity. Additionally, the sensor has shown a linear detection range of 0 to 10^7 vp/mL. Moreover, fiber-optic biosensing platforms were utilized by Lee et al. (2021) [84]. The optical transducer reported was based on SARS-CoV-2 spike antibodies (SSAs) integration with a phase-shifted long-fiber grating (PS-LPEGs), and variation in wavelength separation was used to determine the binding of antibodies to proteins. It was found to be in trend with the protein concentration introduced. In addition to successful detection of SARS-CoV-2, selectivity was confirmed through sensor exposure to highly similar viruses, such as MERS-CoV. The reported LOD of this fiber-optic biosensor was 0.1 ng/mL, showing the promising potential behind such sensors, which is in line with the performance of previously reported LPEG-based optical biosensors.

A study by Ahmadivand et al. (2021) aimed at the development of a method to detect low-level viral presence in samples and mitigate the common drawbacks of conventional diagnostic tools [89]. Plasmonic metasensor technology was investigated for its effectiveness in producing a highly sensitive biosensor that could detect concentrations at the femtomolar level. This technology has been used in several healthcare sectors and modern diagnostics. Toroidal metasurface technology was also implemented to prevent the solo detection of low molecular weight molecules at low densities. This research successfully fabricated a plasmonic immunosensor with monoclonal antibodies specific to the spike protein immobilized on the surface as biorecognition elements. Upon testing the biosensor in synthetic analytes containing spike proteins, an LOD of 4.2×10^{-7} nM was recorded in a duration of 80 minutes. In another

study, a multimode colorimetric/SERS/fluorescence biosensor was developed [90]. The multimode sensor includes gold nanoparticles, around 17 nm in size, which provided an enhanced response time of 40 minutes. All three modes of the biosensor, colorimetric/SERS/fluorescence, have produced similar detection accuracies at the femtomolar level, ranging between 1.6×10^{-4} to 3.95×10^{-4} nM, with the lowest LOD achieved in the colorimetric mode. In this study, the comparison of the outputs of the different modes was suitable for further validation of the biosensor. This detection method based on the use of a multimode sensor offered the added advantage of identifying any false reading in a given test.

In general, the performance of all reported biosensors remains unexplored on wastewater samples. Despite the development of sensors that proved to be much more sensitive than the PCR standard testing, the effect of wastewater complexity on sensor performance is yet to be investigated [72].

Table 3. Studies on biosensors developed for the detection of SARS-CoV-2.

Biosensor type	Biorecognition element	Target	LOD (nM)	Linear range (nM)	Response time (minutes)	Reference
Electrochemical	Molecularly imprinted polymers	SARS-CoV2 nucleoprotein	1.5×10^{-6}	2.2×10^{-8} - 3.33×10^{-4}	-	[77]
Electrochemical	Capture probe	SARS-CoV-2 RNA	200 [a]	10^{-8} - 10^{-4}	-	[81]

Specific nanoplasmonic resonance sensor	SARS-CoV-2 monoclonal antibodies	SARS-CoV-2	370 [b]	0 - 10^{7} [b]	15	[85]
Optical	Aptamer	Spike protein	37	-	-	[83]
Electrochemical	SARS-CoV-2 spike antibody	Spike protein	1×10^{-6} [c]	-	5	[91]
Electrochemical	Spike protein receptor-binding domain	SARS-CoV-2 antibodies	1 [c]	1 - 1000 [c]	30	[79]
Colorimetric/ SERS/ fluorescence triple-mode biosensor	DNA probe	RdRp and E gene	1.6×10^{-4} - 3.95×10^{-4}	1.6×10^{-4} - 1	40	[90]
Electrochemical	Hairpin 1 and Hairpin 2	SARS-COV-2 RNA	2.6×10^{-6}	10^{-5} - 1	-	[80]
Plasmonic	Monoclonal antibody specific to spike protein	Spike protein (S1) of SARS-CoV-2	4.2×10^{-7}	-	80	[89]

(S1)

Fluorescence	-	SARS-CoV-2 DNA	1 [d]	-	< 30	[92]
Colorimetric Electrochemical	SARS-CoV-2 spike monoclonal antibody	SARS-CoV-2 spike antigen	48 [c] 0.001 [c]	- 0.001 - 10 [c]	-	[88]
Optical	SARS-CoV-2 spike antibody	SARS-CoV-2 spike protein	0.1 [c]	-	-	[84]
Electrochemical	Human receptor angiotensin-converting enzyme-2	SARS-CoV-2 spike protein	0.0000021 8 [c]	0.00001 - 100 [c]	4	[86]
Electrochemical	Probe	SARS-CoV-2 RNA segment	0.00001	-	-	[87]
SERS-Based Biosensor	SARS-CoV-2 spike antibody	SARS-CoV-2 spike protein	7.7×10^{-7}	-	-	[93]
Electrochemical	SARS-CoV-2 spike antibody	SARS-CoV-2 spike protein	1×10^{-6}	-	-	[76]

Fluorescence	CRISPR-Cas12	SARS-CoV-2 RNA	10000 [a]	-	< 40	[82]

a: copies/mL, b: vp/mL, c: ng/mL, d: genome equivalent per μL.

3. Future directions and challenges

Wastewater pathogens include bacteria, viruses, protozoa, and parasitic worms [2]. Of the many sources of pathogens in wastewater, domestic waste the main source and the most dominant, as shown in **Figure 2**. The presence of some pathogens in wastewater could be threatening and therefore it is important to treat wastewater appropriately. However, it is essential to recognize the efficacy of actual treatment plants, especially in developing countries. Several efforts have been aimed at evaluating the success of wastewater disinfection processes, including chlorination, ozonation, and ultraviolet (UV) irradiation [94]. In the case of SARS-CoV-2, UV irradiation was found to be effective in eliminating the virus from treated wastewater. SARS-CoV-2 has a specific genomic structure that increases its degradability under UV radiation, which may not be the case for other viruses or pathogenic microorganisms. Therefore, these methods can sometimes be inefficient towards different microorganisms [70], which is why surveillance programs remain highly valuable for recognizing pathogenic constituents in wastewater despite the use of disinfection processes. The continuous monitoring of pathogen-causing diseases provided by surveillance programs could be greatly enhanced with the use of biosensors, rather than conventional methods [95].

Common microorganism assays include culture-based methods, qPCR, and enzyme-linked immunoassay (ELISA). These assays are based on two detection schemes. The first is based on biomolecular recognition, and the second is based on reactions with introduced chemical groups. In either case, these diagnostic tools are often expensive, time consuming and require professional handling of tools in laboratories. Therefore, wastewater biosensors have gained more focus over the years. Biosensors are rapid, sensitive, inexpensive, and portable devices. In principle, biosensors can work at the nanoscale, hence their potential for

miniaturization, which minimizes the materials needed for fabrication without affecting performance. There are several publications on the fabrication of biosensors for the detection pathogens in wastewater. For example, pathogenic biosensors targeting many biomolecules, including human nucleic acids, peptides, and proteins, and antibiotic resistance genes (ARGs) were reported [3]. However, there are still limitations to the real-life applications of these biosensors in wastewater. This is because wastewater provides ideal growth conditions for pathogens, making it difficult to interpret the output signals in the presence of many unknown pathogens, in addition to the fact that targets are often present at very low concentrations in wastewater. Thus, the complexity of wastewater makes it difficult to fabricate a commercial pathogenic biosensor [3].

Nanomaterials are emerging as materials of choice in biosensor diagnostics because of their advancements in properties and their nanoscale size which is comparable with biological materials including enzymes, antibodies, proteins, and nucleotides [96]. This facilitates their use in medical applications with the possibility of detecting minute concentrations of the desired analyte. These materials provide high electrical conductivity, and thus can be used to amplify signals. They are generally used as transducer materials which are major units in biosensor designs [97]. Additionally, using nanomaterials is correlated with an increase in biosensor performance as well as an increase in sensitivities, resulting in low LODs.

At the nanoscale, materials have interesting properties such as high surface area and quantum confinement. Their extremely high surface-to-volume ratio allows for nanomaterials to interact with the environment or other materials strongly, as compared to bulk materials. Moreover, the surface of nanomaterials shows extraordinary catalytic and absorbance activity when reacting with other nanoscale-dimension materials. Additionally, because their particle size is too small or comparable with Bohr exciton radius, the electron mobility is confined. This results in "quantum confinement" of the electron-hole pairs. These confining dimensions will increase or widen the material band gap or energy gap, which is translated as an increase in the band gap luminescence energy.

The introduction of nanomaterials is a very common modification that is often relied on to improve the performance of biosensors. Nanomaterials have been repeatedly studied and developed to become the leading revolutionary elements in several fields of research. According to Falciola et al. (2021), chemical sensors, in particular, have experienced drastic advances due to the utilization of nanotechnologies [98]. Furthermore, nanostructured materials have shown promising potential as novel nanoelectronic biosensors for biomolecular detection; they are extraordinarily sensitive, and their detection schemes are quite simple. Nanomaterials such as CNTs, nanowires, nanoparticles, nanopores, nanoclusters, and graphene were effectively used in the preparation of sensors. Rahman et al. (2021) describe sensors using these materials as nanobiotechnology enabled sensors [75]. That is because in most cases, biorecognition elements are immobilized on a sensor surface, which can only functionalized if an appropriate material is chosen. Among all nanomaterials, graphene and CNTs have been widely used as a result of their properties. These nanomaterials offer several advantages, such as high biocompatibility and size compatibility with living cells/proteins/DNA. On the sensor surface, nanoparticles would be completely exposed to the environment, and thus, small changes in the charge environment can cause drastic changes in their electrical properties. For instance, graphene has an electrical conductivity of 200,000 cm^2/V.s. SWCNTs, on the other hand, provide a convenient interface with micrometer-scale circuitry since SWCNT is composed of carbon which also provides a natural match with organic molecules. This is a major contributor to ultrasensitive biosensing and a promising feature that could facilitate the commercialization installment of pathogenic biosensors in wastewater pipelines, providing real-time date and on-line detection of pathogens. In turn, early warnings of outbreaks of infectious disease outbreaks can be obtained to protect the population from future threats to public health. Even though nanomaterial-based biosensors present a lot of advantages over conventional biosensors, there are some challenges related to miniaturization, automation, and integration of the nanostructured-based biosensors that need to be considered.

In WBE, research has shown various biosensors for the monitoring of inorganic ions, organic pollutants and pharmaceuticals, and biomolecules. On the contrary, there are very limited applications of pathogen detecting biosensors in wastewater. This is because the wastewater matrix is complex and more challenging, despite having an excellent culture medium for pathogens. For that reason, the sensitivity provided by the introduction of nanotechnology

should be utilized to provide biosensors capable of detecting pathogens in wastewater. In fact, in their article, Hui et al. (2020) have already discussed the possible implementation of paper-based wastewater biosensors [99]. Laboratory testing for pathogens in wastewater comprises several restrictions related to sample preparation, sample collection, and transportation along with the risk of exposure to infectious diseases. These factors make conventional methods uncapable of meeting the potential benefits of point-of-care (PoC) devices and lab-on-chip (LoC) systems. Further research on the detection of pathogens in wastewater could open the door for lab-on-chip biosensing technology and possibly online detection of pathogens. It could be especially promising to develop such systems due to their cost effectiveness and possibility of being fabricated with cheap polymers and thin metal electrodes, in addition to miniaturization [100]. A major benefit and contribution that online biosensors can provide are early notifications on the presence of alarming pathogens in water. Hence, proper control strategies can be planned accordingly within appropriate durations to prevent the spread of infectious diseases.

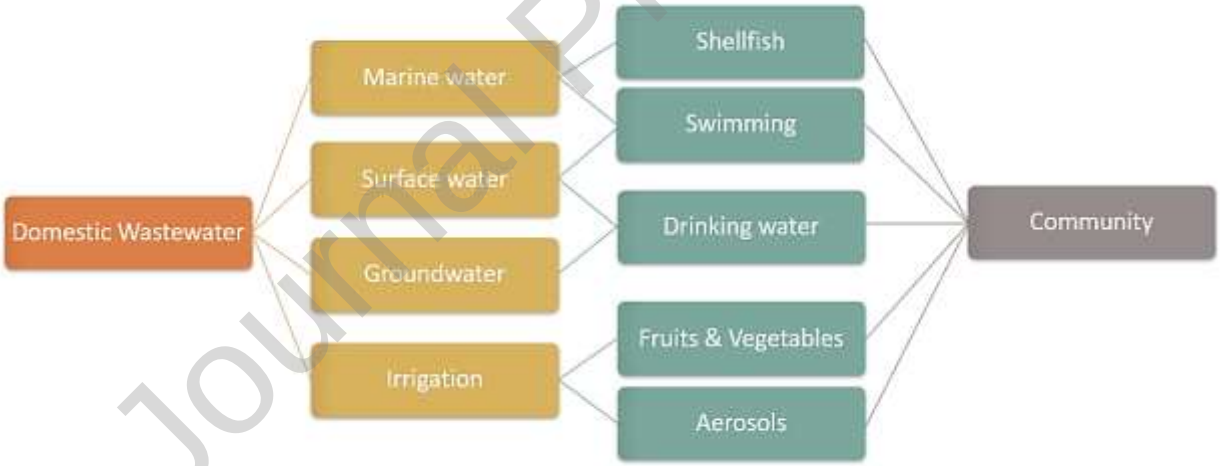

Figure 2. Fate and transmission of enteric waterborne viruses found in wastewater (developed from [101]).

4. Conclusion

An excellent opportunity for monitoring pathogens arises from the fact that they are often present in wastewater through fecal excretions. Conventional methods are still considered the gold standard for screening purposes, with an obvious emphasis on PCR in the case of SARS-CoV-2. In this work, recently developed biosensors for the detection of bacteria, viruses and SARS-CoV-2 were reviewed. These publications have not expanded the scope of their research to include the detection of pathogens in wastewater samples. This is attributed to the difficulty of dealing with complex matrices in wastewater. In addition, research shows that biosensors work better when optimized and integrated with nanomaterials. Therefore, it is recommended that research focus be shifted to biosensing of pathogens in wastewater, rather than conventional detection tools. This provides a potential opportunity for the application of biosensors in online and real-time detection when integrated into wastewater or sewage systems, which could revolutionize the field of screening for currently existing and emerging infectious diseases.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] F. Ejeian *et al.*, "Biosensors for wastewater monitoring: A review," *Biosens. Bioelectron.*, vol. 118, no. July, pp. 66–79, 2018, doi: 10.1016/j.bios.2018.07.019.

[2] C. Chahal, B. van den Akker, F. Young, C. Franco, J. Blackbeard, and P. Monis, *Pathogen and Particle Associations in Wastewater: Significance and Implications for Treatment and Disinfection Processes*, vol. 97. Elsevier Ltd, 2016.

[3] K. Mao, H. Zhang, Y. Pan, and Z. Yang, "Biosensors for wastewater-based epidemiology for monitoring public health," *Water Res.*, vol. 191, 2021, doi: 10.1016/j.watres.2020.116787.

[4] M. Vermi *et al.*, "Viruses in wastewater : occurrence , abundance and detection methods," *Sci. Total Environ.*, vol. 745, p. 140910, 2020, doi: 10.1016/j.scitotenv.2020.140910.

[5] M. Pilevar, K. T. Kim, and W. H. Lee, "Recent advances in biosensors for detecting viruses in water and wastewater," *J. Hazard. Mater.*, vol. 410, no. November 2020, p. 124656, 2020, doi: 10.1016/j.jhazmat.2020.124656.

[6] E. Yoo and S. Lee, "Glucose Biosensors: An Overview of Use in Clinical Practice," *Sensors*, pp. 4558–4576, 2010, doi: 10.3390/s100504558.

[7] M. V. Riquelme, W. Leng, M. Carzolio, A. Pruden, and P. Vikesland, "Stable oligonucleotide-functionalized gold nanosensors for environmental biocontaminant monitoring," *J. Environ. Sci. (China)*, vol. 62, pp. 49–59, 2017, doi: 10.1016/j.jes.2017.08.005.

[8] K. Mao, J. Ma, X. Li, and Z. Yang, "Rapid duplexed detection of illicit drugs in wastewater using gold nanoparticle conjugated aptamer sensors," *Sci. Total Environ.*, vol. 688, pp. 771–779, 2019, doi: 10.1016/j.scitotenv.2019.06.325.

[9] M. S. Kumar *et al.*, "Electrochemical sensing of SARS-CoV-2 amplicons with PCB electrodes," *Sensors Actuators, B Chem.*, vol. 343, no. May, p. 130169, 2021, doi: 10.1016/j.snb.2021.130169.

[10] H. Karimi-Maleh *et al.*, "A critical review on the use of potentiometric based biosensors

for biomarkers detection," *Biosens. Bioelectron.*, vol. 184, no. April, 2021, doi: 10.1016/j.bios.2021.113252.

[11] H. O. Kaya, A. E. Cetin, M. Azimzadeh, and S. N. Topkaya, "Pathogen detection with electrochemical biosensors: Advantages, challenges and future perspectives," *J. Electroanal. Chem.*, vol. 882, 2021, doi: 10.1016/j.jelechem.2021.114989.

[12] H. Y. Zheng, O. A. Alsager, C. S. Wood, J. M. Hodgkiss, and N. O. V. Plank, "Carbon nanotube field effect transistor aptasensors for estrogen detection in liquids," *Am. Vac. Soc.*, vol. 33, no. 6, p. 06F904, 2015, doi: 10.1116/1.4935246.

[13] T. K. Stevik, K. Aa, G. Ausland, and J. F. Hanssen, "Retention and removal of pathogenic bacteria in wastewater percolating through porous media: A review," *Water Res.*, vol. 38, no. 6, pp. 1355–1367, 2004, doi: 10.1016/j.watres.2003.12.024.

[14] A. Ramanujam, B. Neyhouse, R. A. Keogh, M. Muthuvel, R. K. Carroll, and G. G. Botte, "Rapid electrochemical detection of Escherichia coli using nickel oxidation reaction on a rotating disk electrode," *Chem. Eng. J.*, vol. 411, no. December 2020, p. 128453, 2021, doi: 10.1016/j.cej.2021.128453.

[15] Y. Huang, Z. Wu, and G. Zhao, "A Label-Free Electrochemical Immunosensor Modified with AuNPs for Quantitative Detection of Escherichia coli," vol. 48, no. 12, pp. 7960–7969, 2019, doi: 10.1007/s11664-019-07527-6.

[16] X. Mo, Z. Wu, J. Huang, G. Zhao, and W. Dou, "A sensitive and regenerative electrochemical immunosensor for quantitative detection of Escherichia coli O157:H7 based on stable polyaniline coated screen-printed carbon electrode and rGO-NR-Au@Pt," 2019, doi: 10.1039/C8AY02594K.

[17] Y. Lin *et al.*, "Disposable amperometric immunosensing strips fabricated by Au nanoparticles-modified screen-printed carbon electrodes for the detection of foodborne pathogen Escherichia coli O157 : H7," vol. 23, pp. 1832–1837, 2008, doi: 10.1016/j.bios.2008.02.030.

[18] Q. Khue, Q. Huy, N. Phan, T. Anh, T. Thanh, and L. Dang, "A label-free electrochemical biosensor based on screen-printed electrodes modified with gold nanoparticles for quick

detection of bacterial pathogens," *Mater. Today Commun.*, vol. 26, no. September 2020, p. 101726, 2021, doi: 10.1016/j.mtcomm.2020.101726.

[19] K. Nakama, M. Sedki, and A. Mulchandani, "Label-free chemiresistor biosensor based on reduced graphene oxide and M13 bacteriophage for detection of coliforms," *Anal. Chim. Acta*, vol. 1150, p. 338232, 2021, doi: 10.1016/j.aca.2021.338232.

[20] P. Qi, D. Zhang, Y. Sun, and Y. Wan, "A selective near-infrared fluorescent probe for hydrogen sulfide and its application in sulfate-reducing bacteria detection," *Anal. Methods*, vol. 8, no. 16, pp. 3339–3344, 2016, doi: 10.1039/c6ay00054a.

[21] D. Petrovszki, S. Valkai, E. Gora, M. Tanner, and B. Anita, "An integrated electro-optical biosensor system for rapid , low-cost detection of bacteria," vol. 240, 2021, doi: 10.1016/j.mee.2021.111523.

[22] M. Janik *et al.*, "Optical fiber aptasensor for label-free bacteria detection in small volumes," *Sensors Actuators, B Chem.*, vol. 330, no. November 2020, 2021, doi: 10.1016/j.snb.2020.129316.

[23] S. Fang, D. Song, Y. Zhuo, Y. Chen, A. Zhu, and F. Long, "Simultaneous and sensitive determination of Escherichia coli O157 : H7 and Salmonella Typhimurium using evanescent wave dual-color fluorescence aptasensor based on micro / nano size effect," *Biosens. Bioelectron.*, vol. 185, no. April, p. 113288, 2021, doi: 10.1016/j.bios.2021.113288.

[24] A. Sheini, "A point-of-care testing sensor based on fluorescent nanoclusters for rapid detection of septicemia in children," *Sensors Actuators, B Chem.*, vol. 328, no. July 2020, p. 129029, 2021, doi: 10.1016/j.snb.2020.129029.

[25] A. Maria *et al.*, "Performance of an amperometric immunosensor assembled on carboxymethylated cashew gum for Salmonella detection," *Microchem. J.*, vol. 167, no. April, p. 106268, 2021, doi: 10.1016/j.microc.2021.106268.

[26] X. Song, M. Lv, Q. Lv, H. Cui, J. Fu, and Y. Huo, "A novel assay strategy based on isothermal amplification and cascade signal amplified electrochemical DNA sensor for sensitive detection of Helicobacter pylori," *Microchem. J.*, vol. 166, no. April, p. 106243,

2021, doi: 10.1016/j.microc.2021.106243.

[27] R. Cai, Z. Zhang, H. Chen, Y. Tian, and N. Zhou, "A versatile signal-on electrochemical biosensor for Staphylococcus aureus based on triple-helix molecular switch," *Sensors Actuators B. Chem.*, vol. 326, no. August 2020, p. 128842, 2021, doi: 10.1016/j.snb.2020.128842.

[28] R. Wang, L. Wang, J. Yan, D. Luan, J. Wu, and X. Bian, "Rapid , sensitive and label-free detection of pathogenic bacteria using a bacteria-imprinted conducting polymer film-based electrochemical sensor," *Talanta*, vol. 226, no. January, p. 122135, 2021, doi: 10.1016/j.talanta.2021.122135.

[29] Y. Hou *et al.*, "A novel photoelectrochemical aptamer sensor based on rare-earth doped Bi 2 WO 6 and Ag 2 S for the rapid detection of Vibrio parahaemolyticus," *Microchem. J.*, vol. 165, no. March, p. 106132, 2021, doi: 10.1016/j.microc.2021.106132.

[30] Z. Jia, M. Müller, T. Le, M. Riool, M. Müller, and A. Sebastian, "Multiplexed detection and differentiation of bacterial enzymes and bacteria by color-encoded sensor hydrogels," vol. 6, no. April, pp. 4286–4300, 2021, doi: 10.1016/j.bioactmat.2021.04.022.

[31] B. Cui and S. Liang, "Monitoring opportunistic pathogens in domestic wastewater from a pilot-scale anaerobic biofilm reactor to reuse in agricultural irrigation," *Water (Switzerland)*, vol. 11, no. 6, 2019, doi: 10.3390/w11061283.

[32] F. Huang, L. Xue, W. Qi, G. Cai, Y. Liu, and J. Lin, "An ultrasensitive impedance biosensor for Salmonella detection based on rotating high gradient magnetic separation and cascade reaction signal amplification," *Biosens. Bioelectron.*, vol. 176, p. 112921, 2021, doi: 10.1016/j.bios.2020.112921.

[33] H. Jiang, D. Jiang, X. Liu, and J. Yang, "A self-driven PET chip-based imprinted electrochemical sensor for the fast detection of Salmonella," *Sensors Actuators B Chem.*, vol. 349, no. June, p. 130785, 2021, doi: 10.1016/j.snb.2021.130785.

[34] M. R. Ali *et al.*, "Development of an advanced DNA biosensor for pathogenic Vibrio cholerae detection in real sample," *Biosens. Bioelectron.*, vol. 188, no. April, p. 113338, 2021, doi: 10.1016/j.bios.2021.113338.

[35] J. A. Capobianco, C. M. Armstrong, J. Lee, and A. G. Gehring, "Detection of pathogenic bacteria in large volume food samples using an enzyme-linked immunoelectrochemical biosensor," *Food Control*, vol. 119, no. July 2020, p. 107456, 2021, doi: 10.1016/j.foodcont.2020.107456.

[36] X. Ma *et al.*, "Rapid detection of Aeromonas hydrophila with a DNAzyme-based sensor," *Food Control*, vol. 123, no. August 2020, p. 107829, 2021, doi: 10.1016/j.foodcont.2020.107829.

[37] A. Saadati, H. Kholafazad kordasht, M. Ehsani, M. Hasanzadeh, F. Seidi, and N. Shadjou, "An innovative flexible and portable DNA based biodevice towards sensitive identification of Haemophilus influenzae bacterial genome: A new platform for the rapid and low cost recognition of pathogenic bacteria using point of care (POC) analysis," *Microchem. J.*, vol. 169, no. April, p. 106610, 2021, doi: 10.1016/j.microc.2021.106610.

[38] A. Norouz Dizaji *et al.*, "Electrochemical-based '"antibiotsensor"' for the whole-cell detection of the vancomycin-susceptible bacteria," *Talanta*, vol. 234, no. April, p. 122695, 2021, doi: 10.1016/j.talanta.2021.122695.

[39] S. Bu *et al.*, "Ultrasensitive detection of pathogenic bacteria by CRISPR/Cas12a coupling with a primer exchange reaction," *Sensors Actuators B Chem.*, vol. 347, no. August, p. 130630, 2021, doi: 10.1016/j.snb.2021.130630.

[40] J. Hu, F. Tang, L. Wang, M. Tang, Y. Z. Jiang, and C. Liu, "Nanozyme sensor based-on platinum-decorated polymer nanosphere for rapid and sensitive detection of Salmonella typhimurium with the naked eye," *Sensors Actuators B Chem.*, vol. 346, no. August, p. 130560, 2021, doi: 10.1016/j.snb.2021.130560.

[41] L. Yin, N. Duan, S. Chen, Y. Yao, J. Liu, and L. Ma, "Ultrasensitive pathogenic bacteria detection by a smartphone-read G-quadruplex-based CRISPR-Cas12a bioassay," *Sensors Actuators B Chem.*, vol. 347, no. August, p. 130586, 2021, doi: 10.1016/j.snb.2021.130586.

[42] C. Bi *et al.*, "Simultaneous Detection and Mutation Surveillance of SARS-CoV-2 and co-infections of multiple respiratory viruses by Rapid field-deployable sequencing," *Med*,

2021, doi: 10.1016/j.medj.2021.03.015.

[43] B. Li *et al.*, "H7N9 influenza virus surveillance in Gansu , China in 2017," *Virus Res.*, vol. 296, no. January, p. 198335, 2021, doi: 10.1016/j.virusres.2021.198335.

[44] S. Lee, M. Ihara, N. Yamashita, and H. Tanaka, "Improvement of virus removal by pilot-scale coagulation-ultrafiltration process for wastewater reclamation: Effect of optimization of pH in secondary effluent," *Water Res.*, vol. 114, pp. 23–30, 2017, doi: 10.1016/j.watres.2017.02.017.

[45] S. Lee, A. Hata, N. Yamashita, and H. Tanaka, "Evaluation of Virus Reduction by Ultrafiltration with Coagulation–Sedimentation in Water Reclamation," *Food Environ. Virol.*, vol. 9, no. 4, pp. 453–463, 2017, doi: 10.1007/s12560-017-9301-9.

[46] T. Matsushita, N. Shirasaki, Y. Tatsuki, and Y. Matsui, "Investigating norovirus removal by microfiltration, ultrafiltration, and precoagulation-microfiltration processes using recombinant norovirus virus-like particles and real-time immuno-PCR," *Water Res.*, vol. 47, no. 15, pp. 5819–5827, 2013, doi: 10.1016/j.watres.2013.07.004.

[47] S. Jain and A. K. Manocha, "Design and development of smart monitoring module for detection of virus," *Meas. Sensors*, p. 135907, 2021, doi: 10.1016/j.measen.2021.100048.

[48] Y. Wang, Y. Shi, and F. Narita, "Design and finite element simulation of metal-core piezoelectric fiber/epoxy matrix composites for virus detection," *Sensors Actuators A Phys.*, vol. 327, p. 112742, 2021, doi: 10.1016/j.sna.2021.112742.

[49] P. Song *et al.*, "A Microfluidic Field-Effect Transistor Biosensor with Rolled-Up Indium Nitride Microtubes," *Biosens. Bioelectron.*, p. 135907, 2021, doi: 10.1016/j.bios.2021.113264.

[50] J. L. Gogola *et al.*, "Label-free aptasensor for p24-HIV protein detection based on graphene quantum dots as an electrochemical signal amplifier," *Anal. Chim. Acta*, vol. 1166, pp. 1–7, 2021, doi: 10.1016/j.aca.2021.338548.

[51] K. R. Zhao *et al.*, "A signal-switchable electrochemiluminescence biosensor based on the integration of spherical nucleic acid and CRISPR/Cas12a for multiplex detection of HIV/HPV DNAs," *Sensors Actuators, B Chem.*, vol. 346, no. June, p. 130485, 2021, doi:

10.1016/j.snb.2021.130485.

[52] P. F. Liu, K. R. Zhao, Z. J. Liu, L. Wang, S. Y. Ye, and G. X. Liang, "Cas12a-based electrochemiluminescence biosensor for target amplification-free DNA detection," *Biosens. Bioelectron.*, vol. 176, no. January, p. 112954, 2021, doi: 10.1016/j.bios.2020.112954.

[53] Y. Wang *et al.*, "Imidazole metal-organic frameworks embedded in layered Ti3C2Tx Mxene as a high-performance electrochemiluminescence biosensor for sensitive detection of HIV-1 protein," *Microchem. J.*, vol. 167, no. December 2020, p. 106332, 2021, doi: 10.1016/j.microc.2021.106332.

[54] M. Shariati, M. Vaezjalali, and M. Sadeghi, "Ultrasensitive and easily reproducible biosensor based on novel doped MoS2 nanowires field-effect transistor in label-free approach for detection of hepatitis B virus in blood serum," *Anal. Chim. Acta*, vol. 1156, p. 338360, 2021, doi: 10.1016/j.aca.2021.338360.

[55] P. Teengam *et al.*, "NFC-enabling smartphone-based portable amperometric immunosensor for hepatitis B virus detection," *Sensors Actuators, B Chem.*, vol. 326, no. May 2020, p. 128825, 2021, doi: 10.1016/j.snb.2020.128825.

[56] M. Antipchik *et al.*, "An electrochemical biosensor for direct detection of hepatitis C virus," *Anal. Biochem.*, vol. 624, no. March, 2021, doi: 10.1016/j.ab.2021.114196.

[57] L. Wang, K. Liang, W. Feng, C. Chen, H. Gong, and C. Cai, "Molecularly imprinted polymers based on magnetically fluorescent metal–organic frameworks for the selective detection of hepatitis A virus," *Microchemical Journal*, vol. 164. 2021, doi: 10.1016/j.microc.2021.106047.

[58] M. A. Raji *et al.*, "Development of rapid colorimetric assay for the detection of Influenza A and B viruses," *Talanta*, vol. 221, no. April 2020, p. 121468, 2021, doi: 10.1016/j.talanta.2020.121468.

[59] C. Wang *et al.*, "Magnetic SERS Strip for Sensitive and Simultaneous Detection of Respiratory Viruses," *ACS Appl. Mater. Interfaces*, vol. 11, no. 21, pp. 19495–19505, 2019, doi: 10.1021/acsami.9b03920.

[60] M. Cao, Q. Sun, X. Zhang, Y. Ma, and J. Wang, "Detection and differentiation of respiratory syncytial virus subgroups A and B with colorimetric toehold switch sensors in a paper-based cell-free system," *Biosens. Bioelectron.*, vol. 182, no. February, 2021, doi: 10.1016/j.bios.2021.113173.

[61] W. Udos *et al.*, "Label-free surface-plasmon resonance fiber grating biosensor for Hand-foot-mouth disease (EV-A71) detection," *Optik (Stuttg).*, vol. 228, no. October 2020, p. 166221, 2021, doi: 10.1016/j.ijleo.2020.166221.

[62] J. Liu *et al.*, "A lateral flow biosensor for rapid detection of Singapore grouper iridovirus (SGIV)," *Aquaculture*, vol. 541, no. April, p. 736756, 2021, doi: 10.1016/j.aquaculture.2021.736756.

[63] Y. Lee *et al.*, "Fabrication of ultrasensitive electrochemical biosensor for dengue fever viral RNA Based on CRISPR/Cpf1 reaction," *Sensors Actuators, B Chem.*, vol. 326, no. July 2020, p. 128677, 2021, doi: 10.1016/j.snb.2020.128677.

[64] B. Bachour Junior, M. R. Batistuti, A. S. Pereira, E. Maria de Sousa Russo, and M. Mulato, "Electrochemical aptasensor for NS1 detection: towards a fast Dengue biosensor," *Talanta*, p. 135907, 2021, doi: 10.1016/j.talanta.2021.122527.

[65] G. Moattari, Z. Izadi, and M. Shakhsi-Niaei, "Development of an electrochemical genosensor for detection of viral hemorrhagic septicemia virus (VHSV) using glycoprotein (G) gene probe," *Aquaculture*, vol. 536, no. January, p. 736451, 2021, doi: 10.1016/j.aquaculture.2021.736451.

[66] R. Bai *et al.*, "Preparation of IgG imprinted polymers by metal-free visible-light-induced ATRP and its application in biosensor," *Talanta*, vol. 226, no. July 2020, p. 122160, 2021, doi: 10.1016/j.talanta.2021.122160.

[67] P. Teengam, N. Nisab, N. Chuaypen, P. Tangkijvanich, T. Vilaivan, and O. Chailapakul, "Fluorescent paper-based DNA sensor using pyrrolidinyl peptide nucleic acids for hepatitis C virus detection," *Biosens. Bioelectron.*, vol. 189, no. February, p. 113381, 2021, doi: 10.1016/j.bios.2021.113381.

[68] X. Zhao, Z. Zhu, R. Zou, L. Wang, H. Gong, and C. Cai, "An enzyme-free three-

dimensional DNA walker powered by catalytic hairpin assembly for H5N1 DNA ratiometric detection," *Microchem. J.*, vol. 170, no. August, p. 106728, 2021, doi: 10.1016/j.microc.2021.106728.

[69] S. Boonkaew *et al.*, "An automated fast-flow/delayed paper-based platform for the simultaneous electrochemical detection of hepatitis B virus and hepatitis C virus core antigen," *Biosens. Bioelectron.*, vol. 193, no. August, p. 113543, 2021, doi: 10.1016/j.bios.2021.113543.

[70] A. Eftekhari *et al.*, "A Comprehensive Review of Detection Methods for SARS-CoV-2," *Microorganisms*, vol. 232, no. 9, pp. 1–19, 2021, doi: https://doi.org/10.3390/microorganisms9020232.

[71] L. A. Layqah and S. Eissa, "An electrochemical immunosensor for the corona virus associated with the Middle East respiratory syndrome using an array of gold nanoparticle-modified carbon electrodes," *Microchim. Acta*, vol. 186, no. 4, 2019, doi: https://doi.org/10.1007/s00604-019-3345-5.

[72] B. Koo *et al.*, "An isothermal, label-free, and rapid one-step RNA amplification/detection assay for diagnosis of respiratory viral infections," *Biosens. Bioelectron.*, vol. 90, no. November 2016, pp. 187–194, 2017, doi: 10.1016/j.bios.2016.11.051.

[73] S. Suleman *et al.*, "Point of care detection of COVID-19: Advancement in biosensing and diagnostic methods," *Chem. Eng. J.*, vol. 414, no. October 2020, p. 128759, 2021, doi: 10.1016/j.cej.2021.128759.

[74] W. Wang *et al.*, "Detection of SARS-CoV-2 in Different Types of Clinical Specimens," *JAMA - J. Am. Med. Assoc.*, vol. 323, no. 11, pp. 1061–1069, 2020, doi: 10.1001/jama.2020.1585.

[75] A. Rahman, S. Kang, W. Wang, A. Garg, A. Maile-Moskowitz, and P. J. Vikesland, "Nanobiotechnology enabled approaches for wastewater based epidemiology," *TrAC - Trends Anal. Chem.*, vol. 143, p. 116400, 2021, doi: 10.1016/j.trac.2021.116400.

[76] G. Seo *et al.*, "Rapid Detection of COVID-19 Causative Virus (SARS-CoV-2) in Human Nasopharyngeal Swab Specimens Using Field-Effect Transistor-Based Biosensor," *ACS*

Nano, vol. 14, no. 4, pp. 5135–5142, 2020, doi: 10.1021/acsnano.0c02823.

[77] A. Raziq, A. Kidakova, R. Boroznjak, J. Reut, A. Opik, and V. Syritski, "Development of a portable MIP-based electrochemical sensor for detection of SARS-CoV-2 antigen," vol. 178, no. January, 2021, doi: 10.1016/j.bios.2021.113029.

[78] T. Li *et al.*, "Serum SARS-COV-2 Nucleocapsid Protein: A Sensitivity and Specificity Early Diagnostic Marker for SARS-COV-2 Infection," *Front. Cell. Infect. Microbiol.*, vol. 10, no. September, pp. 1–8, 2020, doi: 10.3389/fcimb.2020.00470.

[79] A. Yakoh, U. Pimpitak, S. Rengpipat, N. Hirankarn, O. Chailapakul, and S. Chaiyo, "Paper-based electrochemical biosensor for diagnosing COVID-19: Detection of SARS-CoV-2 antibodies and antigen," *Biosens. Bioelectron.*, vol. 176, no. December 2020, p. 112912, 2021, doi: 10.1016/j.bios.2020.112912.

[80] Y. Peng *et al.*, "An electrochemical biosensor for sensitive analysis of the SARS-CoV-2 RNA," *Biosens. Bioelectron.*, vol. 186, no. April, p. 113309, 2021, doi: 10.1016/j.bios.2021.113309.

[81] H. Zhao *et al.*, "Ultrasensitive supersandwich-type electrochemical sensor for SARS-CoV-2 from the infected COVID-19 patients using a smartphone," *Sensors Actuators, B Chem.*, vol. 327, no. July 2020, p. 128899, 2021, doi: 10.1016/j.snb.2020.128899.

[82] J. P. Broughton *et al.*, "CRISPR–Cas12-based detection of SARS-CoV-2," *Nat. Biotechnol.*, vol. 38, no. 7, pp. 870–874, 2020, doi: 10.1038/s41587-020-0513-4.

[83] N. Cennamo *et al.*, "SARS-CoV-2 spike protein detection through a plasmonic D-shaped plastic optical fiber aptasensor," *Talanta*, vol. 233, no. March, p. 122532, 2021, doi: 10.1016/j.talanta.2021.122532.

[84] S. L. Lee, J. Kim, S. Choi, J. Han, G. Seo, and Y. W. Lee, "Fiber-optic label-free biosensor for SARS- CoV- 2 spike protein detection using biofunctionalized long-period fiber grating," *Talanta*, vol. 235. 2021, doi: 10.1016/j.talanta.2021.122801.

[85] L. Huang *et al.*, "One-step rapid quantification of SARS-CoV-2 virus particles via low-cost nanoplasmonic sensors in generic microplate reader and point-of-care device," *Biosens. Bioelectron.*, vol. 171, no. July 2020, p. 112685, 2021, doi:

10.1016/j.bios.2020.112685.

[86] M. D. T. Torres, W. R. de Araujo, L. F. de Lima, A. L. Ferreira, and C. de la Fuente-Nunez, "Low-cost biosensor for rapid detection of SARS-CoV-2 at the point of care," *Matter*, vol. 4, no. 7, pp. 2403–2416, 2021, doi: 10.1016/j.matt.2021.05.003.

[87] M. Thanihaichelvan *et al.*, "Selective and electronic detection of COVID-19 (Coronavirus) using carbon nanotube field effect transistor-based biosensor: A proof-of-concept study," *Mater. Today Proc.*, vol. 19, no. xxxx, 2021, doi: 10.1016/j.matpr.2021.05.011.

[88] E. Karakuş, E. Erdemir, N. Demirbilek, and L. Liv, "Colorimetric and electrochemical detection of SARS-CoV-2 spike antigen with a gold nanoparticle-based biosensor," *Anal. Chim. Acta*, vol. 1182, 2021, doi: 10.1016/j.aca.2021.338939.

[89] A. Ahmadivand, B. Gerislioglu, Z. Ramezani, A. Kaushik, P. Manickam, and S. A. Ghoreishi, "Functionalized terahertz plasmonic metasensors: Femtomolar-level detection of SARS-CoV-2 spike proteins," *Biosens. Bioelectron.*, vol. 177, no. October 2020, p. 112971, 2021, doi: 10.1016/j.bios.2021.112971.

[90] Y. Gao, L. Qiang, and Y. Wang, "Rapid and sensitive triple-mode detection of causative SARS-CoV-2 virus specific genes through interaction between genes and nanoparticles," no. May, 2021, doi: 10.1016/j.aca.2021.338330.

[91] L. Liv, G. Çoban, N. Nakiboğlu, and T. Kocagöz, "A rapid, ultrasensitive voltammetric biosensor for determining SARS-CoV-2 spike protein in real samples," *Biosens. Bioelectron.*, vol. 192, no. January, 2021, doi: 10.1016/j.bios.2021.113497.

[92] F. E. Chen *et al.*, "Point-of-care CRISPR-Cas-assisted SARS-CoV-2 detection in an automated and portable droplet magnetofluidic device," *Biosens. Bioelectron.*, vol. 190, no. June, p. 113390, 2021, doi: 10.1016/j.bios.2021.113390.

[93] M. Zhang *et al.*, "Ultrasensitive detection of SARS-CoV-2 spike protein in untreated saliva using SERS-based biosensor," *Biosens. Bioelectron.*, vol. 190, no. June, p. 113421, 2021, doi: 10.1016/j.bios.2021.113421.

[94] N. F. Islam, H. Sarma, and M. Narasimhavara, *Emerging disinfection by-products in*

water : novel biofiltration techniques. LTD, 2020.

[95] D. Barceló, "Wastewater-Based Epidemiology to monitor COVID-19 outbreak: Present and future diagnostic methods to be in your radar," *Case Stud. Chem. Environ. Eng.*, vol. 2, no. July, 2020, doi: 10.1016/j.cscee.2020.100042.

[96] V. L. Colvin, "The potential environmental impact of engineered nanomaterials," vol. 21, no. 10, pp. 1166–1171, 2003.

[97] B. D. Malhotra and M. A. Ali, "Nanomaterials in Biosensors Fundamentals and Applications," in *Nanomaterials for Biosensors*, 2018, pp. 1–74.

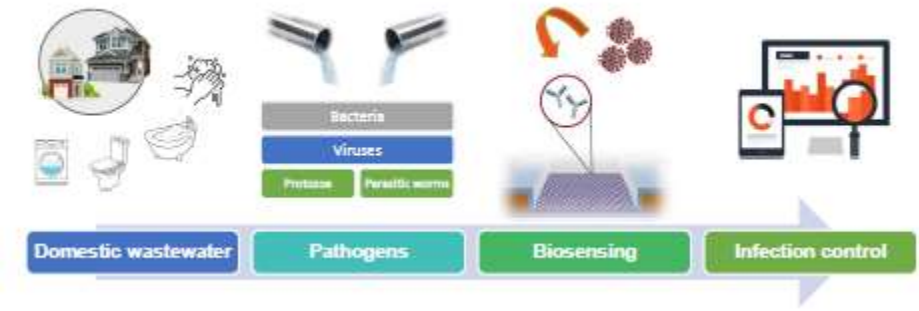
[98] L. Falciola, V. Pifferi, and A. Testolin, *Detection methods of wastewater contaminants*. Elsevier Inc., 2020.

[99] Q. Hui, Y. Pan, and Z. Yang, "Paper-based devices for rapid diagnostics and testing sewage for early warning of COVID-19 outbreak," *Case Stud. Chem. Environ. Eng.*, vol. 2, no. November, p. 100064, 2020, doi: 10.1016/j.cscee.2020.100064.

[100] J. Rainbow, E. Sedlackova, S. Jiang, and G. Maxted, "Integrated Electrochemical Biosensors for Detection of Waterborne Pathogens in Low-Resource Settings," *Biosensors*, 2020, doi: 10.3390/bios10040036.

[101] Y. Ibrahim, M. Ouda, D. Kadadou, F. Banat, and V. Naddeo, "Detection and removal of waterborne enteric viruses from wastewater : A comprehensive review," vol. 9, no. February, 2021, doi: 10.1016/j.jece.2021.105613.

Graphical abstract



Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Wastewater biosensors could revolutionize the field of screening for diseases.
- The complexity of wastewater challenges the real-life application of biosensors.
- Mass testing could be achieved through wastewater biosensors.
- Some biosensors have proven to have lower LODs than conventional detection tools.