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Review

Biosensors for wastewater-based epidemiology for monitoring public health

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ABSTRACT

Public health is attracting increasing attention due to the current global pandemic, and wastewater-based epidemiology (WBE) has emerged as a powerful tool for monitoring of public health by analysis of a variety of biomarkers (e.g., chemicals and pathogens) in wastewater. Rapid development of WBE requires rapid and on-site analytical tools for monitoring of sewage biomarkers to provide immediate decision and intervention. Biosensors have been demonstrated to be highly sensitive and selective tools for the analysis of sewage biomarkers due to their fast response, ease-to-use, low cost and the potential for field-testing. This paper presents biosensors as effective tools for wastewater analysis of potential biomarkers and monitoring of public health via WBE. In particular, we discuss the use of sewage sensors for rapid detection of a range of targets, including rapid monitoring of community-wide illicit drug consumption and pathogens for early warning of infectious diseases outbreaks. Finally, we provide a perspective on the future use of the biosensor technology for WBE to enable rapid on-site monitoring of sewage, which will provide nearly real-time data for public health assessment and effective intervention.

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1. Introduction

Public health issues are gaining increasing attention, including drug abuse, food safety, heavy metal and pesticide exposure, infectious diseases and noncommunicable diseases. Being able to assess these threats in a timely manner and implement the corresponding countermeasures will have a significant impact on public health. Human biomonitoring is a powerful tool for public health assessment by analysis of various biomarkers in human specimens; however, this approach has certain limitations, including sampling biases, extended time requirements, need for complex data elaboration to extrapolate the results to the whole population, high costs and ethical issues (Gracia-Lor et al., 2018).

Wastewater-based epidemiology (WBE) is an effective biomonitoring tool that is receiving increasing attention due to low cost and near real-time response. WBE is an emerging emergingemerging technique related to the extraction, analysis, data processing and interpretation of the targets excreted in faeces/urine and wastewater to provide public health information in certain areas. WBE has been extensively used in the evaluation of drug abuse (Zuccato et al., 2008; Zuccato et al., 2005), food safety (Choi et al., 2019), assessment of exposure to poisons, such as heavy metals (Markosian and Mirzoyan, 2019) and pesticides (Devault and Karolak, 2020), and in other public health aspects, such as prevention of infectious disease outbreaks (Bivins et al., 2020; Murakami et al., 2020; Sims and Kasprzyk-Hordern, 2020). For example, environmental surveillance of poliovirus in wastewater has been performed in Finland (Hovi et al., 2011) and Israel (Roberts, 2013) to assess the levels of poliovirus circulating within the populations. World Health Organization (WHO) has released guidelines for environmental sampling to monitor poliovirus in wastewater (Sims and Kasprzyk-Hordern, 2020). In addition, antiviral drugs have been detected in wastewater (Prasse et al., 2010), and the consumption of antiviral drug (oseltamivir) has been evaluated using WBE (Singer et al., 2013). These approaches can be potentially extended for additional applications for public health assessment.

Rapid development of WBE has increased the importance of appropriate analytical tools for accurate qualitative and quantitative analysis of various targets in wastewater (Choi et al., 2018; Gracia-Lor et al., 2017). The analytical tools used to evaluate these targets are mainly based on expensive instruments that must be located in

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a laboratory and operated by professional technicians, such as inductively coupled plasma mass spectrometry (ICP-MS) for the detection of heavy metal ions, liquid chromatography-tandem mass spectrometry (LC-MS) for the detection of small organic chemicals or their metabolites, and polymerase chain reaction (PCR) for the detection of nucleic acids and genetic information; these methods are characterised by high sensitivity, selectivity and stability (Hernández et al., 2018). However, the requirements for an expensive instrument and restriction of the analysis to a laboratory limit the broad applications of these techniques (Mao et al., 2017). Additionally, in some cases, dynamic changes in pollutants in wastewater have to be measured over time and at different locations. Therefore, traditional laboratory analysis is unable to meet the requirements of on-site wastewater monitoring thus limiting the development of WBE (Ejeian et al., 2018). There is a need to develop rapid and portable assays that enable to perform the analysis at the site of sample collection. Biosensors have recently emerged as effective alternatives that demonstrated potential ability to test sewage in the field (Hernández et al., 2018).

Biosensors are powerful analytical tools that have received considerable attention due to their excellent characteristics and are becoming increasingly important in healthcare, environmental monitoring (Mao et al., 2020b), food safety (Rebe Raz et al., 2010) and drug discovery (Mao et al., 2020a). Biosensors are small devices that utilize biochemical reactions mediated by a biological receptor/biorecognition element, such as nucleic acids, antibodies, enzymes, cells and even microorganisms, and usually detect targets based on optical, electrical, thermal and other signals (Chen et al., 2019). Biosensors are characterized by a fast response, low cost and potential miniaturization with other portable devices that can be operated by nontechnical personnel to measure target parameters using a small number of samples on site (Ejeian et al., 2018). Biosensors have played an important role in quantification of drugs, pharmaceuticals, biomolecules and microorganisms in wastewater for assessment of drug/pharmaceutical consumption in a community and population health. Furthermore, biosensors for analysis of sewage can potentially provide real-time data for government agencies to monitor trends in drug consumption and establish an effective early warning system to prevent community-based disease outbreaks (Mao et al., 2020c). Therefore, the results obtained by biosensors can reasonably reflect the toxic effects of various pollutants in wastewater. This information provides strong technical support for the on-line detection of wastewater and the development of an early warning sensing system.

This paper discussed that WBE-based biosensor technology can be used to assess public health, summarizes the latest progress in the research field, and discusses the feasibility of future applications of the technology in public health. Initially, we briefly introduce WBE as an effective tool for biomonitoring of drug abuse, evaluation of population exposure and other aspects, and discuss the feasibility of monitoring of numerous pollutants in sewage as WBE biomarkers for public health assessment (shown in supporting information). Then, we review various recent progress of biosensors in wastewater analysis and discuss their great potential for the detection of wastewater biomarkers in various areas of public health. Finally, we discuss the possibility and potential of WBE-based biosensor technology for expanded use in public health assessment, including applications relevant to the COVID-19 pandemic.

2. Biosensors for wastewater analysis of potential biomarkers

This section is focused on expanded applications of biosensors for the detection of various pollutants and sewage biomarkers in wastewater that can be potentially used for WBE-based public health assessment.

2.1. Inorganic ions

Heavy metals are not degradable and are frequently detected in wastewater (Markosian and Mirzoyan, 2019). The use of biosensors is becoming a potential choice for the detection of heavy metal ions in wastewater (Shtenberg et al., 2015); a detailed description is provided in Table S1, mainly including Hg (Fig. 1A)(Ezhil Vilian et al., 2017; Lee et al., 2019; Liu et al., 2018a; Liu et al., 2016; Tan et al., 2016), As (Fig. 1B)(Gu et al., 2018; Mao et al., 2020b; Song et al., 2016), Pb (Fig. 1C)(Li et al., 2019), Cr (Tsai et al., 2003; Wu et al., 2017), Cu (Fig. 1D) and several other heavy metal ions (Khan et al., 2020; Mao et al., 2015; Sekhon et al., 2017).

Aptamers are the main recognition elements used in heavy metal analysis in sewage. Aptamers are highly specific nucleic acid sequences that can recognize heavy metals and detect the corresponding heavy metal ions (Liu et al., 2009). At present, the main structures with known affinity to heavy metal ions include T-Hg-T (Wang et al., 2018b), C-Ag-C (Mao et al., 2015) and Pb-G-quadruplex (Yang et al., 2013). Certain nucleic acid sequences for the detection of some other metal ions can be identified by screening using systematic evolution of ligands by exponential enrichment (SELEX) technology; these sequences can be used as aptamers with high affinity to the target ions (Zhou et al., 2017). For example, an aptamer for arsenic is a fragment of a nucleic acid sequence (Mao et al., 2020b).

Previous studies on the analysis of inorganic targets have focused on the detection of heavy metal ions; however, interest in monitoring of nonmetallic ions is also increasing, including a microbial biosensor for sulfide (Liu et al., 2017), a cell biosensor for cyanide (Virender et al., 2018) and a microfluidics device for ammonium levels (Gallardo-Gonzalez et al., 2019). These biosensors rapidly and reliably provide real-time data and demonstrate good robustness and anti-interference performance in wastewater.

Generally, inorganic ion sensors are rarely used in wastewater analysis due to their limits of detection (LOD) because the target inorganic ions are usually present at a very low concentration in sewage. In addition, the complex wastewater matrix may lead to serious interference, which will affect the accuracy of detection. Current efforts investigate the use of these biosensors in wastewater analysis; however, only initial laboratory experiments are being performed, and additional verification is required. Only a few publications are related to these areas, although potential application of biosensors for the detection of various inorganic components in wastewater has been preliminarily demonstrated.

2.2. Organic pollutants and pharmaceuticals

Various organic pollutants have been detected in wastewater. Detection of these pollutants has been reported to involve numerous low-cost, easy and point-of-use sensors using highly specific chemical binding agents coupled to colorimetric, fluorescence or electrochemical biosensing platforms. As shown in Table 1 and Table S2, biosensors are used for the detection of various organic small molecules, and sensors for organic molecules in sewage are mainly focused on pesticides, pharmaceuticals and illicit drugs. Since illicit drug sensors are mainly related to drug abuse and consumption, the application of drug biomarkers in WBE is discussed in Section 3 of this paper, and pesticides and pharmaceuticals are mainly discussed in this section.

Various organic pollutants have been detected in industrial and agricultural wastewater (Rousis et al., 2017) (Table S2). The majority of reported biosensors are pesticide biosensors, such as a biosensor shown in Fig. 2A (Rousis et al., 2016; 2017). Biosensors for pesticides mainly target herbicides (Fan et al., 2016; Saberi et al., 2019) and insecticides (Kardaş et al., 2017; Shetti et al., 2019). The most frequently reported organic pollu-

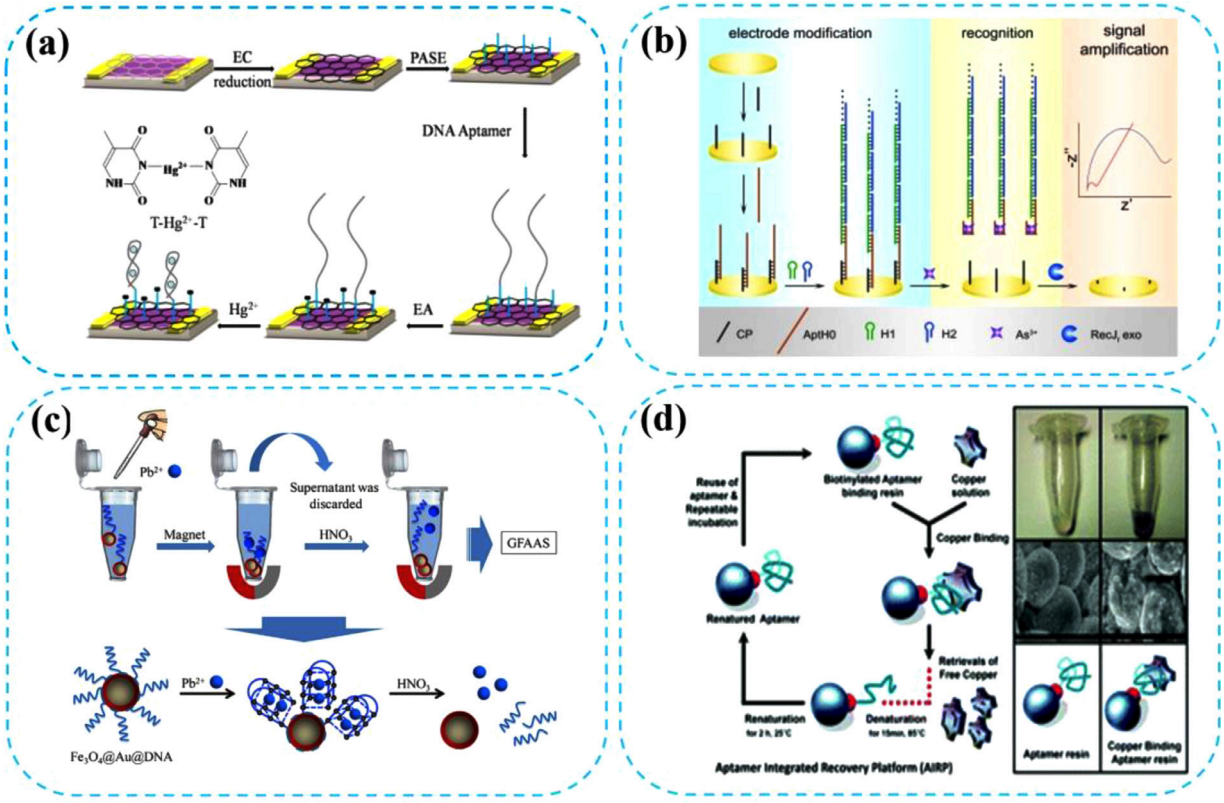


Fig. 1. (a) An electrochemically reduced graphene oxide chemiresistive biosensor for sensitive detection of Hg2+ ions (Reprinted with permission from ref. (Tan et al., 2016)); (b) An electrochemical detector of arsenic contamination based on the hybridization chain reaction and RecJf exonuclease-mediated amplification (Reprinted with permission from ref. (Gu et al., 2018)); (c) Functionalized magnetic composites based on aptamers for trace lead detection (Reprinted with permission from ref. (Li et al., 2019)); (d) Copper-binding aptamotif and aptamer-integrated recovery platform (Reprinted with permission from ref. (Sekhon et al., 2017)).

Table 1
Biosensors for pharmaceuticals in wastewater.

Target	Recognition	Detection signal	Linear range (nM)	LOD (nM)	Ref.
Ofloxacin	Aptamer	Electrochemistry	50.0-2.0 × 10^4	1.0	(Pilehvar et al., 2017)
Ciprofloxacin	Nanomaterial	Electrochemistry	5.0-50.0; 50.0-1.0 × 10^4	5.0	(Gayen and Chaplin, 2016)
	Aptamer	SELEX	-	-	(Reinemann et al., 2016)
Tetracycline	PFPT	Fluorescence	1.6-2.0 × 10^3	1.6	(Malik and Iyer, 2017)
Sulfonamide Antibiotics	MOF	Fluorescence	-	-	(Zhu et al., 2018)
Tetracycline	MOF	Luminescence	0-900.0	30	(Zhou et al., 2018)
Tetracycline hydrochloride	MOF	Luminescence	1.0 × 10^4-3.0 × 10^6	1.0 × 10^4	(Liu et al., 2018b)
FZD and NF	Nanomaterial	Fluorescence	-	-	(Wang et al., 2018a)
Oxytetracycline	Aptamer	Electrochemistry	0.2-1.1	3.8 × 10^{-5}	(Zhou et al., 2019)
Quinine	Aptamer	Personal glucose meter	0-2.0 × 10^3	320.0	(Qiu et al., 2018)
Omeprazole	Microbial, bacterium	Electrochemistry	-	684.4	(Pham et al., 2015)
Lansoprazole	Microbial, bacterium	Electrochemistry	-	257.2	(Pham et al., 2015)
β-Naphthoflavone	Microbial, bacterium	Electrochemistry	-	1.0 × 10^3	(Pham et al., 2015)
Methylcholanthrene	Microbial, bacterium	Electrochemistry	-	311.7	(Pham et al., 2015)
Salbutamol	Antibody	Fluorescence	1.2 × 10^{-2}-9.4	2.7 × 10^{-3}	(Fang et al., 2019)
Acetylsalicylic acid	rGO/AuNPs	Spectroelectrochemistry	880.0 -2.8 × 10^3	260.0	(Prado et al., 2017)
Carbamazepine	Antibody	Colorimetry	4.2- 211.6	4.2	(Ramos et al., 2019)
Diclofenac	Yeast cell	Fluorescence	10.0-5.0 × 10^4	10.0	(Schirmer et al., 2019)
Metformin	Nucleic acids	Fluorescence	2.1 × 10^3-1.0 × 10^4	630.0	(Machini et al., 2019)

PFPT: polyelectrolyte poly[5,5'-(((2-phenyl-9H-fluorene-9,9-diyl)bis(hexane-6,1-diyl))bis(oxy)) diisophthalate] sodium; MOF: metal-organic framework; rGO/AuNPs: reduced graphene oxide with AuNPs; FZD: furazolidone; NF: nitrofurazone.

tant biosensors target phenolic compounds (Akbulut et al., 2015; Mendes et al., 2017; Sanchez-Paniagua Lopez and Lopez-Ruiz, 2018; Sekretaryova et al., 2016; Zhang et al., 2017), mainly including bisphenol A(Allsop et al., 2019; Lim et al., 2018; Qi et al., 2019; Yan et al., 2020; Zehani et al., 2015), catechol (Liu et al., 2019c; Wee et al., 2019) and chlorophenols (Mendes et al., 2017; Yashas et al., 2019). Most of biosensors for phenolic compounds were built based on aptamers (seen in Fig. 2B) (Allsop et al., 2019; Lim et al., 2018; Qi et al., 2019) and bioactive enzymes (mainly laccase and tyrosinase) (Rahimi-Mohseni et al., 2019; Sekretaryova et al., 2016). Additionally, biosensors for organophosphorus pesticides (Bao et al., 2017; Gothwal et al., 2014; Kaur et al., 2015; Montes et al., 2018) and some other pesticides, such as nereistoxin, carbofuran, acephate, ametryn and thiram, have received some attention recently (Grawe et al., 2015; Qian et al., 2014; Takahashi et al., 2018).

Numerous publications described the detection of pharmaceuticals and their metabolites in wastewater, mainly including an-

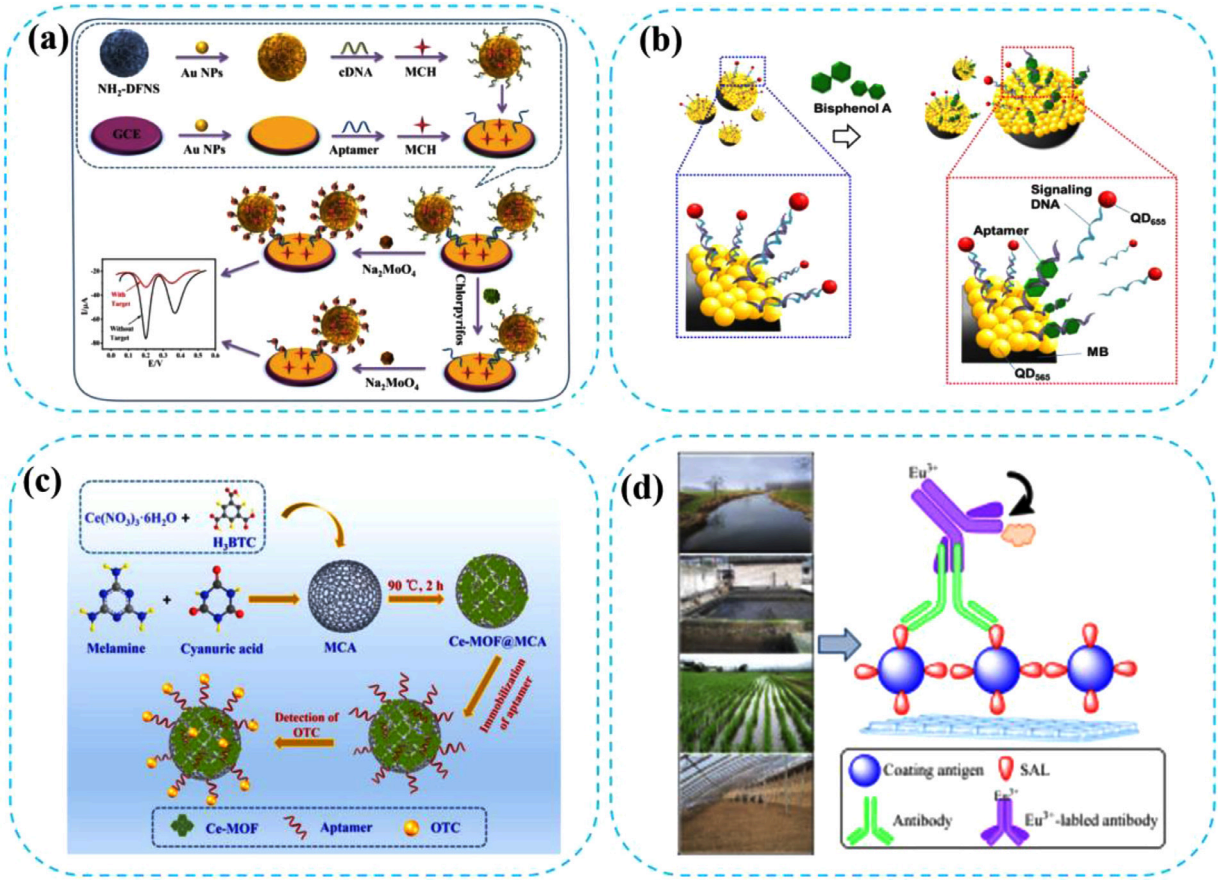


Fig. 2. Biosensors for organic pollutants and pharmaceuticals in wastewater. (a) Fluorometric label-free aptasensor for pesticide acetamiprid that uses cationic carbon dots (Reprinted with permission from (Saberi et al., 2019)); (b) Quantitative analysis of bisphenol A using a nanoaptamer assay (Reprinted with permission from (Lim et al., 2018)); (c) Construction of a MOFs-based label-free aptasensor for the detection of oxytetracycline residues (Reprinted with permission from (Zhou et al., 2019)); (d) A sensitive time-resolved fluoroimmunoassay for the detection of trace salbutamol levels (Reprinted with permission from (Fang et al., 2019)).

tibiotics, benzodiazepines, antidepressants, pharmaceutical opioids, asthma medicines and antihistamines, (Choi et al., 2018; Lai et al., 2011; Petrie et al., 2015; Phung et al., 2017; Thai et al., 2016). Biosensors for monitoring of pharmaceuticals, including drugs, antibiotics and antimicrobials in wastewater are listed in Table 1.

Antibiotics are one the major classes of antimicrobial drugs for the treatment of bacterial diseases and are the most frequently detected traditional pharmaceuticals in sewage. Biosensors are efficient analytical tools for the detection of various antibiotics and antimicrobial drugs in wastewater, such as ofloxacin (Pilehvar et al., 2017), ciprofloxacin (Gayen and Chaplin, 2016), quinine (Qiu et al., 2018), oxytetracycline (Fig. 2C) (Zhou et al., 2019), tetracycline (Liu et al., 2018b; Malik and Iyer, 2017; Zhou et al., 2018), sulfonamide antibiotics (Zhu et al., 2018), quinolone (Reinemann et al., 2016), furazolidone and nitrofurazone (Wang et al., 2018a). The main biological receptors of these biosensors are aptamers due to their specificity and stability (Pilehvar et al., 2017; Qiu et al., 2018; Zhou et al., 2019). As an example, Fig. 2C shows an unlabelled aptamer biosensor used to determine oxytetracycline levels in wastewater (Zhou et al., 2019). Aptamers offer considerable advantages over other biosensors, and various aptamers have been synthesised and screened for the detection of a wide range of antibiotics, such as quinolones (Pilehvar et al., 2017; Reinemann et al., 2016).

The signal detection strategies of biosensors for antibiotics and antimicrobial drugs mainly include electrochemistry (Gayen and Chaplin, 2016), fluorescence (Wang et al., 2018a; Zhu et al., 2018), colorimetry (Ramos et al., 2019) and luminescence (Liu et al., 2018b; Zhou et al., 2018). In addition, Qiu et al. integrated biosensors into portable devices and used a glucose meter for signal detection to improve visibility and provide easy on-site use (Qiu et al., 2018). Some nanomaterials, such as metal-organic frameworks (MOFs) (Fig. 2C), have been widely used in antibiotic biosensors (Liu et al., 2018b; Zhu et al., 2018). Application of these materials improves the analytical performance of the sensors in target detection.

The presence of conventional drugs, such as anticancer, anti-inflammatory, antidiabetic drugs and bronchodilator (Fig. 2D), in wastewater in addition to antibiotics is also a matter of increasing concern (Choi et al., 2018). Biosensors have been developed to measure the levels of these pharmaceuticals in wastewater, including nonsteroidal anti-inflammatory drug diclofenac (Schirmer et al., 2019) and antidiabetic drug metformin (Machini et al., 2019). Cell biosensors (Fang et al., 2019; Pham et al., 2015) and DNA biosensors (Congur et al., 2015; Machini et al., 2019; Prado et al., 2017) are the two main types of conventional pharmaceutical biosensors. For example, Ezgi et al (Bayram and Akyilmaz, 2016) developed a new microbial sensor based on a polyaniline/carboxylated multi-walled carbon nanotubes (MWCNT) complex and *Bacillus subtilis* for the electrochemical determination of paracetamol levels. This biosensor is characterised by rapid detection time and excellent overall performance compared with other biosensors used for paracetamol detection.

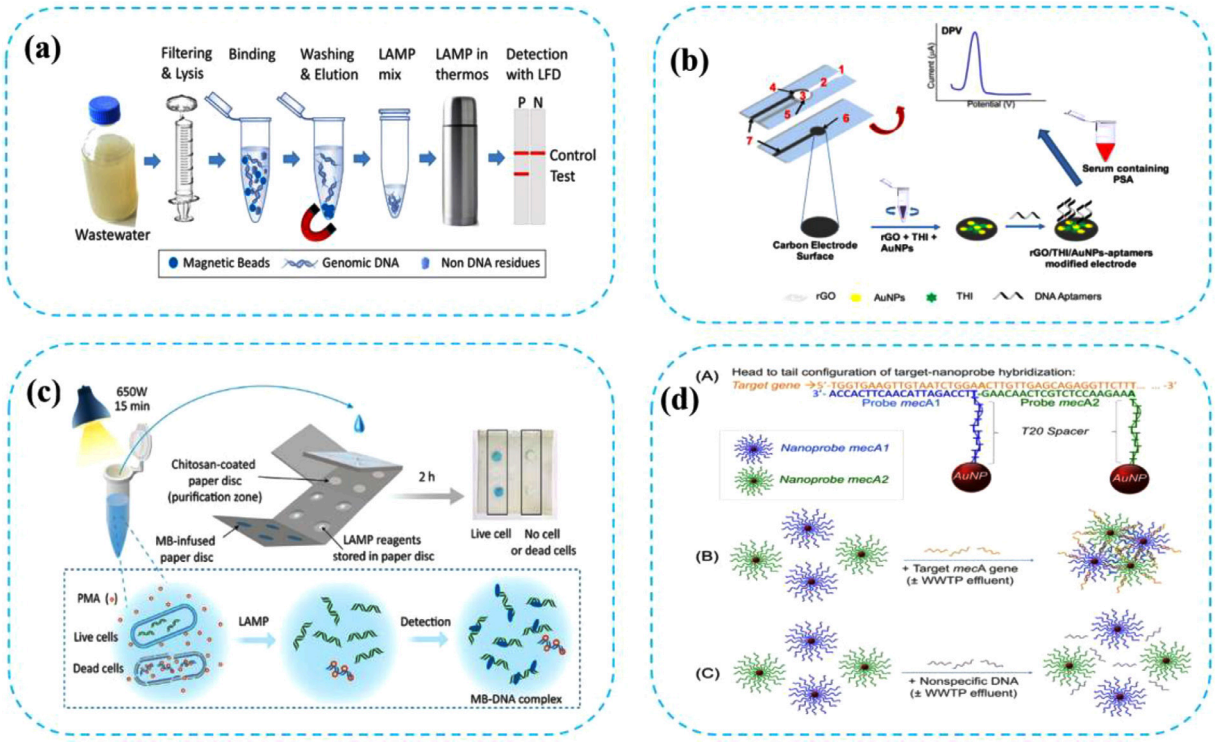


Fig. 3. Biosensors for biomolecules and microorganisms in wastewater. (a) Monitoring genetic population biomarkers for WBE (Reprinted with permission from (Yang et al., 2017)); (b) A graphene nanocomposite-modified electrochemical biosensor for prostate-specific antigen (Reprinted with permission from (Wei et al., 2018)). Biosensors for (c) microorganisms and (d) antimicrobial resistance markers in wastewater (Reprinted with permission from (Riquelme et al., 2017; Trieu and Lee, 2019)).

2.3. Biomolecules

Wastewater represents an ideal milieu for epidemiological studies of biomolecules because it contains a wide range of biomolecules, such as specific human nucleic acids, peptides, proteins and antimicrobial resistance markers (Bartsch et al., 2016; Costán-Longares et al., 2008; Dolejska et al., 2011; Goñi-Urriza et al., 2000; Montazeri et al., 2015; Rodriguez-Manzano et al., 2009; Shannon et al., 2007; Yang et al., 2015b). Currently, an increasing number of biosensors are being used to detect these biomolecules in wastewater.

2.3.1. Human nucleic acids

Although DNA has not been compared with other population marker criteria, scientists have started to detect and quantify the levels of DNA in wastewater nearly in real time using DNA-specific biosensors (Guo et al., 2018; Yang et al., 2015a). Yang et al. described the use of an electrochemical biosensor with a ferrocenyl dsDNA intercalator as a redox marker to monitor human-specific mitochondrial DNA (mtDNA) in wastewater (Yang et al., 2015a). The results confirmed the possibility of the analysis of human DNA in wastewater using designed biosensors. To improve the portability and user friendliness, a rapid visual platform was used for quantitative monitoring of mtDNA in community wastewater (Fig. 3A) based on loop-mediated isothermal amplification (LAMP) with a minimal level of user intervention within 45 minutes (Yang et al., 2017).

2.3.2. Peptides and proteins

Recently, peptides and proteins have attracted increasing attention as human biomarkers in WBE. Prostate-specific antigen (PSA) is a marker widely used to diagnose prostate cancer, and numerous studies focused on the development of a rapid sensing-based assay. Yang et al. initially constructed novel DNA-directed immobilization-based aptamer biosensors for PSA assay (Yang et al., 2015c). Then, for ease of use and portability, this electrochemical biosensor was improved to yield a paper device modified using graphene nanocomposites and aptamers (Wei et al., 2018). The paper device was developed using wax printing to generate hydrophilic and hydrophobic layers that formed a microfluidic channel, and three electrodes were subsequently screen-printed. As shown in Fig. 3B, synthetic nanocomposites were used for aptamer immobilization on working electrodes to enhance the sensitivity of the detection of PSA. A practical sample test indicated that the biosensor can potentially become a sensitive and cost-effective diagnostic platform for prostate cancer through WBE.

2.3.3. Antibiotic resistance genes (ARGs)

Antimicrobial resistance poses a substantial risk to human health (O'Neill and Humphreys, 2005). Resistant microorganisms and their ARGs are frequently present in wastewater, which makes ARGs the most extensively investigated contaminants in wastewater (Berendonk et al., 2015; Michael et al., 2013; Moura et al., 2010; Rizzo et al., 2013; Szczepanowski et al., 2009; Varela and Manaia, 2013). Biosensors have also been applied to ARG analysis. Fig. 3D describes a stable oligonucleotide-functionalized gold nanosensor for monitoring of *mec*A ARG (Riquelme et al., 2017). In practical wastewater analysis, the *mec*A-specific nanosensor was stabile under environmental conditions and at high ionic strength, and demonstrated high selectivity in the presence of target interference. This contribution supported the environmental applicability of a novel, low-cost and field-deployable tool for wide-scale ARG analyses.

In summary, various biosensors, including optical and electrochemical sensors, have been used in wastewater analysis. In general, colorimetric and fluorescent sensors have poorer LODs than electrochemical sensors. Although surface-enhanced Raman scattering/spectroscopy (SERS) has a high sensitivity, it lacks stability,

Table 2
Biosensors for the analysis of biomarkers for wastewater-based epidemiology.

Target	Recognition	Detection signal	Linear range	LOD	Ref.
Oestrogen	Protein	Fluorescence	20.8-476.7 μg/L	1.05 μg/L	(Liu et al., 2019a)
Steroid oestrogen	Aptamer	SERS	0.01-50.0 nM	5.0×10^{-3} nM	(Liu et al., 2019b)
17-β-Oestradiol	Aptamer	Fluorescence	1-1×10^{5} pg/mL	1 pg/mL	(Lee et al., 2017)
Dopamine	Nanomaterials	Electrochemistry	5.0×10^{3}-2.0×10^{6} nM	200.0 nM	(Yuan et al., 2018)
17β-Oestradiol	Aptamer	Electrochemistry	1.0×10^{-6} -9.0×10^{-3}; 1.2×10^{-2}-23.0 nM	5.0×10^{-7} nM	(Rather et al., 2018)
Cocaine	Aptamer	Electrochemistry	10.0-5×10^{3} nM	10.0 nM	(Yang et al., 2016)
Cocaine	Aptamer	Colorimetry	1.0-150.0	3.3 nM	(Mao et al., 2019a)
Methamphetamine	Aptamer	Colorimetry	5.0-200.0 nM	0.5 nM	(Mao et al., 2019a)
mtDNA	Nucleic acids	Lateral flow test	10-10^{5} cells/mL	10.0 cells/mL	(Yang et al., 2017)
mtDNA	Nucleic acids	Electrochemistry	1.0×10^{-2}-100.0 nM	1.0×10^{-2} nM	(Yang et al., 2015a)
PSA	Aptamer	Electrochemistry	0.25-200 ng/mL	0.25 ng/mL	(Yang et al., 2015c)
PSA	Aptamer	Electrochemistry	0.05-200 ng/mL	10 pg/mL	(Wei et al., 2018)

which limits its application in practical wastewater analysis. Colorimetric and fluorescence sensors are based on optical detection and are easily disrupted by coloured or turbid samples. Additionally, fluorescent sensor aptamers need to be labelled, which increases their complexity and cost. Electrochemical aptasensor performance appears to be more reliable in wastewater matrices. These sensors have the lowest LODs and can be reused in some cases after regeneration of the electrode surface. For example, if an aptamer is directly immobilized onto the electrode surface, the chip can be reused after binding the target by inducing careful dissociation of the target. Because recognition of the molecules is based on a conformational change, the process is reversible. However, electrochemical detection often involves immobilization of aptamers onto electrodes, which can cause a reduction in or complete loss of the binding affinity of an aptamer, rendering the sensor ineffective. Although optical and electrochemical sensors are the two main types of sensors, some other sensors, such as piezoelectric, nanomechanical and mass-sensitive sensors, have been reported (Mao et al., 2019b; Yang et al., 2015b).

Biological receptor is another main element of a biosensor that has a critical influence on the selectivity and LOD. Currently, recognition elements mainly include aptamers, antibodies, enzymes and microorganisms. The advantages and disadvantages of various components have been reported in previous publications (Aragay et al., 2011; Fu et al., 2017; Liu et al., 2009; Schirhagl, 2014). In general, sensors assembled from these recognition elements demonstrate good potential in the laboratory; however, most of the currently available biosensors require significant improvements in sensitivity, selectivity, functionality and other characteristics before they can be applied for commercial use in wastewater. Thus, introduction of effective biological receptors (such as whole microorganisms or biomolecules) can improve applicability of biosensors for the detection of the analytes, particularly in complex wastewater samples. Considering increasing complexity and diversification of sewage pollutants, development of additional biological receptors requires high specificity and affinity to detect as many targets as possible.

3. Biosensors for monitoring public health via WBE

This section discusses the available WBE-based biosensor technologies for public health assessment, mainly including the analysis of drug biomarkers, population markers and health markers using biosensors, and the potential use of WBE-based biosensors for surveillance and early warning in infectious disease outbreaks. Details on potential biomarkers are presented in Table 2.

3.1. Evaluation of community-wide drug consumption

Drug abuse is a global problem that is difficult to monitor and evaluate because of its illegality. WBE is the widely used method of drug analysis and evaluation of drug consumption and abuse. Numerous drug biosensors have been described (Kumar et al., 2018; Mokhtarzadeh et al., 2015); however, only a few reports described the use of biosensors for the analysis of drugs in wastewater. In this section, we introduce several biosensors for drugs in wastewater.

In 2016, Yang et al. reported a novel electrochemical aptamer sensor for the assessment of community cocaine consumption through WBE (Fig. 4A) (Yang et al., 2016). The biosensor was used to detect and evaluate the cocaine concentration in wastewater from a city in south-western England for one week. Higher concentrations were measured on the weekend than on workdays; thus, the biosensor was useful for rapid on-site assessment of a drug use trend in the area. A cocaine metabolite benzoylecgonine can be detected in urine-based wastewater by a microbial fuel cell biosensor (Catal et al., 2019), which can be potentially used to monitor the levels of cocaine metabolites in wastewater.

Usually, various illicit drugs (methamphetamine, cocaine and others) are widely distributed and transported in wastewater. Therefore, a rapid duplexed colorimetric detection method for the detection of illicit drugs in wastewater was based on aptamers and noble metal nanoparticles (Mao et al., 2019a). Synthesized Au/Ag nanoparticles were functionalized with DNA reporter probes for methamphetamine and cocaine, respectively (Fig. 4B). Two capture probes for methamphetamine and cocaine were attached to magnetic beads. Higher affinity of an illicit drug to aptamer than that of a reporter probe and aptamer induced disassembly of the sandwich structure in the presence of illicit drugs, resulting in colour changes. The designed biosensor was able to analyse both methamphetamine and cocaine at a trace level within a wide dynamic range, confirming the potential application of WBE to evaluate drug consumption (Mao et al., 2021).

3.2. Population markers

Use WBE for quantitative or per capita evaluation of environmental factors or other health-related information about the exposure requires an estimate of the population size of the target area. In contrast to the usual methods for the estimation of the size of a population contributing to each sample based on the *de facto* population size (Castiglioni et al., 2013; O'Brien et al., 2014), the levels of certain molecules in wastewater, including endocrine substances, nucleic acid biomarkers, peptides and proteins, can be used as alternative markers/biomarkers of the population level. These molecules should possess the following characteristics: (1) ease of measurement, (2) a sewer mass load related to population size, (3) negligible degradation, and (4) a short mean residence time in the sewer network (O'Brien et al., 2017). Therefore, the fabrication of the tools for rapid and effective analytical methods for the detection of these human population markers is important,

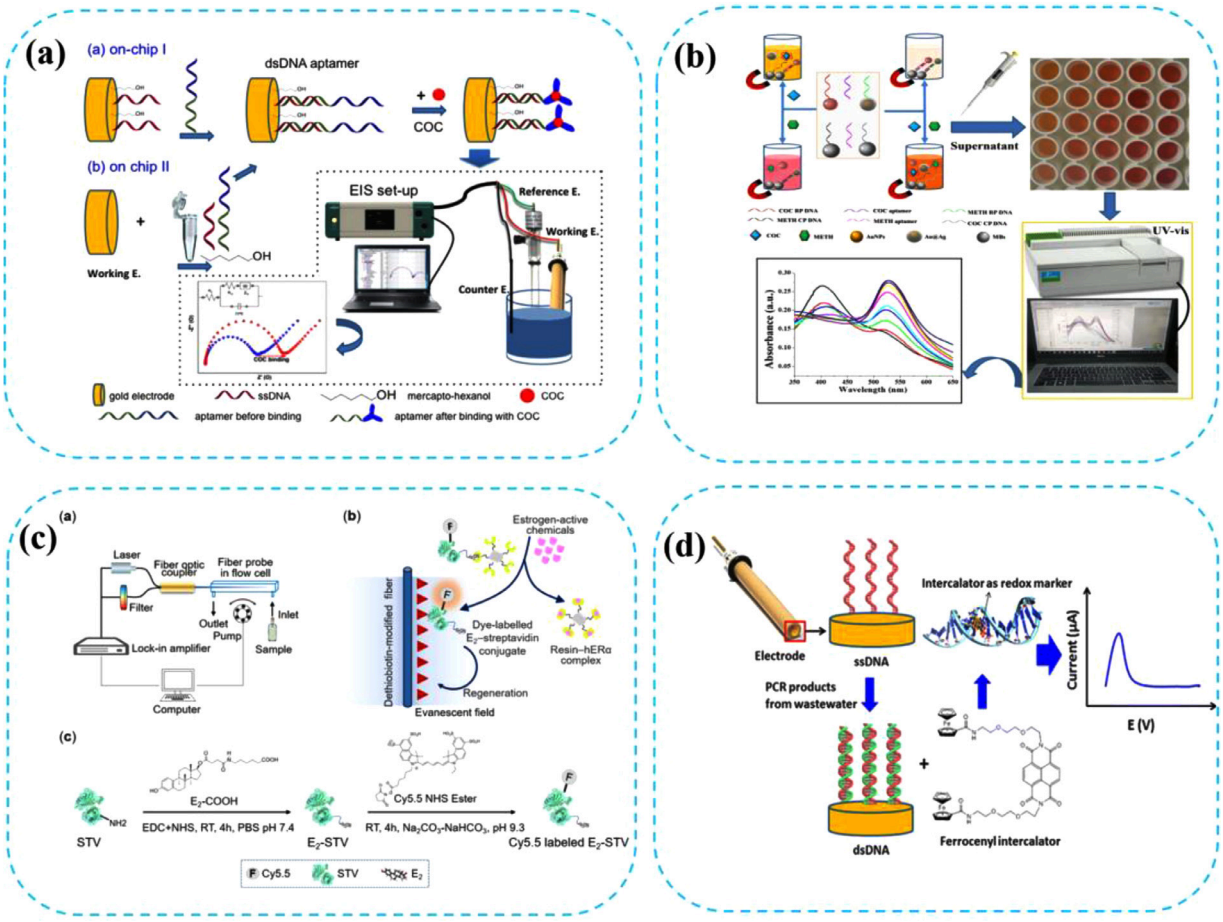


Fig. 4. Biosensors for WBE. (a) An electrochemical aptasensor for cocaine detection in community wastewater (Reprinted with permission from (Yang et al., 2016)); (b) Rapid duplex detection of illicit drugs in wastewater using an AuNP-conjugated aptamer biosensor (Reprinted with permission from (Mao et al., 2019a)); (c) A triple-functional small molecule-protein conjugated optical biosensor for the quantification of oestrogen compounds (Reprinted with permission from (Liu et al., 2019a)); (d) A novel DNA biosensor using a ferrocenyl intercalator for the determination of human health biomarkers in wastewater (Reprinted with permission from (Yang et al., 2015a)).

and scientists are committed to using the advantages of biosensors to detect these biomarkers. In this section, we discuss biosensors used to detect human population markers in wastewater.

3.2.1. Endocrine substances

Ideal markers of the human population level include endogenous substances specific to human metabolism and characterised by homogenous excretion throughout the community and low variance (Been et al., 2014; Liu et al., 2013; Thai et al., 2014). Biosensors have been used to detect these target substances, such as an electrochemical biosensor for the determination of dopamine levels (Yuan et al., 2018). Total oestrogens are ubiquitous endocrine substances that have been detected by SERS-based biosensors in wastewater (Liu et al., 2019b). Assays of single oestrogen substances based on biosensors have also been reported (Fig. 4C). For example, the analysis of 17-β-oestradiol may involve the biological receptors of biosensors, including protein (Liu et al., 2019a) and aptamer (Rather et al., 2018) and fluorescent (Lee et al., 2017) or electrochemical (Rather et al., 2018) biosensor signals. Rather *et al* .fabricated a femtosensitive aptasensor based on graphene-amplified strategy for electrochemical analysis of 17β-oestradiol (Rather et al., 2018).

3.2.2. Exogenous markers

Excretion of exogenous biomarkers, such as carbamazepine, gabapentin, and ibuprofen, is consistent through different populations and can be used as a population marker (O'Brien et al., 2017). Biosensors have been applied to detect these exogenous compounds in wastewater. Ramos et al. developed an automated method for the detection of carbamazepine (Ramos et al., 2019). A miniaturized and automated portable device based on an enzyme-linked immunosorbent assay (ELISA) rapidly detects carbamazepine in sewage. The proposed method combines the advantages of micro-bead injection spectroscopy and a flow-based lab-on-valve platform for automatic immunosorbent renewal, providing a new recognition surface for each sample. In addition, pharmaceuticals have been reported as markers of population size (Lai et al., 2011; O'Brien et al., 2014; Rico et al., 2017). Detailed discussion of the analysis of various pharmaceuticals using biosensors is provided in Section 2.

3.3. Health markers

The monitoring and evaluation of public health is an important component of regional health programs designed to map the current level of health in certain areas and thus help to improve human health. WBE can provide a potentially reliable and rapid evaluation of the overall population health through determination of health markers. In addition to analysis of pathogens, biomarkers are also used to analyse general public health indicators, such as obesity, high blood pressure and diabetes (Yang et al., 2015b). According to a recent report, the obesity level can be predicted by wastewater analysis (Newton et al., 2015).

A number of biosensors have been used to develop efficient analytical methods for the detection of health biomarkers (pathogen and disease biomarkers) in wastewater (Fig. 4D) (Ejeian et al., 2018). Yang et al .reported an efficient point-of-care platform for the detection of genetic biomarkers within communities based on wastewater analysis (Yang et al., 2017). Therefore, certain community sensors can be fabricated and used to evaluate the wastewater profiles and patterns of factors related to health and disease within populations within the framework of WBE. Additionally, biosensors can be miniaturized to fit a portable device for on-site analyses, which may facilitate diagnostics of infectious disease in developing countries with extremely high occurrence of certain diseases (e.g., tuberculosis (McNerney and Daley, 2011) and malaria (Xu et al., 2016)). These biosensors have potential applications in wastewater analysis as sewage sensors to evaluate urinary and faecal pathogens, monitor public health based on WBE and collect the data and information for epidemiological and socioeconomic studies.

Community sewage sensors are widely used to monitor various disease pathogens in a single assay. The use of these sensors may have considerable economic and social impacts, particularly in the areas with limited resources. For example, Yang et al. developed a rapid diagnostic platform for multiple infectious diseases utilizing a paper device (Yang et al., 2018). Moreover, biosensor platforms can collect information about community-wide health to implement effective interventions by health agencies and early prevention measures. Although the selectivity and long-term stability of community sensors and the environmental susceptibility to deterioration of the biorecognition elements are the problems that are yet to be solved, we suggest that efficient monitoring of health in communities will be possible in the future.

3.4. Biosensors for testing sewage for early warning of infectious disease outbreaks

Infectious disease outbreaks have become one of the main threats to public health; for example, the current COVID-19 pandemic caused by SARS-CoV-2 has led to a global disaster (Mao et al., 2020d). WBE is a powerful tool with huge potential for the surveillance and early warning of infectious disease outbreaks and transmission (Orive et al., 2020). The characteristics of infectious diseases in the associated area or community can be monitored by the analysis of infectious disease biomarkers in wastewater samples from WWTP influents and community wastewater (seen in Fig. 5).

Wastewater provides growth conditions for various microorganisms. These microorganisms are frequently detected in wastewater produced from domestic activities and the food industry, and are characterized by substantial changes in organic composition. The levels of pathogenic and nonpathogenic microorganisms vary depending on their sources. A wide range of aquatic microbial diseases cause significant mortality and morbidity worldwide. Moreover, the detection of microorganisms in wastewater is important for the elimination and control of their harmful pathogenic effects. In the current wastewater treatment process, microorganisms are difficult to completely remove from wastewater and are resistant to disinfectants due to their small size and chemical properties. Rapid and sensitive analytical tools are required to detect microorganisms; these tools can assist with the investigations of the outbreaks and development of prevention strategies.

Generally, for infectious diseases, information about a pathogen and host response is very important (Reddy et al., 2018). Pathogens may include viruses and bacteria that cause serious infections. The determination of the pathogens in wastewater influent through WBE can be used to trace the pathogen source. In the case of the COVID-19 pandemic, Mao et al tried to use WBE to analyse SARS-CoV-2 in urban sewage to identify potential virus carriers and provide early warning of COVID-19 outbreaks (Mao et al., 2020d). Important host response information in infectious diseases includes inflammatory processes and immune functions. For example, urinary protein and genetic information related to the diseases has been analysed in wastewater, and these parameters can be potential disease biomarkers for WBE (Mao et al., 2020d). Information about pathogens and host responses obtained in wastewater can be evaluated by WBE, which will provide new insight for public health of a community and may have important implications for potential infectious diseases. However, the complexity of the wastewater matrix makes it challenging to perform the analysis of pathogenic and host response information.

Available techniques for microorganism assays include culture-based methods, ELISA, and PCR (Altintas et al., 2015). These methods can be categorized into two mechanisms of microorganism detection. One of the mechanisms involves specific recognition of an element, such as bacterial aptamers, antibodies, host-guest relationships between bacteriophages and modification with chemical groups. In this section, we provide several examples of recognition of the elements. Abbaspour et al. developed an aptamer-modified silver nanoparticle for the electrochemical dual-aptamer-based sandwich detection of *Staphylococcus aureus*, which causes numerous diseases (Abbaspour et al., 2015). Ertürk et al. used bacteriophages as a recognition element in capacitive biosensors for a phage and host bacteria assay (Ertürk and Lood, 2018). The sensing mechanism depends on the binding of the target phage to specific cavities on the surface of an electrode, leading to a change in capacitance. This method provides a simple and rapid onsite measurement of the levels of a bacteriophage and its host bacteria. Modification with chemical groups has also been reported as a biological receptor for microorganism detection. Rengaraj et al. developed an impedimetric paper-based biosensor by screen printing carbon electrodes onto hydrophobic paper for determination of bacteria in contaminated water (Rengaraj et al., 2018). Due to its cost efficiency, simplicity and biodegradability, this biosensor can be used as a portable test kit that overcomes the limitations of traditional expensive and time-consuming laboratory-based analyses.

Biosensing techniques using smart affinity materials and nanomaterials can be used to measure microorganisms present in wastewater. For example, the levels of the MS2 phage in a water supply affected by wastewater can be determined. Prieto-Simón et al. (Prieto-Simón et al., 2015) developed an immunosensor that fixes a captured antibody on diazonium salt-electrografted SWCNTs through chemical assembly or magnetic capture orientation. The sensitivity of immunosensor was sufficient to determine the presence of MS2 at the level often encountered in the environment affected by wastewater, which is an important step in application of this biosensor for environmental analyses. Subsequently, Reta et al. (Reta et al., 2016) designed an unmarked ultrasensitive electrochemical immunosensor based on a nanoporous silicon membrane for detection of the MS2 phage. This study was the first to report a biosensor based on a nanoporous silicon membrane that uses the blocking effect of a nanochannel to transduce analyte binding.

Another method for the measurement of microorganisms involves the detection of specific nucleic acid sequences of a microorganism because each microorganism possesses a species-specific nucleic acid sequence. Nucleic acid testing is widely employed for environmental monitoring and disease diagnosis to confirm the presence of certain pathogens or microorganisms. The main method for pathogen nucleic acid analysis is nucleic acid-based PCR; however, various PCR inhibitors are present in wastewater and may affect subsequent PCR analysis. In addition, when DNA/RNA is extracted from the PCR inhibitor-rich samples, various commercial extraction kits sometimes show variable efficiency

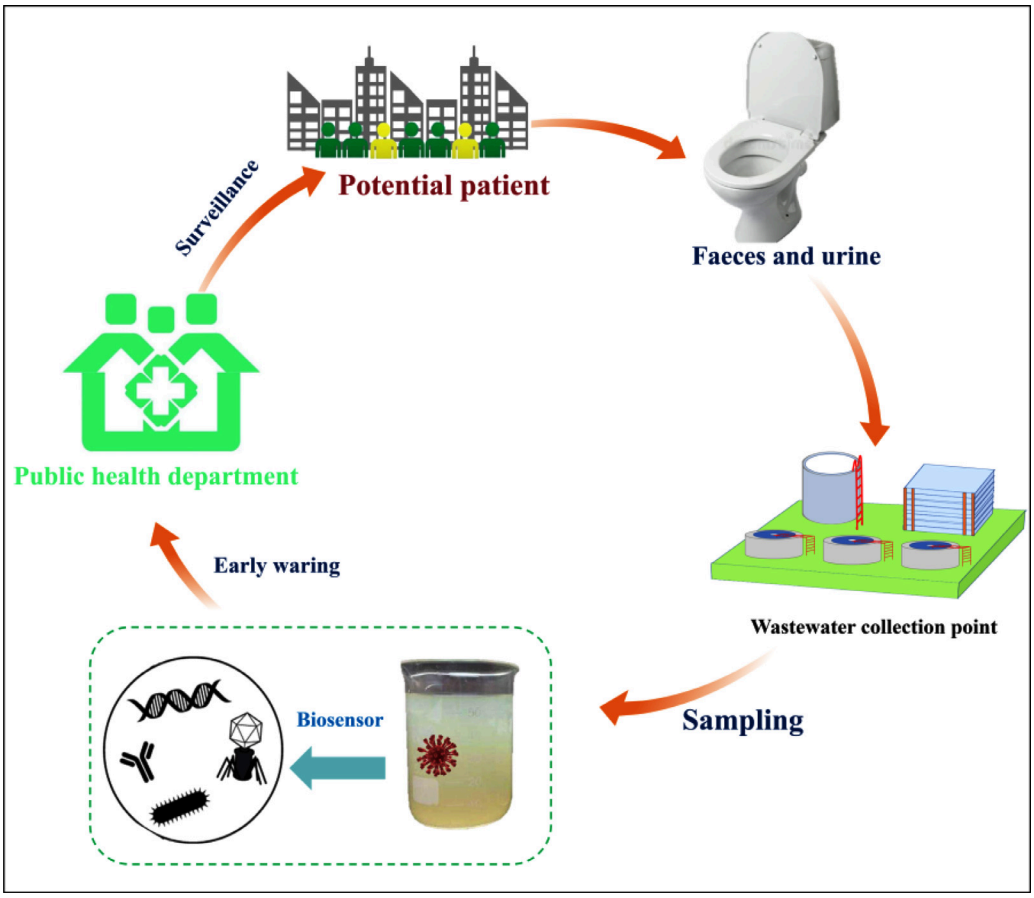


Fig. 5. Biosensors for testing sewage for early warning of infectious disease outbreaks. Adapted with permission from (Mao et al., 2020d).

and consistency (Mao et al., 2020d). This variability challenges meaningful comparisons across the studies and determination of spatiotemporal trends in the pathogens. To solve this problem, progress in the methods of molecular biology has provided new technologies for the analysis of genetic material, including digital PCR (dPCR) and next-generation sequencing. In dPCR, the absolute quantification of the target genes is determined using Poisson distribution statistics via the partitioning of DNA/RNA samples into tens and thousands of reaction wells (Sims and Kasprzyk-Hordern, 2020). This distribution diminishes the effects of PCR inhibitors in wastewater on the results of dPCR (Racki et al., 2014). Next-generation sequencing is another promising technology that provides substantial information about the complex microbial communities in the samples to enable identification of various pathogens (Petrie et al., 2016).

However, the ultimate goal of WBE is to achieve on-site monitoring and provide real-time data, which is required to provide timely early warning of infectious disease outbreaks. Analytical tools have to be simple, rapid, cost-effective, sensitive, selective and multiplexed. The latest developments in nucleic acid-based sensor methods enable field measurements; thus, a system can provide real-time information about infectious diseases and public health. Since the nucleic acid concentration in the samples is typically very low, the amplification protocol is very important for the subsequent detection process. Currently, nucleic acid testing mainly involves nucleic acid extraction, amplification and determination that require large instruments and trained personnel; thus, these tests are costly, time-consuming and inappropriate for point-of-care analysis. Ajonina et al. developed a LAMP-based biosensor for *Entamoeba histolytica* and *Toxoplasma gondii* in wastewater (Ajonina et al., 2018). LAMP is a rapid and stable nucleic acid detection method with ideal characteristics for point-of-care testing because it does not require thermal cycling similar to PCR. Lin et al. modified the LAMP technique and developed a digital LAMP method on a commercial membrane that does not require complex fabrication or specialized equipment (Lin et al., 2019). To facilitate the detection and improve portability, nucleic acid amplification techniques are successfully integrated into portable devices, such as mobile phones and paper devices. Huang et al .fabricated a smartphone-based in-gel LAMP system that enabled rapid quantification of the MS2 coliphage in wastewater samples (Huang et al., 2018). The results of in-gel LAMP were highly tolerant to the inhibitors naturally present in wastewater; in contrast, RT-qPCR was completely inhibited. Simplicity, speed, sensitivity, and versatility of the in-gel LAMP approach indicate its great potential for microbial wastewater quality analysis, particularly in low-income areas.

Development of paper-based microfluidic techniques resulted in recent fabrication of a variety of paper devices for nucleic acid testing. Fig. 3C shows a paper-origami device based on nucleic acid amplification for the point-of-care colorimetric identification of live cells (Trieu and Lee, 2019). However, these analytical methods using paper devices require off-chip reagent storage, complex operation steps and equipment-dependent nucleic acid amplification, restricting their use in point-of-care testing. Tang et al .developed a completely disposable and integrated sample-in-answer-out device for nucleic acid testing by integrating various processes for naked-eye detection into a paper device to overcome these limitations (Tang et al., 2017). This simple device allowed on-chip dried reagent storage and equipment-free nucleic acid amplification with simple operation steps and could be used by un-

trained people. Mao et al. have recently proposed to trace COVID-19 sources through sewage analysis using an efficient and inexpensive paper-based biosensor (Mao et al., 2020c), since paper-based sensors can measure the genetic material in wastewater. A paper sensor is a small analytical device with various functional areas that integrates all processes (extraction, enrichment, purification, elution, amplification and visual detection) required for nucleic acid testing into an inexpensive paper material. The whole testing process can be completed by simple folding of a paper-based device in various steps without a pump or power supply.

Overall, the research in this area is highly demanding; unfortunately, only several research groups have performed related studies (Yang et al., 2015b). This lack of effective analytical tools is an important challenge for the wide application of WBE to provide early warning of infectious diseases, and more efficient biosensors must be developed to acquire enough information about infectious diseases from wastewater.

4. Conclusion and perspectives

A variety of pollutants widely distributed in wastewater can efficiently provide information that reflects drug consumption, chemical exposure, food safety, and public health using WBE. As discussed in this review, biosensors represent a reliable alternative for tracking environmental pollutants by detection of various targets. Simplicity and reliability of detection of various contaminants by biosensors resulted in the development of an increasing number of biosensor-based methods. We have reviewed biosensors with various biological receptors, such as aptamers and antibodies, and various signal strategies, such as optical and electrochemical signals, which have been used to detect various targets in wastewater. Furthermore, interdisciplinary collaborations between analytical scientists and researchers in other areas (e.g., engineering) have provided some good examples of miniaturized and portable biosensors; furthermore, these devices can analyse the targets on site. Critical interdisciplinary success has been reported in this area, including the development of paper device-based and personal glucose meter-based biosensors.

Although biosensors have broad application prospects in wastewater analysis, their application remains a few challenges re. First, the prediction of the physical and chemical properties of various components of wastewater at various time points and conditions is difficult. The complex matrix of wastewater may affect the prediction of various parameters in a sample and interfere with the detection of the required target because this dynamic matrix precludes the inclusion of an adequate blank. Secondly, a new biosensor system with an expanded detection range and the ability to simultaneously detect many components is necessary to detect all types of contaminants in wastewater. Thirdly, the stability of fixed biological elements (such as enzymes, cells, antibodies, tissues, etc.) during shipment, storage and operating conditions is another major consideration. Fourth, this modern technology must also be evaluated by comparison with common analytical methods used to monitor wastewater. Moreover, quantitative and qualitative analytic guidelines must be established and standardized. Thus, certain technical and practical limitations and complexities are associated with this method. In the future, we should overcome these limitations to construct more effective biosensors for various applications. In addition, application of WBE has enormous potential for the analysis and monitoring of the pathogens and pollution health at the community level. Wastewater is a complex matrix, therefore, careful consideration of the microbes present in wastewater and certain standardization of analytical methods will form the basis for future studies and evaluation of wastewater as a tool for monitoring the microbial health and antimicrobial resistance.

Scientists are trying to solve these problems and technical limitations. For example, two major strategies are being developed to overcome the main obstacles caused by the use of biomolecules as the recognition elements to ensure high sensitivity of biosensors and improve their stability during long-term storage. These strategies include (I) the production of recombinant target-specific fragments and definition of the fixed characteristics of antibodies and enzymes to promote the development of tools to quickly and cost-effectively analyse the levels of organic pollutants in wastewater in *in situ* screening and (II) the use of a combination of nanomaterials and nanotechnology to improve the sensitivity and accuracy of biosensors, miniaturization and introduction of multiplexed sensor array devices to improve the detection efficiency. Current progress in nanotechnology can be used to increase the detection performance of biosensors. Wastewater biosensors will become an innovative approach for the simple and rapid monitoring of various targets in wastewater, even to the extent of field detection. These devices will provide strong technical support for the wide application of WBE in assessments of environmental pollution, drug abuse trends and public health and in surveillance and early warning of infectious disease outbreaks. In the future, biosensors for various biomarkers in wastewater must be sufficiently sensitive and selective to overcome the commercialization challenges by demonstrating stability, reproducibility, robustness, ease of use, portability and low cost.

Thus, we have proposed that sewage sensors can achieve the ultimate goal of on-site monitoring and providing real-time data within the framework of WBE. We believe that sewage sensors can be widely and rapidly applied in the near future to obtain high volume of information related to public health, including surveillance and early warning of infectious disease outbreaks, pathogen sources tracking, drug consumption, toxic substance exposure, food safety and disease evaluation.

Declaration of Competing Interest

The authors declare no competing financial interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2020.116787.

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