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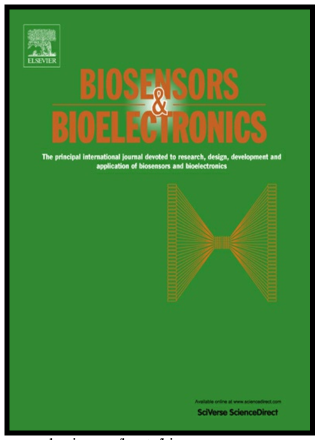

Biosensors for wastewater monitoring: a review

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Biosensors for wastewater monitoring: a review

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Abstract

Water pollution and habitat degradation are the cause of increasing water scarcity and decline in aquatic biodiversity. While the freshwater availability has been declining through past decades, water demand has continued to increase particularly in areas with arid and semi-arid climate. Monitoring of pollutants in wastewater effluents are critical to identifying water pollution area for treatment. Conventional detection methods are not effective in tracing multiple harmful components in wastewater due to their variability along different times and sources. Currently, the development of biosensing instruments attracted significant attention because of their high sensitivity, selectivity, reliability, simplicity, low-cost and real-time response. This paper provides a general overview on reported biosensors, which have been applied for the recognition of important organic chemicals, heavy metals, and microorganisms in dark waters. The significance and successes of nanotechnology in the field of biomolecular detection are also reviewed. The commercially available biosensors and their main challenges in wastewater monitoring are finally discussed.

Keywords:

Wastewater monitoring; Organic materials; Heavy metals; Microorganisms; Biosensor

1 Introduction

Water pollution and habitat degradation are the cause of increasing water scarcity and decline in aquatic biodiversity. While the freshwater availability has been declining through past decades, water demand has continued to increase especially in hot areas with low precipitation rate. According to the recent studies, at least 71% of world's population equivalent to 4.3 billion people faces some degree of water scarcity during some months of the year (Mekonnen and Hoekstra 2016). Although the limiting freshwater resources are identified as a global threat, this is mainly alarming in South East Asia, North Africa and the Middle East (Kummu et al. 2016; Mekonnen and Hoekstra 2016). Massive pollution of freshwater ecosystems has a diverse effect on the quality of well-known water sources (Brown and Matlock 2011).

Wastewater is composed of different harmful materials that have been adversely produced and discharged from domestic, industrial, commercial or agricultural activities (Cornish et al. 1999; Henze et al. 2008). The characteristics of wastewater are composed of their physical appearances, chemical parameters, and abundance of microorganisms. The composition and concentration of substances in wastewater vary widely and can be classified into three broad categories of (i) microorganisms, (ii) inorganic chemicals and (iii) organic pollutants. They can cause adverse environmental effects. For example disruption of endocrine and hormone systems, induction of cytotoxicity and/or genotoxicity and carcinogenic effects (Korostynska et al. 2013).

The concentration of constituents in wastewater is important for selection, design, and functioning of treatment processes, as well as for the reuse of waste. The variable composition of pollutants in wastewater over time and locations caused increasing attention to the development of new technologies to monitor the waste products, using inexpensive and real-time strategies (Michael-Kordatou et al. 2015; Rehman et al. 2015).

Many quality indicators are routinely used to measure the different characteristics of disposing or reused waste fluids. Most of them are based on laboratory techniques, which require frequent data sampling, sample pretreatment and therefore are slow and costly (Chong et al. 2013; Yang et al. 2015). These aspects support the necessity of developing new strategies that are more sensitive, portable and efficient in on-site detection of multi-pollutants consisting of a wide range of materials (Asadnia et al. 2016; Biswas et al. 2017).

Development of biosensors for detection of environmental pollutants has received considerable attention in recent years. Such sensors provide a great advantage to detect the minimal level of a contaminant in complex matrices, such as wastewater. Biosensors are also miniaturized systems that enable the development of portable sensors which can monitor effluent on-site (Tsopela et al. 2016). Considering the wide range of biorecognition methods (including immunochemical, enzymatic, non-enzymatic receptor, molecularly imprinted polymer (MIP), whole-cell and DNA elements), the different types of biosensors are categorized in this paper into (i) electrochemical, (ii) optical, (iii) piezoelectric and (iv) thermal biosensors, based on their transducing mechanisms (Turner 2000).

Over the past few years, a rapidly growing field of study started to develop powerful nanomaterial-based biosensors that exhibit remarkable properties for monitoring chemical reagents and biological phenomena (Li et al. 2013; Zhang et al. 2009).

The impact of biosensors on the evaluation of ground- and river-water quality has been reviewed recently (Biswas et al. 2017; Gumpu et al. 2015; Leonard et al. 2003; Sharma and Sharma 2014). Besides implications in the performance of traditional systems for the detection of pollutions in wastewater, the growing number of initiative actions, based on real-time biosensing of wastewater sources were identified. There are also many studies with a focus on the development of nanotechnological-based approaches in wastewater treatment (Berekaa 2016; Cloete 2010; Henze et al. 2008).

This paper presents a general overview on the latest commercialization and technological advances in the biosensor-based monitoring of three main categories of pollutants in wastewater (organic materials, heavy metals, and microorganisms), with a special focus on the influence of nanotechnology in this field. To provide a better understanding of appropriate sensing mechanisms, we categorize each section based on the biosensing detection methods and their applications. Current crucial issues in wastewater monitoring are identified, and our perspective of biosensor-based strategies for wastewater monitoring is discussed.

2 Biosensors for detection of organic materials

Organic pollutants may have a natural or anthropogenic origin, and a broad spectrum of them can be found in industrial, agricultural and household effluents. Organic chemicals in industrial wastewaters include various hydrocarbons, aromatic substances, chlorine compounds, diphenylmethane, and surfactants. Persistent organic pollutants (POPs) such as polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs) and polychlorinated biphenyls (PCBs) are found in wastewater, especially in the leachate from industrial waste (Weber et al. 2011). Organic pesticides and herbicides are other toxic compounds found commonly in agricultural wastewater that are widely used to control different pests, weeds and unwanted vegetation (Wauchope 1978). Some organic matter such as triclosan has been widely used in household cleaning products as antimicrobial agents that have a potential risk when discharged into wastewater and groundwater (Atar et al. 2015). One other important class of organic waste products are endocrine disrupting compounds (EDCs), which show the binding capability to the endogenous hormone receptors as agonists or antagonists and their sensing in wastewater has become imperative.

Monitoring the precise level of organic matter in wastewater plays a key role in the environment and human health, notably for wastewater treatment and water reclamation processes. There are several issues associated with using organic components in wastewater treatment application such as fouling in membrane filtration systems and the formation of harmful by-products (Tran et al. 2015).

There are two common parameters that are widely measured to analysis organic matter concentration of aqueous environments. First, the oxygen volume consumed by aerobic microorganisms to break down biodegradable organic substances, which is measured as biochemical oxygen demand (BOD) index. Second, the chemical oxygen demand (COD), which reflects the amount of oxygen in the water that is being used by chemical reactions (Wacheux 1998). The traditional technique for BOD characterization is based on the short-term *in situ* measurements or off-line monitoring over a period of 5 or 7 days. Whereas, biosensors provide much faster and precise results for detection of these indexes than conventional method (Bourgeois et al. 2001).

This section focuses on reviewing recent modern biosensing technologies designed to monitor organic components in different wastewater resources with a focus on different types of transducing mechanisms.

2.1 Optical biosensors for detection of organic materials

Table 1 summarizes recent studies on optical-based technologies in this field. Many whole-microorganism-based biosensors have been developed for detection of organic materials in aqueous samples. Among them, genetically engineered cell-based systems, coupled with luminescent as a reporter gene has been widely promoted (Struss et al. 2010). Whole cell biosensors applied for herbicides detection employ non-engineered algae (such as Chlorella) to assess any variation in chlorophyll fluorescence (Védrine et al. 2003).

Some organic pollutants in wastewater, such as triclosan, act as a target for molecular imprinting polymers (MIPs) and can be combined with surface plasmon resonance (SPR) to create a reliable detection technique. Molecular imprinted-SPR nanosensors were designed for the precise detection of some organic matters in wastewater, such as triclosan, through simulation of their biological receptors function (Atar et al. 2015).

Another common mechanism used in optical biosensors for monitoring organic matter is based on the immunoanalytical methods, which require fluorescently labeled antibodies with a high affinity to the target component. This method, known as fluorimetry is commonly used for signal transducing in optical biosensors of organic pollutants. In the past, this technique has been employed to monitor organic, environmental pollutants such as antibiotics, hormones, endocrine disrupting chemicals, and pesticides in groundwater samples (Fig. 1a) (Tschmelak et al. 2005).

Olaniran *et al.* reported an effective system that applied two whole-cell bacterial biosensors that were transformed with pLUX plasmids (*Lux*CDABE) (Olaniran et al. 2008). They showed this method can detect COD in wastewater by measuring changes in bioluminescence response.

2.2 Electrochemical biosensors for detection of organic materials

The detection of organic pollutants in wastewater can also be performed using electrochemical bio-receptors including enzymes, antibodies and whole cells (Table 2). The most successful technique in this category is the amperometric biosensors. Nomngongo *et al.* employed amperometric transduction mechanism in a biosensing system, based on horseradish peroxidase (HRP) bioreceptor to characterize persistent organic pollutants in wastewater samples (Nomngongo et al. 2012). The immobilization was performed by electrostatic attachment of

HRPs on polyaniline (PANi) modified platinum electrode (Fig. 1b). To measure the BOD in wastewater samples, a series of Clark-type oxygen sensors were tailored. As Fig. 1c shows, these systems have a simple common principle, composed of the immobilized microorganisms on a bio-membrane, which is coupled to an amperometric probe to quantify the concentrations of dissolved oxygen (Liu and Mattiasson 2002). Notably, Verma and Singh reported a biosensor that was fabricated by immobilized living microorganisms on cellulose acetate membrane, coupled to an amperometric gold/silver oxygen probe. This device is particularly suitable for analysis of BOD in industrial wastewater due to the fast response time of only 7 minutes, with a detection limit of 1 mg/L (Verma and Singh 2012). A novel electrochemical open-type biosensor has been studied by Yamashita *et al.* for the *in-situ* monitoring of BOD in the wastewater. The open-type anode, in contrast to normal closed-type ones freely exposed to oxygen in an aerated tank containing wastewater (Yamashita et al. 2016). This biosensor provides a high efficiency of detection in non-aerating conditions as well as aerating situations with a high correlation (R2 > 0.9) of generated current to the loading of BOD up to 250 mg/L in wastewater. Regarding the nanomolar level of many organic toxicants in wastewater, kou, *et al.* established a biosensor with a lower limit of detection (LOD) for neutral bisphenol A by using a MIPs based potentiometric sensor (Kou et al. 2013).

In recent studies, several microbial fuel cell (MFC)-based biosensors have been introduced for water quality testing. They are extensively used not only for checking dissolved oxygen (DO), BOD and COD but also in consideration of identifying and analyzing of different organic pollutants in water samples (Zhou et al. 2017). These electro-biosensors are mainly composed of a cathode chamber and an anode chamber (containing anaerobic bacteria), which are separated by a proton exchange membrane.

2.3 Thermal biosensors for detection of organic materials

Yao et al. introduced efficient biosensing system for COD monitoring through measuring heat change in water and wastewater. They used the periodic acid solution as oxidants and a flow injection analysis system to determine the COD values (Yao et al. 2014). This sensing mechanism gave a wide range and a low LOD for COD detection in water samples from various sources. Detected COD values in wastewater samples are correlated with the results from the dichromate method as traditional COD monitoring. The calorimetric COD determination method provides more effective detection results over the traditional approach from the aspect of robustness, long-term stability, and speed.

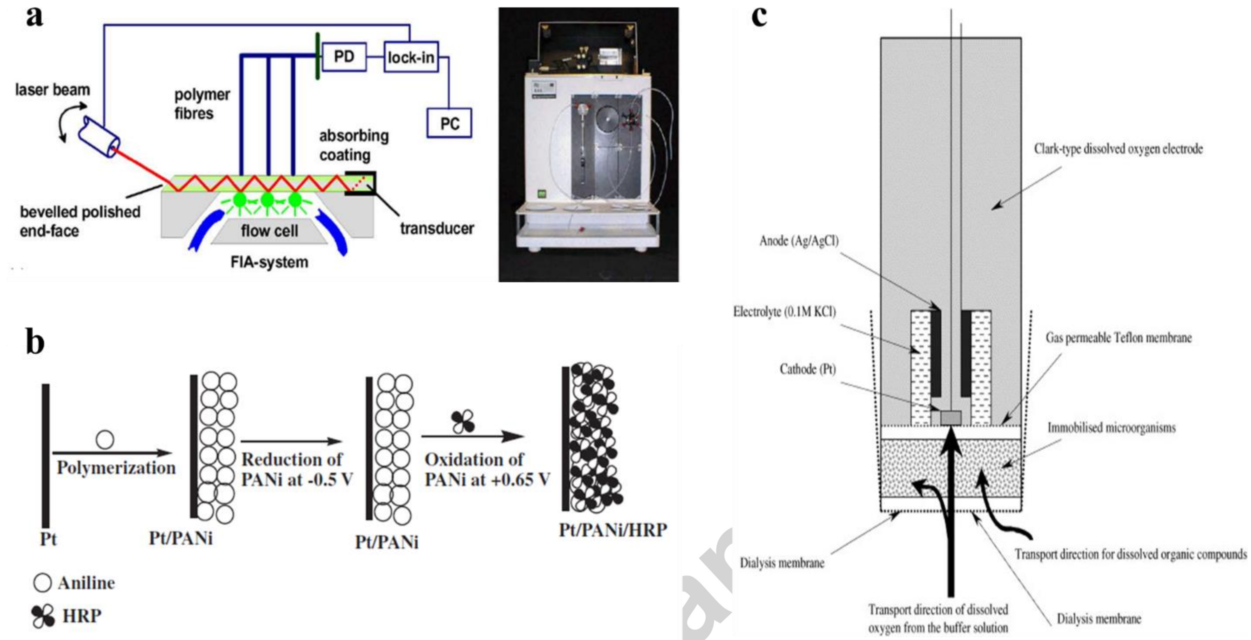

Figure 1. **Illustrations of biosensors for detection of organic pollutants and BOD in wastewater** (a) Schematic description of different parts of fully automated direct optical immunosensor for measurement of several organic component in river samples(Tschmelak et al. 2005), (b) Schematic representation of steps for immobilization of horseradish peroxidase on modified Pt electrode surface to monitor POPs in groundwater (Nomngongo et al. 2012) and (c) Schematic presentation of designed microbial conjugated Clark-type BOD sensor for monitoring of wastewater samples(Liu and Mattiasson 2002).

2.4 Summary and current challenges

This section summarizes the current achievements in the field of a biosensor for recognition and quantification of organic matter as well as the detection of BOD and COD in wastewater samples in direct relation to wastewater treatment. Despite the considerable attention given to biosensors for tracing of organic pollutants in wastewater, current devices are still unable to determine many organic materials such as pesticide and herbicide components in agricultural sewage (Nanekar and Juwarkar 2015). Finding appropriate and cost-effective biorecognition elements, biosensor calibration for organic matters in wastewater sample are the main challenges for improving organic pollutant detection by biosensors in wastewater.

Also, there is special consideration for finding trace amounts of some harmful organic materials in pharmaceuticals wastewater, such as various type of hormones, analgesics, antibiotics, lipid regulators and anti-inflammatory drugs (Das et al. 2017; Radjenovic et al. 2007; Tschmelak et al. 2005; Yuan et al. 2013).

It is clear that applying microorganisms and biomolecules in biorecognition systems can improve the accuracy of detection of organic contaminants in different wastewater resources. However, the limited number of applicable bio-receptors narrowed the range of the contaminants monitoring (Baeumner 2003). Therefore, there is a continuing quest for new bioreceptors.

In the case of whole cell-based biosensors, some secondary metabolites of organic substances in the wastewater leads to anomalously high readings of target organic pollutant. Therefore, a precise correction method that consumes only target organic matter is required. On the other hand, for bacterial-based biosensor constructs, their ability to fast and an *in-situ* indication of the biodegradable organic pollutants are usually not accompanied by quantitatively analysis capacity.

The high amount of organic materials, on the other hand, may hamper the biorecognition elements and lead to attenuation response (Von Sperling 2017). While this problem may be prevented by common pretreatment methods, adding a preparation step affects the simplicity and quickness of monitoring which is as a significant disadvantage of biosensing systems.

3 Biosensors for detection of heavy metals (HMs)

Heavy metal pollutions in wastewaters are commonly associated with agricultural sullage, chemical manufacturing, mining, cosmetic products, nuclear wastes, metal processing, painting, and other industrial effluents (Sayari et al. 2005). Biochemical reactions in living organisms depend on a certain threshold levels of essential HMs including Co, Cu, Fe, Mn, Mo, Ni, Se, and Zn. While, nonessential HMs, like lead (Pb), chromium (Cr), cadmium (Cd), mercury (Hg), arsenic (As) and antimony (Sb) are non-biodegradable, they are highly toxic and carcinogenic even at a trace level. They can bind to the surface of microorganisms and transport inside the food chain which may cause various human chronic diseases (Jaishankar et al. 2014).

Analytical methods such as atomic absorption spectroscopy, ultraviolet−visible spectroscopy, and chromatography for HMs detection have been widely used for controlling these pollutants. Although the already noted methods provide a sufficient level of sensitivity and selectivity, their uses are limited due to their time- and cost-consuming sample preparation and pre-concentration procedures (Gumpu et al. 2015). Recently, biosensors are introduced as an impressive alternative for conventional sensing systems due to their high sensitivity, simplicity, and detectable signals. Herein, we report several biosensors and nanobiosensors for monitoring HMs.

3.1 Optical biosensors for detection of heavy metals

Several microbial biosensors were designed to emit bioluminescence signals at the presence of low concentration of HMs (Jouanneau et al. 2012; Martín-Betancor et al. 2015; Olaniran et al. 2011). The bacterial biosensors use recombinant *Escherichia coli* strains that were developed based on their fluorescent protein expression. When these bacteria are in contact with the HMs, the expression of fluorescent proteins are enhanced to report the hazardous amount of HMs in wastewater samples (Raja and Selvam 2011; Ravikumar et al. 2012).

Enzyme-based biosensors function on the basis of inhibitory effects of HMs. To improve the efficiency of detection, based on their specific optical properties, porous silicon (PS) structures have been widely used as the enzyme immobilization substrate to monitor chromium ion in water and wastewater (Biswas et al. 2017). In the past, two optical fiber biosensors based on immobilized urease and acid phosphatase were employed to detect trace levels of HMs (Gani et al. 2010; Prakash et al. 2008; Tagad et al. 2016). Also, a rapid and effective β-galactosidase (β-GAL)-based colorimetric bioactive paper sensor was constructed, that was able to measure Ag^{+}, Ni^{2+}, and Cr^{4+}, in parts per million (ppm) concentration level. The changes in color intensity can be estimated by the naked eye and do not require external instrumentation (Fig. 2a) (Hossain and Brennan 2011).

With considering the fine progression created in the fields of DNA-based devices and nanotechnology, the development of high precision nanobiosensor based on fluorescence resonance energy transfer (FRET) present an effective method for multiplexed heavy metal detection. Xudong and co-workers designed a new innovative FRET sensor based on this technique. The receptor of this nanobiosensor consists of quantum dots (QDs) were conjugated with a thin silica layer that covalently coupled to DNAzymes and two quenchers. This can improve the selectivity and specificity of the system, owing to their high photostability. Against various metal ions, the QD reflected different fluorescence signals and exhibited differential between various metal ions (Fig. 2b) (Wu et al. 2010). Some multidimensional aptasensors also provide promising opportunities for simultaneous determination of multiple HMs in water samples, by taking advantage of Au- or Ag-NPs (Song et al. 2016; Tan et al. 2016; Zhan et al. 2015).

3.2 Electrochemical biosensors for the detection of heavy metals

Enzyme-based electrochemical biosensors are the most common biosensors for monitoring HMs. In this regard, a wide variety of enzymes such as glucose oxidase, urease, tyrosinase, peroxidase, etc. have been studied (Bachan Upadhyay and Verma 2013; Dong et al. 2018; Maleki et al. 2017; Moyo and Okonkwo 2014; Rao et al. 2014; Soldatkin et al. 2012; Wei et al. 2018). Furthermore, electrochemical aptasensors have been considered as promising tools in HM monitoring, due to their strong and stable interaction with very low levels of metal ions (Hayat and Marty 2014; Wu et al. 2012b; Zhang et al. 2018; Zhang et al. 2013).

Recently, single chamber batch-mode cube microbial fuel cell (CMFC) and double-chamber microbial fuel cell (DMFC) and sediment microbial fuel cell (SMFC) as new electro-biochemical sensors were designed for real-time HMs monitoring in wastewater influent (Zhao et al. 2018). These biosensors are based on the changes in voltage signal through the activity of immobilized electrogenic bacteria. According to the low detectable concentration of some HMs (such as Cr^{6+}, Cd^{2+}, Cu^{2+} and Zn^{2+}), these biosensors can be introduced as sensitive sensors for wastewater quality monitoring (Liu et al. 2014; Tao et al. 2017).

Integrating the electrochemical biosensors with nanomaterials could significantly improve the device performance (Nguyen et al. 2018; Song et al. 2018). For example, Chen et al. showed that the sensitivity of an electrochemical aptasensor fabricated for Cu^{2+} detection enhanced by applying gold nanoparticles (GNPs) (Chen et al. 2011b). The other example of the new generation of electrochemical nanobiosensor for quantitative detection of Hg^{2+} in industrial effluents takes advantage of guanine nanowire-based amplification (GWA) method to reach picomolar detection limit (Fig. 2c) (Huang et al. 2016). The biosensing process is based on the direct growth of guanine wire structures according to the formation of strong and stable T–Hg^{2+}–T mismatches in the presence of Hg^{2+}. The generated guanine nanowire on the gold electrode surface triggers the electrochemical H_2O_2-mediated oxidation of 3,3',5,5'-tetramethylbenzidine (TMB), which in turn results in a great increment in the electrical signal.

The applicability of modified Au electrode with multi-walled carbon nanotube (MWCNT)/peptide for co-detection of Cd^{2+} and Hg^{2+} was validated using industrial wastewater samples. The modified MWCNT electrode in accompanied with the peptides led to the enhancement of peak-current density, reaction rate, and high sensitivity. The LOD of these systems is below the mentioned level in environmental protection agency (EPA) guideline for Cd^{2+} and Hg^{2+} in drinking water (Rahman et al. 2012).

Ganjali *et al.* reported a nanobiosensor based on L-cysteine functionalized reduced graphene oxide (rGO) on glassy carbon (GC) electrode. It was used to detect multiple ions like Cd^{2+}, Pb^{2+}, Cu^{2+}, and Hg^{2+} in industrial effluents under optimized conditions. Regarding to the world health organization (WHO) guideline, this platform provides the opportunity to simultaneously measuring metal ions with acceptable LOD values. (Ganjali et al. 2011).

3.3 Piezoelectric biosensor for the detection of heavy metals

With the covalent attachment of metallothionein (MT) onto a quartz crystal, a piezoelectric biosensor was designed to monitor Zn^{2+} and Cd^{2+} in aqueous media (Saber and Pişkin 2003). Chen et al. established a piezoelectric nanobiosensor for precise detection of Hg^{2+}, using quartz crystal microbalance with dissipation monitoring (QCM-D) technology (Chen et al. 2011a). The sensing element of this system consisted of three types of DNA probes, in which the reporter DNA probe was coupled to GNPs. Through Hg^{2+} loading, the specific T–Hg^{2+}–T complex was assembled, which could not be hybridized with functionalized GNPs, and this resulted in dropping off the frequency and dissipation changes. The higher concentration of Hg^{2+} leading to the more hairpin formation by linker DNA and subsequent reduction in the GNP-mediated amplified QCM-D response(Fig. 2d) (Chen et al. 2011a). Likewise, a DNAzyme–based QCM-D biosensing instrument provided high sensitivity and selectivity toward Pb^{2+} ions (Teh et al. 2014).

Recently, ion sensors based on AlGaN/GaN HEMTs have been developed to monitor various ions. These devices are sensitive to charge characteristics at the surface and have the distinct advantage that a reference electrode is not required for operation, unlike many other types of ion sensors. Asadnia et al. showed that such devices coated with plasticised poly(vinyl chloride) (PVC)-based membranes containing an ionophore can be used to detect Hg^{2+} (Asadnia et al. 2016) and Ca^{2+} (Asadnia et al. 2017) ions in water.

3.4 Microfluidic devices for detection of heavy metals

Recently portable microfluidic devices have been extremely efficient for monitoring HMs in different fluids with the potential applicability for dark waters. As an innovative example, the integration of microfluidic approach to an optical transducing system formed a cost-effective biosensor for monitoring lead concentration. The sensing mechanism relies on the inhibition of immobilized urease in the presence of Pb^{2+}, which is followed by reducing substrate hydrolysis and can be quantified through the colorimetric changes of a pH-sensitive indicator (Verma et al. 2016).

Peng *et al.* developed a fluorescent-based microfluidic chip using magnetic multi-walled carbon nanotubes (MWCNTS) for tracing small amounts of Cr(III) and Cr(VI) in various water samples, with a detection limit of 0.094 nM (Peng et al. 2017). In the other way, a microelectromechanical system (MEMS) was fabricated through a bio-inspiration strategy that mimics shark's olfactory sensing system for on-site determination of lead (Wang et al. 2016). Cropek and coworkers also designed a highly sensitive microfluidic Pb^{2+} biosensor by immobilization of a lead-specific catalytic DNA on the polymethylmethacrylate (PMMA) microchannel (Dalavoy et al. 2008).

Several recent studies introduced microfluidic-based devices that utilize a field-effect transistor (FET) as its signal-transducing system (Kao et al. 2017; Vourdas et al. 2017). The sensing microchannel structure was composed of protein-functionalized rGO thin-films that can detect metal ions in various aqueous solutions (Sudibya et al. 2011). Furthermore, the plasmonic properties of AuNPs and high affinity of T-containing oligonucleotides to Hg^{2+} used to design a new microfluidic device, based on oligo-T/AuNPs. This device is fully portable and rapidly responds to Hg^{2+} within microfluidic channels at room temperature (Fig. 2e) (He et al. 2008).

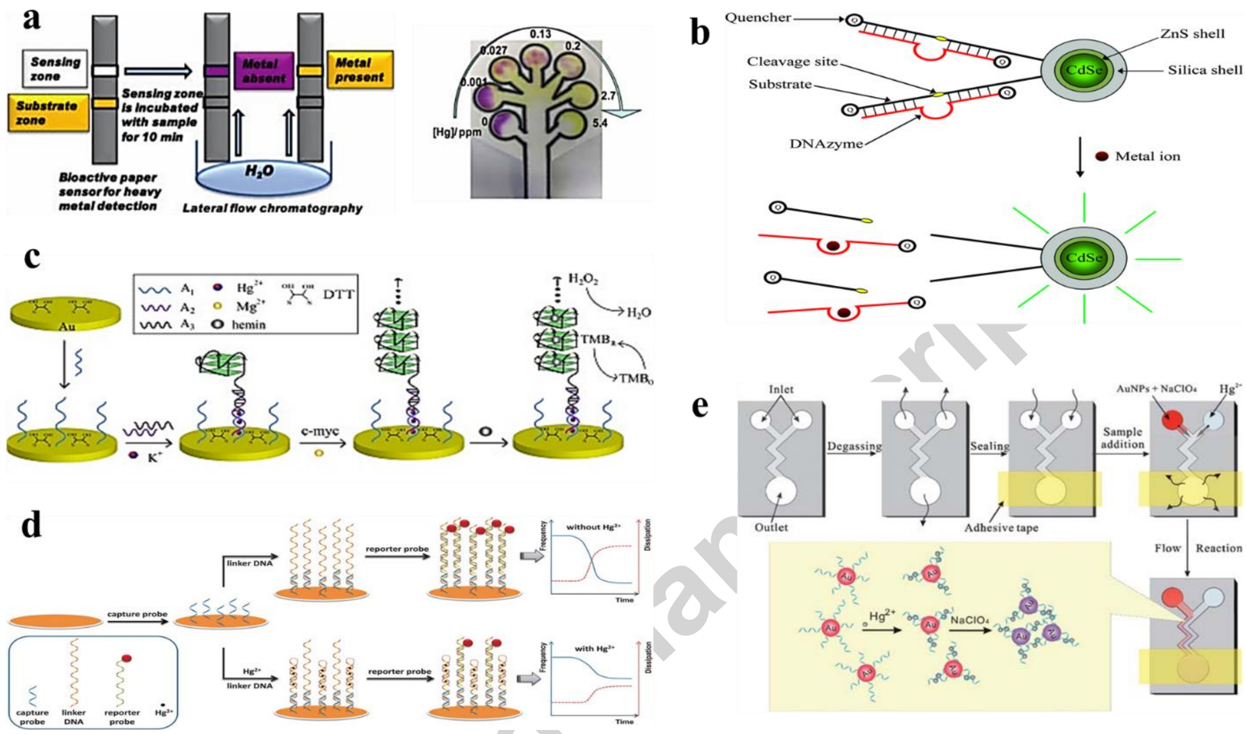

Figure 2. Illustrations of biosensors for detection of heavy metals (a) Schematic description for detection principle of β-GAL based optical biosensor for sensing of Hg ion with various concentrations (Hossain and Brennan 2011), (b) Schematic diagram QDs-DNAzyme based FRET nanobiosensor for detection of metal ion (Wu et al. 2010), (c) Schematic plane of the working mechanism of a label-free guanine nanowire amplification-based Hg^{2+} biosensor (Huang et al. 2016), (d) Schematic presentation of GNPs-enhanced QCM-D sensor for Hg^{2+}, composed of a DNA capture probe, a linker DNA , and a DNA reporter sequence tagged with GNPs (Chen et al. 2011a), and (e) The schematic presentation of a power-free biosensing system for calorimetrically detection of Hg^{2+} by using modified Au nanoparticles (He et al. 2008)

3.5 Summary and current challenges

While a considerable amount of heavy metals in real-world wastewater samples form stable complexes with organic materials, unfortunately in most cases biosensors were designed for detection of free HM ions. Also, wastewater samples may contain particulates, or organic materials can interfere with the sensing signals (Koedrith et al. 2015; Li et al. 2013). Consequently, the samples may require pretreatment in the laboratory before sensing.

When the sample is transparent (has a turbidity of less than one nephelometric turbidity unit (NTU)) and single phase, it can be analyzed directly. Nevertheless, for dark waters, the filtration of the sample or convenient digestion procedures are suggested. Nowadays, the majority of these biosensing systems are still described as scientific reports, and only a few of them are commercially available for large-scale monitoring of water and wastewater effluents.

4 Biosensors for detection of microorganisms

The large spectrum of waterborne microbial diseases causes notable mortality and morbidity all over the world (Henze et al. 2001). Sewage provides an ideal growth condition for different types of microorganism such as bacteria, viruses, protozoa, algae, fungi, and yeasts. They are mainly released to wastewater from household activities and the food industries, which are characterized by a great variation in their organism's composition. The level of pathogenic, nonpathogenic, saprophytic, autotrophic and heterotrophic forms of microorganisms vary with their sources (Gerardi 2003).

Non-pathogenic fecal bacteria (like *E. coli*, total coliforms, and RNA bacteriophages) that are routinely found in wastewater has little effect on human health. On the other hand, the concentration of parasites and pathogenic viruses like *Cryptosporidium* and *Giardia* in wastewater are directly associated with the disease rate in population, which may reflect the public health issues (Grøndahl-Rosado et al. 2014). Also, the minimal infective dose is different for various microbial pathogens. For example, based on U.S. Environmental Protection Agency policy (U.S. EPA, 1992), most bacteria are hazardous when there are more than 10^3 infectious cells, but viruses and protozoa can cause infection with less than ten infectious particles

Conventional methods for microbial pathogens detection need to use an artificial medium or microscopic methods, which are accompanied with several technical limitations such as low detection accuracy, sensitivity, sample processing complexities and time-consuming. For instance, coliform assays are traditionally used to assess coliform bacteria in the environmental samples instead of overall monitoring of microorganisms (Lee et al. 2006).

Due to the mentioned drawbacks of the conventional methods, developing more effective techniques for finding the trace amounts of pathogens in dark waters have received considerable attention. Recently, several molecular methods such as polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) and fluorescent *in-situ* hybridization (FISH) have been applied in this field (see Table 4). Applying biosensor-based strategies to detect microorganisms in water and wastewater has become a very attractive research field (Ramírez-Castillo et al. 2015; Rengaraj et al. 2018; Yu et al. 2017). Utilizing biosensors have provided real-time identification of microbiological contamination, whereas traditional techniques take several days to obtain the result.

4.1 Optical biosensors for detection of microorganisms

Optical biosensors are known to be selective, sensitive, and accessible for real-time monitoring of toxicants, drugs and pathogen bacteria in wastewater (Bae et al. 2004; Lazcka et al. 2007). To sense pathogens, several optical techniques such as surface plasmon resonance (SPR) (Guo et al. 2018; Qiu et al. 2018) and resonant mirror (Daghestani and Day 2010; Kassal et al. 2018) have been used. Immobilization of specific antibodies against *Salmonella enteritidis* and *Listeria monocytogenes* on a gold SPR surface provide a detection limit down to 10^6 cell/ml (Koubova et al. 2001).

It was confirmed that surface-enhanced Raman spectroscopy (SERS) molecular probes could detect *E. coli* and *Listeria* (as representative of gram-negative, gram-positive bacteria, respectively) in a very specific binding manner in relatively short detection time of bacterial specific antigens (Xiao 2010).

Sensitive eco-friendly chemiluminescence (CL) immunosensor was developed by Zhang *et al.* applying a two steps sandwich measurement strategy (Zhang et al. 2014). The sensor was designed based on a capture antibody against *E. coli* O157: H7, which was immobilized onto the magnetic bead (MB) surfaces and a Biotinylated detection antibody. Avidin-conjugated glucose oxidase (GOx) was coupled and catalyzed the conversion of glucose into gluconic acid and H_2O_2. The intensity of CL signals through the catalytic activity of laccase on luminol reflected the level of produced H_2O_2 and in turn showed the concentration of *E. coli* O157: H7 (Fig. 3a).

Yildirim *et al.* established a portable optical fiber biosensor to indirectly sense *E-coli O*157: an H7 strain in wastewater samples by applying a fluorescently–labeled specific aptamer (Yildirim et al. 2014). The number of free aptamers could be measured through hybridization with their complementary DNA probes that are immobilized on the surface of an optical electrode fiber. The higher fluorescent signal could relate to the lower level of *E-coli* in evaluated samples. The developed system is low- cost, rapid, highly sensitive and highly portable for on-site wastewater testing.

Noble metal nanoparticles (such as silver and gold) are being noticed as an impressive substrate for utilizing in SERS pathogen biosensors (Singh et al. 2009; Tripp et al. 2008). Nanomaterials may also mediate microbial DNA detection via metallic nanoparticles. The colorimetric changes of gold nanoparticles, which conjugated to single-stranded DNA probes, after hybridization with their complementary DNA could eliminate the need for expensive and complex fluorescent labeling. Hence, Au NPs are widely used in bacterial sensing instruments, especially for monitoring the trace amounts of organisms in dark water samples (Jyoti et al. 2016).

Wu *et al.* reported a sensitive and simple aptasensor based on the colorimetric assay for detection of *E.coli* O157: H7 and S. typhimurium (Wu et al. 2012a). In this approach, aptamers with high affinity to target bacteria are adsorbed on the surface of AuNPs. Presence of targeted bacteria leads to the conformation change of aptamers, which was resulted to aggregation of AuNPs and consequently red to pinkish/purple color change that can be recognized with the naked eye (Fig. 3b). This method is simple, fast, specific and can directly measure the whole bacteria without specialized instrumentation and pretreatment.

Another notable approach uses carboxyl functionalized graphene quantum dots (cf-GQDs) to label a specific antibody against *E. coli* O157: H7 via the primary amines on the antibody. This system can specifically recognize *E. coli* O157: H7 in different sources such as water and food to generate a strong fluorescent signal with the minimum detection limit of 100 cfu/mL (Yang et al. 2018).

4.2 Electrochemical biosensors for detection of microorganisms

Recently, many portable devices are designed to amperometrically monitor various microorganisms by their electrooxidation or electroreduction phenomena (Kokkinos and Economou 2017). It is known that 97 % of E.coli strains have β-D-Glucuronidase (GLUase) activity, Rochelet *et al.* used the paminophenyl β-D-glucuronide substrate for the detection of GLUase activity of *E.coli* with disposable single-use carbon screen-printed electrodes to monitor *E.Coli* in wastewater samples. In spite of poor sensitivity of this method, it could be adapted to applications in the fields of water and wastewater analysis (Rochelet et al. 2015).

The rapid, inexpensive and sensitive amperometric biosensor measuring trace amounts of *Bacillus cereus* and *Mycobacterium smegmatis* was reported by Yemini *et al.* Due to the generic structure, the amperometric biosensor can be applied for the assessment specific enzymes of other organisms on the electrodes in less than eight hours (Yemini et al. 2007). This method can be used for the fast detection of the small amount of the actual microorganisms (Borisova et al. 2018).

Although the potentiometric method is not a common choice for sensing bacterial contamination, the success of measuring *Escherichia coli,* DH5a in water with a very sensitive, fast, inexpensive and compact potentiometric alternating biosensor has been established by Ercole, *et al.* (Ercole et al. 2002). An electrochemical dual aptasensor for the detection of *Staphylococcus aureus* (S. aureus) in aquatic samples was proposed by Abbaspour *et al.* (Abbaspour et al. 2015). Detection process was carried out with a primary aptamer, which was immobilized on magnetic beads through the specific biotin-streptavidin reaction. The secondary aptamer -conjugated AgNPs was used to report the presence of S. aureus via the sandwich immunosensing system. At the final step, dissolved AgNPs in the acidic solution formed a differential pulse stripping voltammetric signal that indirectly exhibited S. aureus quantification in samples (Fig. 3c).

Substrate modification of the graphite electrode by a bionanocomposite of polypyrole (PPy)/AuNP/MWCNT/Chitosan developed a sensitive voltammetric immunosensor. Through this platform, MWCNT and AuNPs provided an extensive surface area for immobilization of anti-*E. coli* O157: H7 monoclonal antibody. This modified electrode system supported highly effective monitoring of *E. coli* O157: H7 in environmental and food quality control. However, lack of regeneration ability of antibodies for subsequent detections caused serious limitation for applying this biosensor in real-world samples (Güner et al. 2017).

As the impedance biosensor consists of two real and imaginary parts, it depends on heavy mathematical formulation for its projection. So, despite label-free recognition and simplified sensor preparation, a limited number of researches have been reported on microorganism detection by impedance biosensors. Tahir and Alocilja reported a single-use impedimetric device for *E. Coli* detection (Muhammad-Tahir and Alocilja 2003). Using anti- *E. coli* antibody on the self-assembled monolayer of gold electrode presented an impedance immunosensor for finding *E. coli* in river water samples that have the potential for wastewater monitoring. The specific binding of *E. coli* to the electrode resulted in the increment of electro-transfer resistance, which was directly reflected the bacterial concentration (Geng et al. 2008).

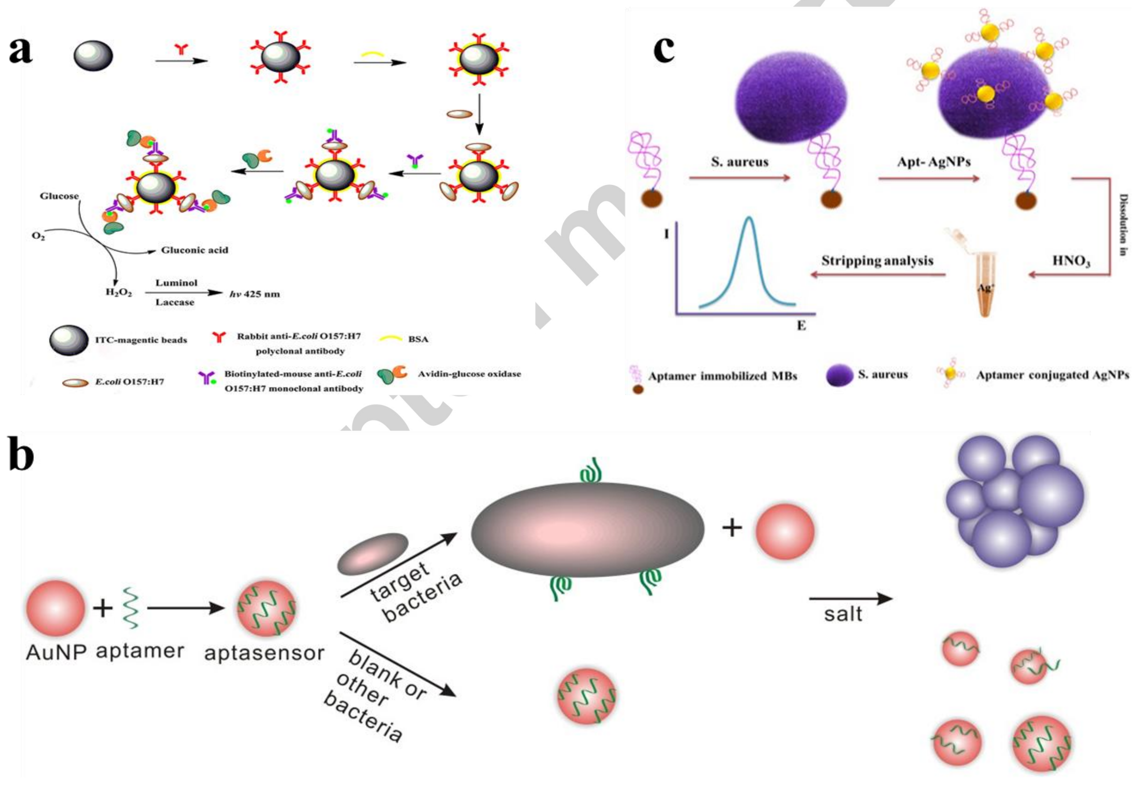

Figure 3. Biosensors in microorganism detection (a) The schematic view of production and function of a sandwich-type chemiluminescence immunosensor for monitoring E. coli O157:H7 (Zhang et al. 2014), (b) Schematic presentation of the principle of a colorimetric aptasensor with the high affinity to *E.coli* O157:H7 and S. typhimurium (Wu et al. 2012b), (c) Schematic description for

electrochemical dual aptasensor for the detection of S. aureus using magnetic nanoparticles and AgNPs (Abbaspour et al. 2015)

4.3 Summary and current challenges

Despite the success of biosensing methods, some important challenges must be addressed to see commercial application and development of bacterial biosensors for environmental wastewater samples. Some of the limitations include the need for sample pretreatment and reach to multiplexing microorganism detection in a single sample (Bazin et al. 2017; Theron et al. 2010). There are plenty of SPR-based devices successfully established for identifying pathogens in aqueous conditions, but the complexity, cost and large size of equipment limit their application. While some limited studies in this field presented single-step sensing mechanisms, they are not applicable for detection of whole cells. Targeting efficiency of nanomaterials to specific species of bacteria should be improved to enhance SERS signals strongly and achieve the fingerprinting peaks for intended organisms. Also, developing sensors with multi-structures can provide a good opportunity for simultaneous detection of various organisms (Xiao 2010; Zhang et al. 2018). The use of potentiometric biosensors for microbial detection is limited to issues related to biomolecule immobilization methods, poor detection restrictions, linear range, low reproducibility potential and insufficient stability (Lazcka et al. 2007; Liu et al. 2017).

5 The importance of nanotechnologies in wastewater biosensing

Using nanomaterials for biosensor construction enhances miniaturization of different parts of instruments (Lv et al. 2018). The small size of the sensor enables the integration of several processes into a single device to design real-time monitoring of multiple materials, which is very useful for monitoring the composition of wastewater. Furthermore, the use of nanomaterials has a positive effect on their physical and chemical features and improves the sensitivity, decreasing analysis time, increasing measurement reliability and demonstrating high-throughput multiplex detection (Ansari 2017; Kim et al. 2015; Rodriguez-Mozaz et al. 2006).

The potential applications of nanotechnology are accompanied by the reduction of cost and labor intensity of biosensing instruments. The efficiency of nanobiosensors depends on the selectivity interaction of small biomolecules as biorecognition elements and hypersensitivity of nanostructures when they are exposed to pollutants (Ghasemzadeh et al. 2014). The unique feature of nanoparticles like large extinction coefficients, pronounced photostability, luminescent and catalytic capacity proposed them as ideal choices for monitoring of environmental specimens (Kaittanis et al. 2010).

A recent interesting application of nanotechnology in the field of biosensors is employing nanomaterials to modify and functionalize electrodes, particularly in microfluidic platforms to provide high throughput screening. Moreover, using nanomaterials in accompanied to the freeze-drying method can make a great facility to reach to higher retention period for portable microbial biosensors (Lim et al. 2015). Recently, nanomaterials have gained importance in the detection of toxic compounds in water due to their small size and unique physicochemical characteristics (Dasgupta et al. 2017).

The current progress in the integration of nanotechnology with biologically sensing mechanisms have been greatly noticed, specifically for environmental monitoring (Justino et al. 2017; Reverté et al. 2016; Stoytcheva et al. 2018). In fact, recently numerous innovative biosensors have been reported, which applied nanomaterials to improve the accuracy and efficiency of sensing pollutants in wastewater samples. In this regard, we previously mentioned several examples of nanobiosensors had been established for detection of organic components (Aragay et al. 2012; Atar et al. 2015; Zehani et al. 2015), heavy metals (Chen et al. 2011b; Huang et al. 2016; Tan et al. 2016; Zheng et al. 2013) and microorganisms (Abbaspour et al. 2015; Güner et al. 2017; Wu et al. 2012a) in dark water resources.

Overall, it should be mentioned that merging nanotechnology with precise biorecognition mechanisms and various transducing strategies can explore several interesting lines of investigation toward the improvement of biosensors for environmental wastewater monitoring. Shortly, growing research studies in this area can lead to new possibilities for nanostructured biosensors in integration with strong signal-processing instruments or microfluidic devices.

6 Transition to commercialization

In contrast to the medical biosensors, a few commercial biosensors are available for environmental monitoring applications. Most of the currently available biosensors need to be significantly improved regarding sensitivity, selectivity, functionality, etc. before they can be applied for commercial use in wastewater plants. In this regard, introducing effective bio-recognition elements (such as whole microorganisms or biomolecules) can improve the applicability of biosensors to detect the fewer amounts of analytes, particularly for turbid samples like wastewaters. Table 5 summarizes some of the commercially available biosensing instruments have been used for detection of pollutants in wastewater.

BOD biosensors are the most popular sensing devices for water and wastewater quality sensing. To the best of our knowledge, a BOD biosensor was commercially presented for the first time by Nisshin Denki (Electric) Co. Ltd. in 1983 (Iranpour et al. 1997). Commercial BOD biosensor based on electrochemically- microbial fuel cell technology was presented by Korbi Co., Ltd. (Jouanneau et al. 2014). Recently a biosensor based on mediator-less microbial fuel cell has been introduced for monitoring of biodegradable organic matter in wastewater, which has a great potential to be used shortly as a commercial biosensor for BOD measurement (Anam et al. 2017). Several commercial products for sensing of toxicants is established through quenching the bioluminescence activity of luminescent bacteria such as *Vibrio fischeri* or *Photobacterium phosphoreum*.

Various commercially available optic biosensors are designed to measure the level of organic materials and heavy metals in different environmental conditions. Surface plasmon resonance (SPR) biosensors are widely marketed for monitoring the environmental samples, like β-SPR biosensor (SENSIA, Spain) for detection of Carbaryl. Texas Instruments Inc. also has developed Spreeta 2000 as a cheap and portable SPR-sensor for tracing of various biomolecule pollutants in soil or water, taking advantage of nanotechnology (Chinowsky et al. 2003).

Besides the important role of nanotechnology in the development of biomolecular detection, label-free cantilever sensors, like VeriScan 3000 and Biocom, are designed for the monitor of over 100 different compounds through environmental samples. Many operational systems were manufactured by various companies for the measurement of microorganisms in physiological and environmental fluids, which can be extended for wastewater samples.

It is of particular importance to note here some promising patents that presented a remarkable potential for commercialization. To sense and eliminating copper nanoparticles in industrial level, Corrèa and his colleagues patented two novel biosensing systems by using dead biomass of *Rhodotorula mucilagin, Hypocrea lixii* and *Trichoderma koningiopsis* (Corrèa et al. 2014; Corrêa et al. 2014). A patent also granted for monitoring organic materials in water and wastewater fluids through sensing methane produced from biodegradation of organic components. This invention applied an anaerobic methanogenic microorganism in a bio-electrochemical system composed of at least one anode-cathode containing reactor (Silver et al. 2017). The process of measuring of wastewater concentration was disclosed by Dooley and Burns through selectively sensing the level of dissolved oxygen with two precise oxygen sensor (Dooley and Burns 2017). Indeed, recently a highly sensitive patent issued to recognize the very low level of various macromolecules and even different pathogens. It was carried out by non-covalently adding specific bioreceptors on a substrate, which was comprised of single-walled carbon nanotubes (SWNT) and a pair of both electrodes (April et al. 2018).

7 Concluding remarks

Water quality extensively affects all aspects of human life, including health, food, energy, and the economy. Besides the rapidly developing area of wastewater reclaiming, lots of complications in traditional approaches for characterizing once-used waters, such as low efficiency, high cost and time consumption has been conducted. This is related to technical limitations of conventional monitoring systems and incapability to providing financial capacity over them. Biosensors, considered as a reliable alternative for tracing environmental pollutants through real-time and in-situ strategies for management of freshwater resources.

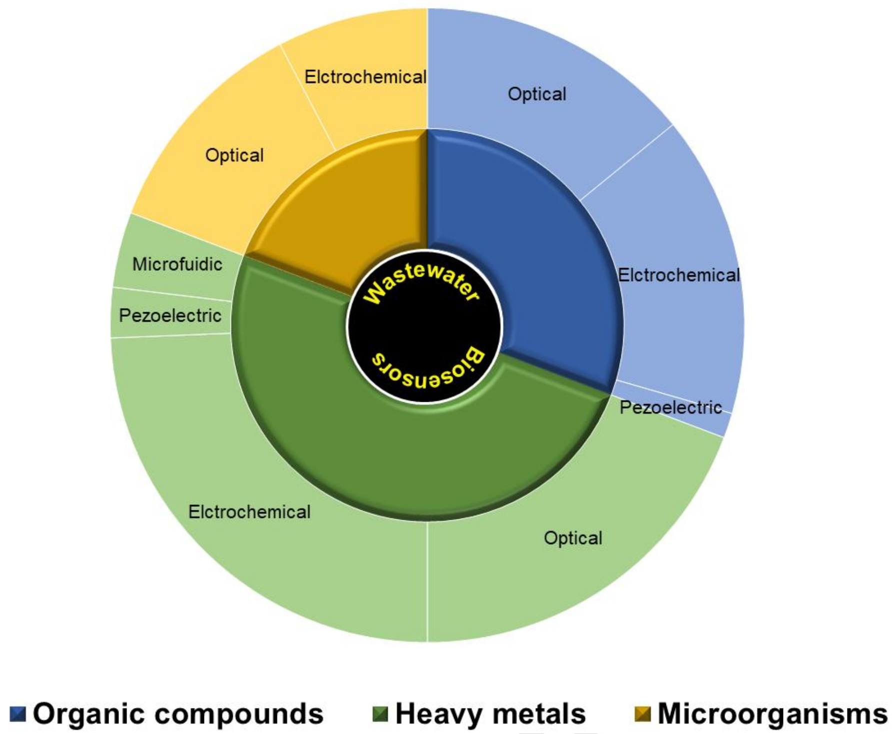

Figure 4. The Pie chart of the contribution of biosensors with potential application in wastewater monitoring

As we reviewed some biosensing mechanisms in this paper, increasing number of biosensor-based strategies were established, due to their simplicity and high selectivity for monitoring different types of environmental pollutants (Nepomuscene et al. 2007). Figure 4 presents an overall view of the contribution of three common types of biosensors with the potential capability for detection of a harmful component in dark water. Despite their promising applications in wastewater monitoring, there are many limitations that restrict their use in the real-world test to effluents and sewage (Kröger and Law 2005). As can be seen in Figure 4, biosensors for the detection of HMs have been considered more the subject of research and development whereas microorganisms and organic matters are also important as much as HMs.

Varying compositions of wastewaters over different time and situations create difficulties in the prediction of their physicochemical properties, like pH, conductivity, and turbidity. Indeed, the complex matrix of dark waters could affect the predicted parameters in samples and interfere with the detection of the desired elements. Stability of the immobilized biological elements (such as enzyme, cell, antibody, tissue, etc.) through shipment, storage and application condition is the other main aspect that should be considered. Some studies propound that an effective biosensor should be stable for at least six months under normal condition (Brecht 2005; Kissinger 2005).

The modern techniques need to be evaluated and compared to common analytical systems monitoring of water and wastewater. Both quantitative and qualitative validation guidelines should be established to assess their different aspects (Cárdenas and Valcárcel 2005; Trullols et al. 2004).

7.1 The perspective of a biosensor in wastewater monitoring

Recently, much effort has been made to reach to an effective strategy for wastewater monitoring. Several pieces of evidence support the special role of biosensors in plans for the administration of water resources. Concerning this fact, there are some technical and practical limitations and complications, which should be overcome to develop more efficient and commercial applicable biosensors. To achieve this goal for the detection of a broad range of wastewater pollutants, it is necessary to design novel sensing systems with larger detection range and capability for the simultaneous detection of several compounds. Additionally, the limit of detection in the lower concentration range should be possible, and also biosensor should have long-storage stability.

Two main strategies are in progress to overcome major obstacles for using biomolecules as the recognition elements, that is: (i) production of recombinant target-specific fragments and site-directed immobilization of antibodies and enzymes to promote rapid, cheap and *in-situ* screening of organic pollutant in wastewater, and (ii) The incorporation of nanomaterials to improve sensitivity and precision of biosensors, in addition to increasing detection efficiency by miniaturization and introducing multi-sensor array devices.

Even though most of the previously reported studies have concentrated on the detection of HM ions, like Hg^{2+}, Pb^{2+}, $Cd^{2+,}$ and Cu^{2+}, there are increasing interest in monitoring other inorganic elements. Development of commercial bacterial biosensors for wastewater monitoring can be rather complicated. It is important to have the strain and species selectivity without pre-enrichment processes, and the ability to separately sense live and dead microorganisms, alongside with the low detection limit. These issues should be handled through a portable device for the *in-situ* application.

Furthermore, standard calibration curves and limit of detection for all mentioned materials are different for different types of water resources. Since many studies were performed utilizing biosensors for the measuring toxicants in groundwater and river water samples, it is suggested that more researches are required for better understanding of these concepts in wastewater, principally for organic and heavy metal pollutants, as well as microorganisms.

Current advancements in the field of nanotechnology show great promises in improving some aspects of biosensors and can open up new opportunities to achieve better detection performance (Aragay et al. 2012).

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Table 1. Organic materials and optical biosensors for their detection

Analyte	Bioreceptor	Transducer	LOD	Ref
Octane	Whole-cell: *Escherichia coli* DH5	Light emission/ bioluminescence	0.34 µg L^{-1}	(Sticher et al. 1997)
4-chlorobenzoic acid	Whole-cell: *Escherichia coli*	Light emission/ bioluminescence	60 mg L^{-1}	(Rozen et al. 1999)
Phenol	Whole-cell: *Acinetobacter* sp. DF4	Light emission/ bioluminescence	2.5 mg L^{-1}	(Abd-El-Haleem et al. 2002)
Triclosan[H]	MIP	SPR	0.017 ng mL^{-1}	(Atar et al.

				2015)
Atrazine[H] **Simazine**[H] **Isoproturon**[H]**,** **Diuron**[H] **DNOC**[H]	Whole-cell: *Chlorella vulgaris* entrapped on a quartz microfibre filter	Fluorimetry	0.25 µg L^{-1} 0.5 µg L^{-1} 0.025 µg L^{-1} 5 µg L^{-1}	(Védrine et al. 2003)
Atrazine[H] **Bisphenol A**[E] **Estrone**[E] **Isoproturon**[H]	labelled antibodies	TIRF	0.002 µg L^{-1} 0.005 µg L^{-1} 0.019 µg L^{-1} 0.016 µg L^{-1}	(Tschmelak et al. 2004b)
Propanil[H]	Polyclonal IgG antibody	Fluorimetry	0.6 ng L^{-1}	(Tschmelak et al. 2004a)
Carbaryl[P]	Monoclonal antibody	SPR	1.38 µg L^{-1}	(Mauriz et al. 2006)
Bisphenol A[E]	labeled antibody	Fluorimetry	0.014 µg L^{-1}	(Rodriguez-Mozaz et al. 2005)
Naphthalene **Phenanthrene**	DNA/Sol–gel array	Fluorimetry	-	(Doong et al. 2005)
Estrone[E]	labelled antibody	Fluorimetry	0.20 ng L^{-1}	(Tschmelak et al. 2005)

LOD, Limit of detection; [H], Herbicide; DNOC, Dinitro-ortho-cresol; [E], EDC; [P], Pesticide; TIRF, Total Internal Reflection Fluorescence

Table 2. Organic materials and electrochemical biosensors for their detection

Analyte	Bioreceptor	Transducer	LOD	Ref
PBDE-100 **PCB-28** **PCB-101**	Enzyme (peroxidase) / immobilised polyaniline-modified Pt electrode	Amperometry	0.014 μg L^{-1} 0.016 μg L^{-1} 0.019 μg L^{-1}	(Nomngongo et al. 2012) (Nomngongo et al. 2012)
Atrazine[H]	biotinyl-Fab fragment K47 antibody	Impedance spectroscopy	20 ng mL^{-1}	(Hleli et al. 2006)
Paraoxon[P] **Methyl parathion**[P]	Enzyme (organophosphorous hydrolase)	Amperometry/ carbon nanotube-modified	0.041 mg L^{-1} 0.21 mg L^{-1}	(Deo et al. 2005)
Dichlorvos[P] **Parathion**[P] **Azinphos**[P]	Enzyme (acetylcholinesterase)	Sonochemically/ micro-electrode array	0.2×10^{-14} g L^{-1} 0.3×10^{-13} g L^{-1} 0.3×10^{-13} g L	(Law and Higson 2005)
Paraoxon[P] **Carbofuran**[P]	Enzyme (acetylcholinesterase)	Amperometry/ multielectrode	0.2 μg L^{-1}	(Bachmann and Schmid 1999)
Catechol	Enzyme (cellobiose dehydrogenase) (glucose dehydrogenase)	Amperometry	 0.85 μg L^{-1} 0.77 μg L^{-1}	(Nistor et al. 2002)
SDS[S]	whole-cell: *Pseudomonas* and *Achromobacter*	Amperometry	0.25 mg L^{-1}	(Taranova et al. 2002)
LAS[S]	whole cells: *Trichosporon cutaneum*	Amperometry/ Clark oxygen electrode	0.2 mg L^{-1}	(Nomura et al. 1998)

PCBs	DNA	Chronopotentiometry	0.2 mg L^{-1}	(Marrazza et al. 1999)
Daunomycin			0.3 mg L^{-1}	
Aflatoxin B1			10 mg L^{-1}	
EE[E]	Enzyme (tyrosinase)	Amperometry	4.4 mg L^{-1}	(Dempsey et al. 2004)
Oestradiol[E]			4.06 mg L^{-1}	
Phenol[E]			0.94 mg L^{-1}	
Nonylphenol[E]			2.2 mg L^{-1}	
Bisphenol A[E]			5.2 mg L^{-1}	
Bisphenol A[E]	Enzyme (tyrosinase)	Amperometry/ MWCNT modified	2.2 ng L^{-1}	(Zehani et al. 2015)
Aldicarb[P]	Enzyme (Acetylcholinesterase)	Amperometry	0.024 mg L^{-1}	(Arduini et al. 2006)
Carbaryl[P]			0.025 mg L^{-1}	
Paraoxon[P]	Enzyme (Butyrylcholinesterase)		0.002 mg L^{-1}	
Chlorpyrifos-methyl oxon[P]			0.0005 mg L^{-1}	

LOD, Limit of detection; PBDE, Polybrominated diphenylether; PCB, Polychlorinated biphenyl; PBB, polybrominated biphenyl; [H], Herbicide; [E], EDC; [P], Pesticide; [S], Surfactant; SDS, Sodium dodecyl sulfate; LAS, Linear alkylbenze-ne sulfonates; EE, Ethinyloestradiol; MWCNT, Multi-walled carbon nanotube

Table 3. Heavy metals and biosensors for their detection

Analyte	Bioreceptor	Transducer	LOD	Ref
Cr^{6+}	Urease	Conductometric	0-50 ppm	(Nepomuscene et al. 2007)
Hg^{2+}	Urease	Optical	10^{-4} µM	(Prakash et al. 2008)
Cu^{2+}	DNAzymes/ (CdSe-ZnS)	Fluorescent	0.5 nM	(Wu et al. 2010)
Pb^{2+}			0.2 nM	
Cu^{2+}	Cysteine/ CNT	ASV	0.015 mg L^{-1}	(Li et al. 2013)
Pb^{2+}	L-cysteine/ rGO	DPASV	0.416 µg L^{-1}	(Muralikrishna et al. 2014)
Cu^{2+}			0.261 µg L^{-1}	
Hg^{2+}			1.113 µg L^{-1}	
Cd^{2+}			0.366 µg L^{-1}	
Cd^{2+}	Peptide/ MWCNT	Electrochemical (Potentiometric)	2.749 × 10M	(Rahman et al. 2012)
Hg^{2+}			9.068 ×10^{-10} M	
Hg^{2+}	DNA/ GNPs	Piezoelectric (QCM-D)	4-7 nM	(Chen et al. 2011a)
Hg^{2+}	Protein/rGO	FETs	1 nM	(Sudibya et al. 2011)
Pb^{2+}	guanine-rich (G-rich) aptamer/ MWCNTs/GNPs	Electrochemical (Impedance)	4.3×10^{-15} M	(Zhu et al. 2014)
Cr^{6+}	Bacteria	Electrobiochemical (Potentiometric)	1 mg/L	(Liu et al. 2014)
Ag^{1+}	β-Galactosidase	Colorimetric	0.002 ppm	(Hossain and Brennan 2011)
Ni^{2+}			0.230 ppm	
Cr^{4+}			0.150 ppm	
Cd^{2+}	Phosphatase and esterase/BSA/AuNPs	Conductometric	10^{-20} M	(Tekaya et al. 2014)

Hg^{2+}			10^{-20} M	
Hg^{2+}	*Escherichia coli* and lusiferase	Bioluminescence	0.05 nM	(Lyngberg et al. 1999)
Ag^{1+}	DNAzyme/GO	Fluorescent	5.0×10^{-9} M	(Wen et al. 2011)
Hg^{2+}	DNA/GO	Fluorescent	5.0×10^{-10} M	(Zhang et al. 2012)
Cr^{4+}	ascorbic acid/carbon dot	IFE	10^{-4} M	(Zheng et al. 2013)
Hg^{2+}	MPA−HCys/GNP	Bio-inspired colorimetric	0.005 mg L^{-1}	(Darbha et al. 2008)
Ag^{1+}	Laccase and HRP/ $PSiO_2$ and Psi nanostructures	FFT	0.003 µM 0.009 µM	(Shtenberg et al. 2015)
Cu^{2+}				
Ag^{1+}	Cytosine rich aptamer/ QD/GO	FRET	0.1 mg L^{-1}	(Chang et al. 2014)
As^{3+}	GSH/ AgNPs	SERS	0.00076 mg L^{-1}	(Li et al. 2011)
As^{3+}	Aptamer/ GNPs	Colorimetric	0.0006 mg L^{-1}	(Wu et al. 2012b)

LOD, limit of detection; rGO, reduced graphene oxide; ASV, anodic stripping voltammetry; DPASV, differential pulse anodic stripping voltammetry; CNT, carbon nanotube; MWCNT, multi-walled carbon nano tube; CV, cyclic voltammetry; GNT, gold nanoparticle; QCM-D, quartz crystal microbalance with dissipation monitoring; BSA, bovin serum albumin; GO, graphene oxide; IFE, inner filter effect; MPA, mercaptopropionic acid; HRP, horseradish peroxidase; FFT, fast fourier transform; AgNP, silver nanoparticles; SERS, surface enhanced Raman spectroscopy; GSH, glutathione

Table 4. Molecular techniques for detection of pathogenic microorganisms (Berekaa 2016; Rose and Grimes 2001)

Methods	Characteristics and Advantages	Disadvantages
Immunological methods	Specific for the target organism (Environmental samples)	Unable to distinguish between live and dead organisms
Nucleic acid hybridization	Specific differentiation of virulent from nonvirulent strains	Require culturing
Polymerase chain reaction (PCR)	Rapid and specific quantitative detection	Unable to distinguish between live and dead organisms
DNA microarrays	Sensitive and rapid detection	Lake of sufficient sensitivity for environmental approaches Require sample pre-enrichment
Biosensors	Real-time, fast detection	Affected by environmental changes in pH, mass, temperature, *etc* in
Quantum dots (QDs) and dye-doped nanoparticles **Metal nanoparticles**	Wide adsorption spectrum Higher conductivity Multiplexed diagnostics	
Carbon nanotubes	Sensitive, high stability and surface area	

Table 5. Commercial instruments for detection of pollutants in wastewater and environmental fluids

Instrument	Analyte	Transducing system	Manufacturer	Ref
BOD-2000	BOD	Electrochemica (Amperometric)	Nisshin Denki Kosaku KK, Japan	(Iranpour et al. 1997)
BOD biosensor 7842-Microbe Sensor Series	BOD	Electrochemical (Potentiometric)	DKK-TOA Corporation, Japan	(Praet et al. 1995)
ARAS BOD sensor	BOD	Electrochemical (Amperometric)	Dr. Lange GmbH, Germany	(Riedel et al. 1993)
BOD-Module	BOD	Electrochemical (Amperometric)	Prufgeratewerk Medingen GmbH, Germany	(Reiss et al. 1998)
HABS-2000	BOD	Electrochemical (Amperometric)	Korbi Co., Ltd., Korea	(Jouanneau et al. 2014)
Microtox	Organic materials, HMs	Bioluminescence	Azur Environmental, US	(Environmental 1995)
ToxAlert	Different toxicants	Bioluminescence	Merck, Germany	(Farré and Barceló 2003)
LUMIStox	Different toxicicants	Bioluminescence	Dr Lange GmbH, Germany	(Monarca et al. 2000)
Spreeta 2000	Different toxicicants	SPR	Texas Instruments Inc., US	(Chinowsky et al. 2003)
β-SPR	Carbaryl	SPR	SENSIA, Spain	(Mauriz et al. 2006)
CellSense TM	HMs	Amperometric	Outotec oyj, Finland	(Wang et al. 2008)
VeriScan 3000 System	Adaptable for different molecules	Microcantilever	Protiveris Inc., US	(Rodriguez-Mozaz et al. 2006)
BioCom chip	Adaptable for different molecules	Microcantilever	Kalinex Inc., US	(Carrascosa et al. 2006)

BIAcore series	Sulfonamides Microorganisms	SPR	Biacore AB, Sweden	(Rodriguez-Mozaz et al. 2004)
SPR-CELLIA	Microorganisms	SPR	Nippon Laser & Electronics Lab., Japan	(Rodriguez-Mozaz et al. 2004)

Highlights:

- Conventional water pollution monitoring is costly and time-consuming.
- The emerging biosensors have recently gained a significant attention for environmental application.
- Nanotechnology can improve greatly the performance of biosensors for water quality monitoring.
- This review provides readers with a general overview of biosensors for water pollution monitoring.
- The contribution of nanotechnology in biosensor development for water applications is reviewed.