Biosensors for on-line water quality monitoring – a review

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ABSTRACT
Biosensors are analytical tools that convert a bio-signal into a measurable response. The applicability of these devices is rapidly expanding and their fabrication for continuous and real-time detection is gaining increased attention. Current research continues to further improve their features. Some of the major advantages of these devices are that they are portable and easy to operate, provide a rapid response, can be miniaturized, have a long-life cycle and a short response time and possess a high sensitivity, selectivity and specificity. Biosensors are more efficient and economical than conventional detection methods for a wide range of applications. This article is a tutorial review on the basic concepts of biosensors and their application in water quality monitoring, especially on-line detection systems. The following topics are discussed: (i) basic concepts of biosensors, (ii) biosensing elements and transducers, (iii) biosensor types and performance criteria and (iv) specific applications on water quality monitoring. In addition, this review highlights the various aspects of portable paper-based sensors for on-site detection of pesticides, heavy metals and bacteria in water. Online sensing technologies for water quality monitoring are preferable than offline/onsite detecting technologies, considering that online systems provide real-time data.

1. Introduction
The demand for freshwater has increased rapidly due to massive urbanization, industrialization, reliance on irrigated agriculture, and rising living standards, especially due to the water use for luxury baths and swimming pools (Duffy, Woods, Walsh, & Kane, 2010). Wastewater can introduce many pollutants (e.g. inorganic/organic compounds, heavy metals, acidity, basicity, organophosphates and pesticides), including pathogenic *Escherichia coli* to the environment. Freshwater has become scarce globally not only due its dwindling quantities, but also due to the deteriorating quality of freshwater. Thus, reducing hazardous effluents from entering the environment is a priority in many countries (Dhewa, 2015; Robarts, Barker, & Evans, 2007). Clean water is a global goal as waterborne diseases are a major health concern. According to the World Health Organization (WHO) “safely managed drinking water services” is a global goal as water-borne diseases are considered a major health issue (“Drinking-water,” n.d.). In the USA alone, about 76 million cases of waterborne illnesses are reported each year (Xiao, 2010). In Bangladesh, one in five deaths is attributed to waterborne poisoning (Bullough, Fenech, & Bridle, 2013). Hence, the determination of physical-chemical properties, pollutants (chemicals or biologicals) in water is the first step in enabling effective remediation of freshwater resources and the identification of threats to health and safety and prevention of waterborne deceases (Dhewa, 2015; Pranjali, Suniti, Amrita, Deepa, & Brijesh, 2012). Several analytical methods based on UV spectroscopy, high performance liquid chromatography (HPLC), gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS), wet-chemistry and electro-chemistry are available for monitoring water quality. Even though some of these techniques (e.g. GC, GC-MS) are ultra-sensitive and precise, almost all of these methods require sample pre-treatment and qualified personnel, and they are labour intensive and costly. Moreover, these methods are not suitable for applications requiring quick results such as when monitoring situations demanding urgent action or in remote areas and developing countries. Simple and handy assays capable of screening toxic chemicals are important for such applications. In addition, aforementioned methods are not suitable for quick online detection of water contamination. Besides, even though most *E. coli* strains are harmless, some serotypes can spread dangerous diseases and even cause death. Thus, the recognition of *E. coli* as a
faecal indicator organism is imperative (Edberg, Rice, Karlin, & Allen, 2000). However, the classical incubation method used to determine the presence of this indicator organism takes about 18–72 h to produce results (Rompré, Servais, Baudart, de-Roubin, & Laurent, 2002). In such situations, the application of biosensors has been shown to be effective, as they are portable, sensitive and have the potential for automation and online use (Dhewa, 2015; Robarts et al., 2007). As such, the number of scholarly publications on the topic of biosensors has witnessed a significant increase recently (Olson & Bae, 2019). Briefly, a biosensor is produced by coupling a bio-element with a transducer (Figure 1). Electrochemical transducers detect the gradient of current or voltage; optical transducers detect the gradient of fluorescence intensity, absorbance or reflectance; and acoustic transducers detect the gradient of frequency. The human nose can be considered as a good example of a biosensor, which is very sensitive and selective. Human nose can detect many chemicals qualitatively and even can provide a general idea of the quantity, with low detection limits. Generally, the smell is sensed by the olfactory receptors (acting as the sensing element) of nostrils. The signals of the olfactory membranes are then transformed into electrical responses by the olfactory nerve cells (acting as the transducer), which pass the signals to the brain. Here, the brain (acting as a microprocessor) interprets and converts the responses into a feeling or impression.

However, the typical biosensor design is not universally suitable for monitoring many target analytes (Xiao, 2010). Also, establishing a detailed network of monitoring stations based on biosensors in very large countries, e.g. USA (Robarts et al., 2007), is not financially feasible. Alcock (2004) has studied biosensors that can be applied for quick field evaluation of water contamination. Online and offline sensors are available for measuring heavy metals, temperature, polycyclic aromatic hydrocarbons (PAHs), acidity, oxygen, toxicity, genotoxicity, pesticides, mixtures of benzene, toluene, ethylbenzene and xylene (BTEXs), nutrients, endocrine-disrupting chemicals (EDCs) and pathogens (Alcock, 2004; Barbulescu, Duteanu, Negrea, & Ghangrekar, 2018; Di Nardo et al., 2018; Hernandez-Vargas et al., 2018; Kumar, Hu, Singh, & Mizaikoff, 2018; Lukyanenko et al., 2019; Montes, Céspedes, Gabriel, & Baeza, 2018; Thapar, Malik, & Salooja, 2018; Xu, Obodo, & Yadavalli, 2019). Notwithstanding the developments in the field, a significant effort is still required to develop robust, quick, selective and sensitive devices applicable in remote areas (Lagarde & Jaffrezic-Renault, 2011).

This review has two parts: (i) A section on basic concepts of biosensors, types of biosensors, and their performance criteria; and (ii) A section on the application of biosensors for water quality monitoring with a special focus on online biosensing technologies. Several potential ideas for the advancement of online water quality determination are discussed. Bioactive paper sensors for on-site detection of toxins and bacteria in water are also included. As real-time data can be obtained using online devices, online water quality monitoring approaches are much better than offline/onsite monitoring approaches. However, a review on the application of biosensors for online water quality monitoring has not been published. Hence, the aim of this tutorial review is to fill this gap and describe these biosensor processes in detail.

2. Basic concepts of biosensors

The field of biosensors is a fast-expanding field as they provide higher sensitivity and selectivity than current conventional sensors. The two main parts of a biosensor are: (i) a biological element and (ii) a transducer, which are discussed briefly in the following sections.

2.1. Biological elements

Biological elements are used to sense the existence and concentration of a substance. The most common biological elements are enzymes, antibodies, nucleic acids, receptors and whole cells. Enzymes are fast acting biological agents producing sensitivity improvement of biosensors when bonded to a specific substrate through their catalytic effect for a particular reaction. They are used in their pure form or may be available in microorganisms or in slices of undamaged tissues. Antibodies are cleverly combined with a substrate to provide a signal for a
transducer to sense. Unlike enzymes, they do not have any catalytic effect and they bind especially with the matching antigen to remove it from the sphere of activity. Biosensor sensitivity is highly improved when antibodies are employed as the biological element. Typically, nucleic acids (DNA or RNA) react with the specific substrate/element due to their base pairing characteristics. They are highly suitable for diagnosing genetic disorders. Receptors have molecular recognition properties and are proteins housed in the plasma membrane made of a phospholipid bilayer. They cannot be isolated easily but bind solutes with a high affinity and specificity similar to antibodies. Whole cells are excellent sensors for contaminant detection. Many biosensors incorporating whole microbes as the antigen element have been designed for the fast and impressive detection of inorganic pollutants and heavy metals (Amaro, Turkewitz, Martín-González, & Gutiérrez, 2011; Olaniran, Hiratal, & Pillay, 2011). A reagentless platform in which biocompatible agarose is used as an entrapment agent, with cell sensors and growth media mounted within the gel matrix, has also been developed (Lin, Zhang, & Yeh, 2019).

2.2. Transducers

A transducer is considered the main stage of a biosensor. A transducer converts a physical change caused by a reaction into a suitable signal for processing in a subsequent stage. Biological elements or biomaterials can be either linked to or integrated with a transducer. Biomaterials can be incorporated into a support or bound chemically or physically to the transducer surface, which depends on the immobilization method of the biomaterial on the transducer. Immobilization methods include adsorption, entrapment, covalent attachment, microencapsulation, inclusion, cross-linking etc. (Korotkaya, 2014). Among the several types of available transducers, calorimetric, potentiometric, amperometric, conductivity-based, optical, acoustic and piezoelectric transducers are commonly used. A brief outline of these transducers is provided in the following sections.

2.2.1. Calorimetric transducers

Calorimetric transducers involve the measurement of the heat produced (or absorbed) by a reaction (1). The heat change can be determined using thermistors and hence be correlated to the analyte concentration to be measured. For example, heat generated by exothermic reactions catalysed by an enzyme (biological element) may be used to determine the analyte concentration by determining the rate of reaction. Such a device is considered the most common type of enzyme-based biosensor. The changes of temperature are generally determined by means of thermistors placed upstream and downstream of the bed columns containing immobilized enzymes. Thermistors used to determine temperature changes operate based on a variation of the electrical resistance with temperature, according the following relationship:

\[
\frac{R_1}{R_2} = e^{B(T_1 - T_2)}
\]  

(1)

where \(R_1\) and \(R_2\) denote the resistance of the thermistors at \(T_1\) and \(T_2\) in kelvin (K), respectively, and \(B\) denotes the thermistor constant.

2.2.2. Potentiometric transducers

Ion-selective electrodes can be used as potentiometric transducers for transducing the bio-signal into an electrical one. Ion-selective electrodes involve the measurement of the emf (potential) produced by changes of charges or ions in a cell at open circuit (Brian, 2008; Sethi & Lowe, 1990). It is important to note that the emf is directly proportional to the logarithm of the concentration of the substance being measured. The signal of an ion-selective electrode is described by the following equation:

\[
E = E_0 + \frac{RT}{zF} \ln([i])
\]

(2)

where \(E\) denotes the measured potential (in volts), \(E_0\) designates the characteristic constant for the ion-selective/external electrode system, \(R\) denotes the gas constant, \(T\) is the temperature in K, \(z\) designates the signed ionic charge, \(F\) is the Faraday constant, and \([i]\) denotes the concentration of the analyte of interest.

Several types of ion-selective electrodes are currently available. They include the following: (i) glass pH electrodes, (ii) glass electrodes for cations and (iii) solid-state electrodes. Glass pH electrodes are based on membranes that are selective and permeable for gases such as \(\mathrm{CO}_2\), \(\mathrm{H}_2\mathrm{SO}_4\) or \(\mathrm{NH}_3\). These gases penetrate the membrane leading to a change in the pH of the electrode solution, which is then measured. Glass electrodes for cations are based on a very thin hydrated glass membrane. The latter produces a differential electrical voltage caused by the dose-dependent competition between the cations for selective binding spots. The selectivity of the membrane is a function of the constituents of glass. The fact that the sensitivity to \(\mathrm{H}^+\) is greater than that of \(\mathrm{NH}_4^+\) is well known. Solid-state electrodes are not glass membrane electrodes. They are based, instead, on thin membranes of specific ion conductors made of a silver sulphide-halide combination. An example of a reaction involving the release of \(\mathrm{H}^+\) that may be employed in potentiometric biosensors is shown by Equation (3).
\[ D - \text{glucose} \ + \ O_2 \xrightarrow{\text{GOD}} D - \text{glucono-1,5lactone} \ + \ H_2O_2 \xrightarrow{H^+} D - \text{gluconate} \ + \ H^+ \]  

where the GOD is the enzyme glucose oxidase.

As shown in Figure 2, potentiometric biosensors use a pH-meter probe. The thin sensing glass probe is coated with an immobilized enzyme membrane, where the enzyme-catalysed reaction absorbs or generates \( H^+ \). This causes a pH change which can be read directly from the display of the pH meter.

### 2.2.3. Amperometric transducers

The well-known Clark electrode used to measure the concentration of oxygen in blood is a good example of a sensor based on an amperometric transducer. This electrode, as any other amperometric transducer, can be converted into a biosensor to detect a specific analyte. Clark produced the blood glucose biosensors by immobilizing an enzyme (e.g. glucose oxidase [GOD]) in gel on a gas permeable membrane coating the electrode. This biosensor consists a silver/silver chloride reference electrode along with a platinum cathode at which oxygen is reduced. The principle of operation, which also applies to others amperometric biosensors, is illustrated in Figure 3. Briefly, glucose reacts with GOD to form gluconic acid with two electrons and two protons, thus reducing glucose oxidase. The latter, in the presence of oxygen, electrons and protons are converted into \( H_2O_2 \). Glucose oxidase reacts again with glucose. Therefore, a higher glucose content leads to more oxygen consumption and thus the amount of oxygen detected by the electrode is directly proportional to the glucose concentration. For better performance, a mediator is used as the oxidant instead of oxygen. Amperometric and potentiometric biosensors have almost identical performance in terms of their time response, frequency response and sensitivities. The above types of electrochemical transducers can be miniaturized using silicon-based field-effect transistors (FET). Figure 4 shows an ion sensitive field effect transistor with an enzyme layer placed on it.

### 2.2.4. Optical transducers

With the advances in optical fibres and biosensors based on laser technology, optical transducers are currently receiving considerable attention. Although a substantial amount of research has been conducted, measuring changes in light absorption between the reactants and products of a reaction and determining the light output by a luminescent process are two of the major areas of optical biosensors which require improvement. The main types of photometric techniques used in biosensors are: (i) Ultraviolet-visible absorption, (ii) Fluorescence (phosphorescence) emission, (iii) Bioluminescence/chemiluminescence, (iv) Internal reflection spectroscopy, (v) Surface plasma spectroscopy and (vi) Laser light scattering. Ultraviolet-visible absorption spectroscopy is based on the Beer-Lambert law described by Equation (4).

\[ \log(I/I_0) = \varepsilon CL \]  

where \( I_0 \) denotes the incident light intensity, \( I \) is the transmitted light intensity, \( \varepsilon \) denotes the extinction coefficient, \( C \) denote the analyte concentration and \( L \)
designates the path length of light through the solution. The light generated may be sensed photometrically using expensive, high-voltage photomultiplier tubes or low-cost low-voltage photodiode techniques. In fluorescence spectroscopy, the light absorbed at a short wavelength by the analyte (solution) is re-emitted at a longer wavelength, intensity of which depends on the concentration of the analyte. Bioluminescence/chemiluminescence is a process where light is emitted from a chemical or biological reaction without excitation by light. The intensity of the light emitted is proportional to the analyte concentration. The methods based on total internal reflectance (Figure 5) make use of the evanescent wave fraction of incident light on the interface at a certain angle penetrating a short distance into the solution of lower refractive index (RI). Thus, a change in RI or absorptivity of the reflecting surface due to the presence of the analyte causes a reduction in light transmission through the sample. The intensity of light, which is proportional to the analyte concentration, is measured against that of the incident light. The surface plasmon resonance (SPR) technique is illustrated in Figure 6. The device consists of a prism with a RI $n_1$. The prism is coated with a very thin metal layer (gold or silver) with an RI of $n_2$ and the sample layer (analyte) of an RI of $n_3$ ($n_1 > n_3$) is deposited on the metal layer. The intensity of the plasmon (i.e. free electron on the metal surface) is influenced by the environment of the metal surface (e.g. antibody–antigen binding). The changes of the plasmon due to antibody–antigen binding can be measured as a change in the angle of incidence.

The continuous improvement in fibre optics technology has led to greater design flexibility and miniaturization of optical sensors (Hisham, 2018). Optical fibres made of polymeric materials have become an indispensable option to transform a photometric assay into a sensor. Optical fibres behave as a guide for light from the source to the sensing location. The propagation of light waves along the fibres depends on the principal of total internal reflection (TIR). The latter is related on the angle of the incident beam of light on the interface between two media with different refractive indices as expressed by Snell’s law described by Equation (5).

$$\frac{\sin \theta}{\sin \varphi} = \frac{n_2}{n_1} = n$$

where $\theta$ denotes the incident angle, $\varphi$ denotes the refractive angle, $n_1$ denotes the incident refraction index, and $n_2$ denotes the refraction index of refractive medium.

In biosensors, optical fibres can operate in the extrinsic mode or intrinsic mode. In the extrinsic mode, the fibre simply transmits light from the light source to the light collector. The absorption of light can be expressed by Beer-Lambert law (Equation (4)), and thus, is a function of the measured intensity of light and the thickness of the sample. While in the intrinsic mode, the light characteristics of the measured light are subjected to change within the penetration distance of the evanescent field adjacent to the fibre optic guide. Examples of biosensors based on optical fibres operating in the intrinsic mode and extrinsic mode are shown in Figures 7 and 8, respectively.

### 2.2.5. Piezo-electric transducers

These devices are made of crystals (e.g. quartz) which convert vibration on their surface into an electrical voltage. The frequency of vibration is altered by the material adsorbed on its surface or the mass of material, which could be related to changes in the measured frequency (Brian R. Eggins, 2008; Fraser, 1995a, 1995b). This frequency change, which can be easily detected using low cost electronic devices, is expressed by Equation (6).

$$\Delta f = \frac{Kf^2 \Delta m}{A}$$

where $f$ denotes the resonant frequency, $\Delta f$ denotes the change in resonant frequency (Hz), $K$ designates a constant for the particular crystal, $\Delta m$ denotes the mass difference of adsorbed material (g) and $A$ denotes the adsorbing surface area (cm$^2$).

### 3. Biosensor types and performance criteria

Biosensors are categorized depending on the transducer detection method. Table 1 lists several types of biosensors with their significant features.

The performance of a biosensor assessed using several features. Some of the important desirable features are listed below:

- **Selectivity**: Bioelements are normally highly specific for the analytes, stable under normal
storage environment, and show compatibility over many assays.

b. Accuracy: The signal must be precise, accurate, reproducible and linear over the desirable range (Turner, Karube, & Wilson, 1986).

c. Biocompatibility: Implantable sensors should be biocompatible; the sensor should work satisfactorily in vivo.

d. Durability: The biosensors should be durable. Currently available biosensors have a short lifespan and do not tolerate severe environmental conditions (Korotkaya, 2014).

e. Contamination: Must ensure that bioelements and chemicals do not leak from devices.

f. Cost and size: A biosensor must be small, cheap, portable, user friendly and capable of being used without the need of skilled personnel.

g. Others: Effectiveness and environment friendly nature.

Developments in the field of biosensors in the past two decades have resulted in massive improvement in all of the above features, especially biocompatibility and the lifetime of biosensors (Jain et al., 2010). However, several major challenges, including making them cost-effective, easy to use and effective, still remain unresolved. Effectiveness of a biosensor is the most beneficial parameter to compare technologies during their developmental phase. The dynamic range (DR) and the limit of detection (LOD) are the key features of effectiveness. Biosensors can be designed to provide two types of data: namely, transient ("kinetic") data and endpoint ("equilibrium") data. Transient data indicate the way the sensor response varies with time and careful evaluation of mass transfer may be utilized to determine the kinetic features of the surface binding reaction. A single point for every experiment is recorded in endpoint data so that the sensor response after the elapse of a specific time or once a specific event has taken place can be evaluated. The latter event takes place when a stable state has been achieved indicating that saturation or an equilibrium in the reaction occurring at the biosensor surface has been reached. LOD is basically an intuitive quantity indicating the least concentration at which a biosensor can develop a signal that can be easily differentiated from the noise in the measurement. Currently, a signal-to-noise ratio (SNR) of more than one is a clearly distinguishable signal from noise. When the SNR value is below one, it is difficult to distinguish the signal from noise. If a biosensor produces an SNR of one at a concentration level of 10 fm, analyte concentration down to 10 fm can be quantitatively calculated. However, differences among reactions can lead...
to a higher LOD. Hence, multiple trials should always be performed to obtain the correct LOD for a sensor. The LOD can be determined using both transient data and endpoint data.

4. Applications

Biosensor technology serves a very wide field with an enormous impact on healthcare, agriculture, food and water quality control (Bhalinge et al., 2016; Hernandez-Vargas et al., 2018; Mehrotra, 2016). Research and development (R&D) on biosensors is increasing faster than their commercialization. This is mainly due to the cost and lifetime of the devices (Bogue, 1996). While the medical field dominates the biosensor market, their utility for environmental monitoring has only been investigated in the last decade (Bogue, 1996). Currently, the most lucrative biosensor is the one used for monitoring blood sugar in diabetic patients, which is based on the electrochemical amperometric transduction protocol. On the other hand, for laboratory use, an optical monitoring system seems to be more viable (Mongra, 2012). In the food processing industry, despite the stringent FDA regulations, some food hazardous to humans still reach the market. Biosensors (e.g. enzymatic and potentiometric) are simple, selective and inexpensive alternative devices which can be used to monitor these food items, especially the aging of beer (Ahmadi et al., 2011), presence of pathogens in food, e.g. *E. coli*, and other substances (Mehrotra, 2016; Rapini & Marrazza, 2017). Some thermophilic organisms in milk and milk products can also be monitored using biosensors (Thapar et al., 2018). Many other uses have been studied and/or commercialized for environmental monitoring, waste water quality monitoring, forensics and chemical/biological warfare detection (Ejeian et al., 2018; Korotkaya, 2014). The analyses are for process monitoring, increasing product yields, energy optimization and raising the process automation level (Korotkaya, 2014). Overall, the benefit of biosensors is their capability to detect extremely low concentrations, and to monitor molecular interactions at a cellular level, e.g. protein interactions, or cell and virus interactions. In this review, the application of biosensors in water quality monitoring is described in greater detail.

4.1. Offline sensing technologies for monitoring water quality

Scientific literature is abound with different biosensor technologies for offline evaluation of biological and/or ecological quality elements or for the monitoring of chemical contaminants (inorganic and organic). Even though contaminants are generally classified based on their chemical structure, they can also be classified based on their nature of action, such as endocrine disruption, carcinogenicity,

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Table 1. Types of biosensors based on the transducer measurement method (Amini & Kraatz, 2015; Lagarde & Jaffrezic-Renault, 2011; Saleem, 2013)

| Type       | Variation         | Method                                                                                     |
|------------|-------------------|---------------------------------------------------------------------------------------------|
| Electrochemical | Amperometric     | Measures the electrical current resulting due to an enzyme redox reaction. Example: Blood glucose biosensor. |
| Potentiometric | Conduct-metric   | Measures the change in the potential using ion-selective electrodes. Example: pH electrode (ISFET). |
| Thermometric | Thermal           | Measures the heat produced from biological reactions. Examples: Glucose, urea, uric acid, and penicillin G. |
| Optical    | Fiber optic lactate | Measures the change in oxygen concentration by identifying fluorescent dye reduction by oxygen |
|            | Colorimetric      | Measures the intensity of dye colour produced by glucose. Example: glucose strip.           |
|            | Luminescent       | Measures the quantity of light released. Example: The detection of urine microorganisms.     |
| Piezoelectric | Acoustic         | Measures the change in resonance frequencies resulting from the adsorption of analytes on piezoelectric crystals. |
cytotoxicity, genotoxicity or mutagenicity. In this section, some of these aspects are discussed. For the determination of toxicity and genotoxicity, cellsense (an amperometric sensor that integrates bacterial cells, *E. coli*) is used for rapid toxicity measurement (Rodriguez-Mozaz, Alda, & Barcelo, 2006; Saleem, 2013). A similar configuration was used by Kvatsinsky, Leibowitch, and Farkas (2008). Also, a multi-channel two-stage mini-bioreactor based on genetically modified bioluminescent bacteria is used to detect the toxicity of some EDCs (Long, Zhu, & Shi et al., 2013; Rodriguez-Mozaz et al., 2006). Enzyme-based sensors have also been developed for measuring toxicity (Saleem, 2013). Enzyme-based sensors have also been developed for measuring toxicity (Saleem, 2013). Enzyme-based sensors have also been developed for measuring toxicity (Saleem, 2013). Enzyme-based sensors have also been developed for measuring toxicity (Saleem, 2013).

Biosensors for measuring biochemical oxygen demand (BOD) were developed in the early stages of the emerging technology. Clark and Lions (1962) developed an amperometric sensor for the measurement of dissolved oxygen. Based on this sensor, fast measurement of BODs using biosensors relied on the determination of the bacterial respiration rate near a transducer (Rodriguez-Mozaz et al., 2006). An optical fibre biosensor based on yeast has been developed for monitoring low BOD values in river waters in 15 min (Dhewa, 2015; Rodriguez-Mozaz et al., 2006). Several biosensors have been developed to monitor trace amounts of nitrogen compounds in water. One of the latest sensors was developed by Xiao (2010), which was a field-deployable nanosensor for onsite detection of nitrite and microorganisms (Dhewa, 2015; Xiao, 2010). Velasquez (2015) successfully tested a microbial fuel cell (MFC) biosensor to measure nitrate and pathogens in ground water in Tanzania (Velasquez, 2015). The signal time was as low as 2.8 min (Di Lorenzo, Thomson, Schneider, Cameron, & Ieropoulos, 2014; Velasquez, 2015). A biosensor can determine surfactants by detecting the dissolved O₂ uptake, which can change the current produced by the oxygen electrode (Saleem, 2013). Recently, a photosynthetic MFC (photoMFC) device was reported for onsite monitoring of toxicants in water (Chouler, Monti, Morgan, Cameron, & Di Lorenzo, 2019). The MFCs are simple to operate and use microalgae to form a sensitive, portable and cost-effective bioelectrochemical sensor. This photoMFC has been reported to be more efficient than other MFCs for onsite detection of formaldehyde and other contaminants, such as herbicides and pharmaceuticals, which pose a global environmental concern.

Multiple studies have resulted in different types of biosensors for the determination of pesticides, hormones, PCBs, dioxins, phenols, bisphenol A, LAS, APEs, alkanes, PAHs, antibiotics and toxins (Dhewa,
Bullough et al. (2013) have developed different methods of measuring arsenic using genetically modified whole-cell biosensors (Bullough et al., 2013). The Sensicore WaterPOINT 870 Multi-Parameter Optical Water Quality Analyzer, one of the most versatile devices available, can determine up to 24 different physical-chemical parameters in just a few minutes (Robarts et al., 2007). Table 2 summarizes a list of biosensors for the measurement of organic and inorganic compounds, including pathogens, E. coli, etc.

4.2. Bioactive paper sensors for on-site monitoring of water quality

Rapid, sensitive, on-site monitoring of toxins, heavy metals and bacteria in water and food samples without using advanced instrumentation and/or trained personnel is an acute need in clinical applications, situations requiring a quick response and resource-limited areas. Paper-based biosensors, which meet the above requirements, may be the ideal solution for this purpose (Peixoto, Machado, Oliveira, Bordalo, & Segundo, 2019). Considering that it is abundant, cheap, disposable, sustainable and easy to use, paper has received considerable attention of researchers as a solid-phase platform for the improvement of biosensors. Several types of paper that can be used for the fabrication of paper-based devices, such as cellulose filter paper (e.g. Whatman® filter paper Grade 1 and Grade 2), cellulose chromatography paper (e.g. Whatman® chromatographic paper Grade 1 and Grade 2), nitrocellulose membranes (e.g. Millipore Hi-Flow Plus HF240) and printing paper (e.g. Fabriano 5HP paper, Boise® Aspen® 30 multiuse recycled copy paper) are available. The papers are classified according to properties, such as particle retention, pore size, thickness and flow rate. The selection of a paper is dependent on the type of device as well as its specific application. Over the last decade, important studies have been conducted on the development of paper-based biosensors for on-site monitoring of water quality (Chouler, Cruz-Izquierdo, Rengaraj, Scott, & Di Lorenzo, 2018; Klug, Reynolds, & Yoon, 2018; López Marzo, Pons, Blake, & Merkoçi, 2013; S. Peixoto et al., 2019; Scala-Benuzzi, Raba, Soler-Illia, Schneider, & Messina, 2018; Scala-Benuzzi, Takara, et al., 2018; Vijitvarasan, Oaew, & Surareungchai, 2015). The lead author of this review has developed novel reagentless bioactive paper (lab-on paper) sensors for monitoring pesticides (e.g. paraoxon, bendiocarb and carbaryl), heavy metals and E. coli in...
water and food (Hossain et al., 2012; Hossain et al., 2009b). The sensors were fabricated using Grade 1 Whatman® filter paper. The paper was inkjet printed with a substrate zone and a sensing (biomaterial) zone. The substrate and biomaterial (enzyme) were entrapped within a silica matrix in a sandwich configuration (Figure 9). The colorimetric signal intensity of the sensing zone was either evaluated using the naked eye or quantified using an office scanner/cell phone with ImageJ software. This sensor can detect pesticides rapidly (≤5 min) and with a high sensitivity (LOD: paraoxon ~1 nM; bendiocarb ~1 nM; carbaryl ~10 nM; malathion ~10 nM) without the use of an instrument or reagents. The paper sensor can also detect heavy metals (Hg(II), Cu(II), Ag(I), Cd(II), Cr(VI), Pb(II), Ni(II), etc.) in 10 min, with low detection limits. Paper based sensors have also been developed for the simultaneous detection of a pathogen (e.g. *E. coli* 0157) and total coliform bacteria in water, food and beverage samples. The colorimetric detection using the naked eye enables the use of the paper sensors in remote areas without access to advanced instruments and trained personnel.

### 4.3. Online sensing technologies for monitoring water quality

Many biosensors have been proposed for continuous measuring protocols involving online measurements (Bhattacharyya et al., 2005; Han et al., 2002). Development and verification of predictive models are essential to understand and manage water quality in real-time. Development of predictive tools may also help to prioritize contaminant sources and to identify the relative importance of the many factors that influence water quality on different geographical scales (Robarts et al., 2007). Models can also be used to estimate the probabilities that the concentration of a specific compound exceeds guidelines/standards for drinking, agricultural and recreational water. Generation of real-time data allows the validation and verification of the model, concomitantly reducing the uncertainty of the output (Robarts et al., 2007). Predictive tools may also be used for mapping and predicting the extent of contamination in case of accidental spills or pollution events (Allan et al., 2006). In this regard, a novel early-warning system (EWS) has been developed to measure the toxicity of water effluents (Gu, Kim, Cho, & Hansen, 2001; Long et al., 2013; Tschmelak et al., 2005). More importantly, the EWS is a combined approach for detecting, interpreting, analysing and communicating data, in which real-time measurements are conducted by biosensors and a generic alarm is produced when a pollutant is identified in a sample.

Recently, Di Nardo et al. (2018) have developed an integrated smart meter with sensors connected to a cloud computing system for smart management of a water network, allowing the efficient online monitoring of water quality. Pasternak and his co-authors have reported a self-powered, autonomous BOD biosensor for online water quality measurement based on signal frequency (Pasternak, Greenman, & Ieropoulos, 2017). The schematic diagram and the working protocol of this sensor are presented in Figure 10. Contamination of water with urine can be detected using this sensor. As electroactive microorganisms are used to produce this biosensor, it is self-powered and can operate autonomously for five months. Galang, Bayliss, Marshall, and Sinnott (2012) have developed BeagleBoard- and Arduino-based biosensors that can automatically monitor animal behaviour responses to determine contamination in the area, along with other information such as...
temperature, turbidity and pH (Galang et al., 2012). Their work, however, require further validation, as it is totally dependent on a built-in video capture system. Meghwani (2017) have developed an online biosensor for water quality monitoring and control in a swimming pool. The measurement and control system is based on National Instruments hardware and its software package LabView, as shown in Figure 11. The device controls the disinfectant (calcium hypochlorite) dosage, water temperature, pH and turbidity, in order to continually maintain the pool water disinfected. Thomas et al. (2015) have described a concept of an online biosensor, which monitors selected pollutants and algal toxins in seawater for about six-months continuously using wireless data transmission. This biosensor is designed to be installed on buoys or similar platforms (Thomas et al., 2015). Shi et al. (2013) have demonstrated an automated online optical biosensing system (AOBS) for issuing quick warnings and the rapid detection of microcystin-LR (MC-LR) (Shi et al., 2013). This system has been successfully used for continuous long-term monitoring and early warning of MC-LR, which is a toxin formed by cyanobacteria, in Lake Tai (China). Researchers have also developed a prototype online fibre-optic biosensing system based on immobilized bioreporter bacteria for monitoring water pollutants (Eltzov, Slobodnik, Ionescu, & Marks, 2015). A schematic diagram of this biosensor and its principle of operation are shown in Figure 12. Scientists have also developed a marine MFC-biosensor with an integrated electro-chemical cell, which can be used to determine assimilable organic carbon (AOC) in seawater in real-time (Bee Quek, Cheng, & Cord-Ruwisch, 2015). Figure 13
Figure 13. Schematic diagram of the computer-feedback-controlled MFC-biosensor with an integrated electrochemical cell. The cell is divided into two chambers by a membrane. Seawater is continuously circulated through the anodic chamber of the O2 removal cell. The DO and pH probes measure the DO and pH of the influent and effluent using LabViewTM interfaced with a DAQ (adapted from Bee Quek et al., 2015).

Figure 14. Components of the real-time optical biosensor for automated bacterial colony counting using chip microscopy. (a) The ePetri consists of a CMOS chip, a thermoelectric cooler, and a camera. (b) The bacterial suspension is loaded onto the CMOS chip with adequate growth media. (c) The ePetri is placed in an incubator to take the images of colonies. (d) A bacterial growth curve is obtained from microscopy (adapted from Jung & Lee, 2016).

Table 3. Summary of online biosensors for detecting E. coli (adapted from Zhang, Jiang, Hao, & Qu, 2019).

| Category                     | Description                                                                 | References                                      |
|------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------|
| Optics (non-imaging)         | Measuring bacterial growth by detecting changes of optical signals using photometers | Maugeri, Lychko, Sobral, and Roque (2019)        |
| Optics (imaging individual cell) | Measuring physiological, morphological, metabolic or structural features of bacteria with cameras integrated with microscopy | Chen et al. (2017); Syal et al. (2017)          |
| Optics (imaging population)  | Measuring population of bacteria in liquid with imaging                     | Fredborg et al. (2013); K. et al. (2017)        |
| Electrochemistry (sensor)     | Measuring bacterial growth by detecting changes of electrochemical features of the electrodes and analytes | Ahmed, Rushworth, Hirst, and Millner (2014); Maugeri et al. (2019) |
| Electrochemistry (biosensor)  | Measuring bacterial growth by monitoring changes of electrochemical features of cells or metabolites using bio-elements immobilized on electrodes | Ahmed et al. (2014); Syal et al. (2017)          |
| Electrochemistry (contactless sensor) | Measuring E. coli growth by detecting changes in conductivity with CöD or other contactless sensor | Zhang et al. (2018)                            |
| Microcalorimetry             | Monitoring heat generation by growing bacterial cells                        | Bonkat et al. (2012)                           |
| Resonant mass                | Quantifying cell number by measuring changes of the mass of individual cells using a small channel of cantilever | Godin et al. (2010); K. et al. (2017)           |
| Gene analysis                | Measuring E. coli growth by detecting genes via augmentation with PCR         | Syal et al. (2017); Zhang et al. (2017)         |
shows the schematic diagram and working principle of the computer-controlled integrated sensor.

*E. coli* are considered as indicator bacteria for faecal pollution in water. Recently, researchers have developed electrochemical biosensors for on-line monitoring and measurement of *E. coli* pollution in surface water, groundwater and drinking water (Ettenauer, Zuser, Kellner, Posnicek, & Brandl, 2015; Kellner, Ettenauer, Zuser, Posnicek, & Brandl, 2016). The *E. coli* detection is based on intracellular β-galactosidase activity. This biosensor works as a standalone equipment with online data transfer for detecting and controlling the water quality as well as enhancing water treatment procedures. The instrument is capable of detecting at least two colony forming units (CFU) of *E. coli* in 8 h. Jung and Lee (2016) developed an optical biosensor for automated, real-time monitoring of microbial colony formation and growth in water (Figure 14) (Jung & Lee, 2016). The system can dynamically detect individual microcolonies using sub-pixel sweeping microscopy with a high-resolution. Similarly, Hassan et al. (2019) have reported the real-time detection of heavy metals and other toxic chemicals in water using a sulphur-oxidizing bacteria (SOB)-based biosensor. Generally, in nontoxic water, SOB oxidizes elemental sulphur (S(0)) to sulphuric acid (H2SO4) resulting in a decrease of the water pH, while SO4-2 increases the electrical conductivity (EC) of water. However, in the presence of contaminants, activity of SOB is inhibited, which results in an increase of the pH and a decrease of EC. Therefore, changes in EC can be used as an indication of toxicity that can be determined using the following equation.

\[
\text{Toxicity} = 1 - \frac{EC_{\text{out},t} - EC_{\text{out}}}{EC_{\text{ss}} - EC_{\text{in}}} \tag{7}
\]

where ECss denotes steady state EC, ECin denotes the influent EC, ECout,t denotes the EC at time ‘t’ after injection of toxic chemical and ECout denotes the EC of the effluent after the injection of the toxic chemical. Table 3 summarizes the general features of biosensors based on bacteria (optics- and electrochemistry-based) as well as some other advanced sensors.

### 5. Conclusion

In this article, the basic concepts of biosensors and the principles of their operation are discussed. Depending on the types of biological elements and transducers employed a wide variety of biosensors are available. Biosensors can be used for the rapid monitoring of water quality with a high specificity to a wide range of analytes, both as online and offline systems. Online systems are preferable for obtaining continuous real-time data. They can also provide early warnings of the spread of diseases by monitoring toxins or bacteria in the samples. Commercial-scale application of biosensors is, however, still limited due to various factors such as the size, cost, detection range, leakage of biological elements, selectivity and online deployment. In order to bring about a novel analytical device revolution, the scientists must consider these factors when designing biosensors. Advances in nanotechnology, biotechnology, microelectronics, microfluidics, telecommunication and other fields are promising and can be used to improve biosensor technologies. Successful integration of nanomaterials with biomolecules on the surface of electrodes or nanofilms may lead to the evolution of a novel generation of biosensors. Such devices can play a crucial role in clinical diagnosis, process control, food analysis and environmental measurement in the coming years. Even though, there are substantial advances in this field, a lot more remains to be accomplished, with a long way to go and explore.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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