Chapter 7 Development of Environmental Biosensors for Detection, Monitoring, and Assessment

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7.1 Introduction

Whatever that can be around us comprises our environment. Environment is everything that boosts our ability to live on the planet earth including the chemical, physical, and various other natural forces. It makes up our surroundings along with all the nonliving and living things like the water covering most of the earth's surface, the air we breathe, the soil, the animals and plants near us, the insects, and much more. Living things such as human beings constantly interact with each other and with their environment and try to adapt themselves as per its conditions in order to survive, forming an ecosystem $-$ a functional unit of environment (Health $\&$ Science [2011\)](#page-16-0). Since several years, scientists have been carefully analyzing this interaction and examining the different ways in which the humans affect their environment. Human beings are playing with natural environment to make their own lives' comfortable. As we know, every coin has two sides, and a rose has also a thorn: the same is true with modernization. There is no doubt that modern techniques play a significant role in making humans' lives easy, but they are also associated with major drawbacks that affect our environment badly. Scientists have observed increasing cases of air and water pollution, acid rains, deforestation, change in climate such as global warming, and many other problems which are dangerous not only to the earth but also to ourselves (Fact Monster [2017](#page-15-0)). Figure [7.1](#page-1-0) shows the effect of human activities on the environment. There have also been substantial occurrences of various natural calamities like cloud bursting, earthquakes, tsunamis, etc. in the past few years. So, proper monitoring of environment is the

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Fig. 7.1 Impact of human activities on environment

need of the hour so as to protect ourselves and the whole planet in general from such life-threatening situations and also to prepare ourselves well in advance in order to fight and survive such situations (Fact Monster [2017;](#page-15-0) Health & Science [2011\)](#page-16-0).

Environmental monitoring basically refers to the ways by which we can interpret the overall condition of our planet indicating how healthy it is. Global management of environment firstly requires the thorough investigation of various pollutants that are contaminating the air, water, food, soil, etc., which ultimately pose greater ecological threats, and then to find and adopt ways to eliminate these pollutants completely in the environment (Koedrith et al. [2015](#page-16-1)). Air pollutants originate from various sources such as power plants, refineries, laboratory and industrial processes, vehicle emissions, etc. Sulfur and nitrogen dioxide, volatile organic compounds, carbon monoxide, particulate matter, etc. are some of the major air contaminants. Water and soil pollutants are generally categorized as either radioactive elements (such as tritium), or microbiological (such as coliform) or inorganic compounds (like arsenic), or synthetic organic elements (like pesticides, herbicides, weedicides, etc.). Some volatile organic compounds like benzene also contribute in increasing water and soil pollution. Nowadays, food safety and biothreats are other major concerns gaining much attention (Singh [2017\)](#page-18-0). Figure [7.2](#page-2-0) specifies the various ways needed to be adopted in order to deal with these several environmental contaminants.

Strict regulations have been imposed by the US Environmental Protection Agency (US EPA) on the concentrations of various air and water contaminants present in the environment. However, traditional detection and monitoring techniques (like different chromatographic techniques (Justino et al. [2017;](#page-16-2) Lang et al. [2016;](#page-16-3)

Fig. 7.2 Different ways to reduce the impact of environmental pollutants on health

Hassani et al. [2017](#page-15-1)) are complex, time-intensive, and costly and require skilled labor and proper laboratory facilities with existing limitations in analytical and sampling methods (Ho et al. [2005;](#page-16-4) Looney and Falta [2000](#page-16-5); Koedrith et al. [2015\)](#page-16-1). Moreover, these conventional techniques also lack the efficiency when used for in situ measurements essential in emergency situations such as in the cases of acute poisoning, epidemics, or accidental release of biothreats or pesticides (Arduini et al. [2013;](#page-14-0) Guo et al. [2017;](#page-15-2) Zhang et al. [2014](#page-18-1)). Thus, in order to overcome the magnification of various environmental issues, there is urgent need of highly sensitive, rapid, cost-effective, miniaturized, portable, and easy-to-operate techniques and devices that can detect and monitor various contaminants responsible for the destruction of ecosystem (Zhang et al. [2014](#page-18-1); Guo et al. [2017](#page-15-2); Arduini et al. [2013\)](#page-14-0). Advancements in nanotechnology and biosensing techniques have made it possible to design such devices that can achieve all the aforesaid objectives. The analytical performance such as limit of detection, sensitivity, and specificity, especially for in situ applications, has been improved to a greater extent with the development of smart and rapid biosensors employing various nanocomposites or nanomaterials to detect distinct environmental pollutants (Maduraiveeran and Jin [2017;](#page-16-6) Zhao et al. [2013](#page-18-2)).

The subsequent sections of this chapter describe the various available biosensing techniques and explore their potential for the monitoring and detection of environmental pollutants.

7.2 Biosensing Techniques

The term "biosensor" is short form of "biological sensor." A biosensor is an analytical device that is made up of a biological component with a physicochemical detector. The biological element (e.g., an enzyme, cell receptors, organelles, a nucleic acid, an antibody, etc.) interacts with the analyte being tested producing a biological response, and then the transducer converts this biological response mostly into an electrical signal (Robertson [2019](#page-17-0)) which can be more easily measured and quantified. The detector element or the transducer works in either one of the following physicochemical ways: optical, piezoelectric, electrochemical, electrostatic, or electromechanical. Depending on their specific application, biosensors are also known as optrodes, immunosensors, resonant mirrors, biochips, chemical canaries, biocomputers, and glucometers (Robertson [2019;](#page-17-0) Monošík et al. [2012\)](#page-17-1).

7.2.1 Biosensor System

Every biosensor comprises a bio-recognition component, bio-transducer component, and electronic system which includes a signal amplifier, processor, and display. A typical biosensor system with its working principle is shown in Fig. [7.3](#page-3-0).

7.2.2 Classification of Biosensors

As shown in Fig. [7.3](#page-3-0), biosensors can be designed to detect and identify different bio-recognition components using different transduction principles. So, they can be classified based on these two criteria as shown in Fig. [7.4](#page-4-0) (Monošík et al. [2012](#page-17-1)). The brief description of each biosensor type is given below:

Fig. 7.3 Working principle of biosensors. (Vargas-Bernal et al. [2012](#page-18-3))

Fig. 7.4 Classification of biosensors

7.2.2.1 On the Basis of Bio-recognition Element

Biosensors can be used to detect either natural biological materials (such as microorganisms, antibodies, nucleic acids, enzymes, cell receptors, tissues, organelles, other natural products, etc.), or biologically derived materials (like aptamers, various engineered proteins including DARPins (designed ankyrin repeat proteins), bones, biomass, recombinant antibodies, etc.), or biomimics (such as synthetic receptors, combinatorial ligands, biomimetic catalysts, imprinted polymers, etc.) (Hashemi Goradel et al. [2018;](#page-15-3) Justino et al. [2017](#page-16-2); Lu et al. [2017\)](#page-16-7). Based on these recognition elements, biosensors are classified as:

Immunosensors

Immunosensors make use of the specific binding feature of an antibody and antigen (Salvador et al. [2007](#page-17-2)). In these types of biosensors, antibodies are used as recognition receptors. Antibodies are organic compounds that have the capability to control the grafting process and physicochemical superficial properties so as to improve the detection specificity and sensitivity (Vargas-Bernal et al. [2012\)](#page-18-3). Such biosensors are highly selective in detecting various analytes owing to the availability of a wide range of affinities. Further, monoclonal antibody technology (Peterson [2005\)](#page-17-3) if used with immunosensors can result in better screening of antibodies with defined biological and chemical properties at affordable costs.

Enzymatic Biosensors

These biosensors use enzymes as recognition elements owing to their easy availability, varied functionality, and high specificity (Hashemi Goradel et al. [2018\)](#page-15-3). Enzymatic biosensors are catalytic biosensors based on the principle of the conversion of a non-detectable substrate into an electrochemically or optically detectable product (Salvador et al. [2007\)](#page-17-2). Inhibitors, substrates, and modulators of catalytic reaction can be detected using these sensors. This process allows the detection of substrates, products, inhibitors, and modulators of the catalytic reaction. Such biosensors can operate in either direct mode or indirect mode. Direct mode involves the monitoring of concentrations of analytes and products formed during enzymatic reactions, whereas indirect mode monitors the enzyme inhibition by the analyte (Nigam and Shukla [2015;](#page-17-4) Rebollar-Pérez et al. [2016\)](#page-17-5). For the detection of various environmental pollutants, indirect mode is more favorable. For the successful biosensor design, immobilization of enzyme on the solid surface is one of the crucial steps. Immobilized enzymatic biosensors are more advantageous in comparison with the free enzyme-based sensors as they are rapid, require minimal amounts of sample, and are more sensitive as interference by the differential mode is less in these biosensors (Vargas-Bernal et al. [2012](#page-18-3); Nigam and Shukla [2015\)](#page-17-4).

Whole-Cell Based Biosensors

Whole cells (fungi, yeast, bacteria, or eukaryotic cells) can also be used as biorecognition element as they are better sources of enzymatic activities than isolated enzymes (Salvador et al. [2007;](#page-17-2) Gutiérrez et al. [2015](#page-15-4)). Whole-cell-based biosensors, like yeast biosensors, microbial biosensors, etc. utilize the immobilization techniques in order to immobilize the cells on transducer electrode. Such sensors are useful especially when detection of noncommercially available or expensive enzymes is needed (Ahmed et al. [2014;](#page-14-1) Hashemi Goradel et al. [2018\)](#page-15-3).

Genosensors

In these types of biosensors, bio-recognition component is a class of small biomolecules or biopolymers known as nucleic acids. Nucleic acids include DNA and RNA which are very large and complex forms of organic molecules and contain the genetic information and code of an organism. These are composed of nucleotides and are essential to all known forms of life (Antonio Blanco [2017\)](#page-14-2). The working of DNA-based biosensors relies on the principle of hybridization of complementary strands of DNA in a highly specific manner. Selection of probe and immobilization plays a significant role in the construction of a genosensor (Hashemi Goradel et al. [2018\)](#page-15-3). Genosensors have a wide range of applications in various fields like in medical diagnostics for the detection of genetic diseases, in forensic medicine, in environmental monitoring to control food and water contamination, for the detection of various pathogens, and so on (Valle [2011](#page-18-4); Orozco et al. [2016](#page-17-6)).

Aptasensors

Aptasensors are also DNA-based sensors in which selected DNA plays the role of a highly specific receptor of chemical or biological species (Hashemi Goradel et al. [2018\)](#page-15-3). Aptasensors use aptamer, an artificial nucleic acid ligand, as bio-recognition agent. Aptamers can be generated against proteins, amino acids, drugs, and other molecules (O'sullivan [2002\)](#page-17-7). The utilization of aptamers in biosensors for the detection of biomolecules has many advantages when compared with the use of antibodies due to the chemical stability, cost-effectiveness, and smaller size of aptamers (Lim et al. [2010](#page-16-8)).

Genosensors and aptasensors are the affinity-based DNA biosensors in which a DNA probe is used to capture an analyte or target. The other category of DNA biosensors based on the catalytic capability of DNA is termed as catalytic-based DNA biosensors. DNAzymes and aptazymes fall under this category of DNA-based biosensors (Hashemi Goradel et al. [2018](#page-15-3)). DNAzymes are not found in nature and are basically catalytically active DNA molecules that are obtained and isolated via in vitro selection (Zhou et al. [2017\)](#page-18-5). Aptazymes are ligand-activated self-cleaving ribozymes containing integrated aptamer domains and generated by combining DNAzymes, used for chemically modifying nucleic acids, and aptamers, required for binding a broad range of molecules (Zhou et al. [2017](#page-18-5); Tang et al. [2017](#page-18-6); Hashemi Goradel et al. [2018\)](#page-15-3).

Biomimetic Biosensors

In these biosensors, non-biological receptors are used as bio-recognition element as these receptors have the capability of mimicking the behavior of natural biomolecules. Biomimetic biosensors can be used to analyze the sense of taste, an indispensable perception of human sensory organ. Sense of taste refers to the five basic tastes including bitterness, sourness, sweetness, umami, and saltiness (Lu et al. [2017\)](#page-16-7). These biosensors also find applications in medical diagnostics, in monitoring environment and food safety, etc. (Kozitsina et al. [2018;](#page-16-9) Huffman et al. [2017](#page-16-10)).

7.2.2.2 On the Basis of the Transduction Principle

The transducer is an imperative part of a biosensor since it is responsible for the conversion of a biological or biochemical response into an analyzable and measurable electrical signal. Selection of a transducer for a particular application is crucial because appropriate transducers have a dramatic role in providing the optimum detection with high sensitivity and specificity (Salvador et al. [2007\)](#page-17-2). Different transduction mechanisms are available for designing a biosensor, and according to these signal transduction principles, biosensors can be classified in the following ways:

Electrochemical Biosensors

The electrochemical transducers are one of the most developed biosensors available in the literature (Rebollar-Pérez et al. [2016\)](#page-17-5). An electrochemical biosensor exploits the various physicochemical properties of electroactive substances and detects these properties in order to realize the bio-recognition so as to provide a measurable signal such as electrical voltage, current, impedance, or superficial charge (Vargas-Bernal et al. [2012\)](#page-18-3). Depending on the measured electrical signal and mode of electrical measurement, electrochemical biosensors can be subdivided into different categories such as potentiometric (measurement of voltage), amperometric (measurement of current), chronoamperometric, conductometric, impedimetric (resistive, capacitative), coulometric, and field-effect transistors (Vargas-Bernal et al. [2012;](#page-18-3) Salvador et al. [2007;](#page-17-2) Hashemi Goradel et al. [2018;](#page-15-3) Semantic Scholar [2013\)](#page-18-7). In chronoamperometric transduction technique, the working electrode potential is stepped which produces the current as a function of time by using faradaic processes. This technique is useful for analyzing the kinetics of chemical reactions, for monitoring the adsorption and diffusion processes, and for measuring protein and protease activity (El Harrad and Amine [2017](#page-15-5)). Conductometric technique depends on the current produced by ions in solution, while impedimetric transduction refers to the measurement of impedance (voltage as a function of the current). The performance of both these biosensing methods is highly influenced by a sensing layer placed between the two electrodes (Salvador et al. [2007\)](#page-17-2). Field-Effect Transistor (FET) based Biosensor mechanism is based on the measurement of current which is varied due to the potentiometric effect at a gate electrode (Guiseppi-Elie and Lingerfelt [2005](#page-15-6)). Coulometric biosensing principle is based on the exhaustive electrolysis of analyte by which we can determine the unknown analyte concentration in a solution. This is done by converting the analyte completely from one oxidation state to another (Chemicool Dictionary [2017](#page-14-3); David Harvey [2019](#page-15-7)).

Optical Biosensors

Optical biosensors rely on the principle of detecting the alterations in various optical properties such as adsorption, reflectance, refractive index, scattering of light, absorbance, luminescence (bioluminescence, chemiluminescence), fluorescence, etc. (Mishra et al. [2017](#page-17-8)). Optical techniques can be used for the quantification of an optical spectrum and for interpreting the changes in a mechanical measure and are required for overall biological or chemical analysis (Fortin [2009\)](#page-15-8). The detection of analyte is usually done by employing electromagnetic radiations, like ultraviolet (UV), infrared (IR), or other radiations (Hashemi Goradel et al. [2018\)](#page-15-3).

Piezoelectric Biosensors

Piezoelectricity is an electrical effect caused by the variations in the material strain (Fortin [2009](#page-15-8)). Piezoelectric biosensors are sensitive to changes in density, mass, or viscosity of samples in the vicinity of its active surface (Hashemi Goradel et al. [2018\)](#page-15-3). Acoustic wave biosensors employ the piezoelectric transduction mechanism for the detection of biomolecules. Such sensors are based on an oscillating crystal resonating at the fundamental frequency. The bio-recognition element needed to interact selectively with the target analyte is coated as a layer on the crystal element, and when the binding of analyte on sensing surface takes place, the mass of crystal changes which eventually changes the resonance frequency (Monošík et al. [2012\)](#page-17-1). Such biosensors are easy to operate and offer label-free on-line analysis at affordable costs. Cantilever biosensors are the other emerging class of mass-sensitive biosensors that can achieve lower limit of detections when compared with conventional methods (Salvador et al. [2007](#page-17-2)).

Thermometric Biosensors

Quantification of the release of thermal energy or the evolution and absorption of heat during a biochemical reaction is the fundamental principle of thermometric biosensors (Monošík et al. [2012;](#page-17-1) Hashemi Goradel et al. [2018](#page-15-3)). In these biosensors, immobilized enzyme or cell is directly attached to a thermistor, and the resistance of transducer changes as a result of the temperature variations in reaction medium (Salvador et al. [2007;](#page-17-2) Monošík et al. [2012](#page-17-1)).

Magnetic Biosensors

Magnetic biosensors utilize magnetic particles (such as paramagnetic or superparamagnetic particles or crystals) for detecting biological interactions (Nabaei et al. [2018\)](#page-17-9). The size of these particles ranges from micro- to nanometers, and their surfaces are modified and functionalized in order to recognize and identify specific biomolecules by measuring the variations in magnetic properties (magneto-optical properties, resistance, etc.) or by analyzing magnetically induced effects such as coil inductance alterations, Hall effect, giant magnetoresistance (GMR), etc. (Monošík et al. [2012](#page-17-1); Li and Kosel [2013](#page-16-11); Rife et al. [2003](#page-17-10)).

7.3 Environmental Biosensors

Environmental monitoring is the need of the hour as it is required to protect the whole ecosystem from various toxic pathogens and pollutants that are present and can be released into different media including water, soil, food, and air. These

environmental contaminants are associated with various diseases and thus are harmful to human health. Such pollutants in environment can be categorized into two groups: organic and inorganic compounds. Organic pollutants include pesticides, bisphenol A, surfactants, polycyclic aromatic hydrocarbons, hormones, linear alkylbenzene sulfonates, polychlorinated biphenyls, phenols, dioxins, antibiotics, alkanes, and toxins. Inorganic phosphates, metals, and nitrate are the types of inorganic pollutants. In recent years, various biosensors including genosensors, aptasensors, immunosensors, and enzymatic biosensors employing different transduction mechanisms (Monošík et al. [2012\)](#page-17-1) as mentioned in the previous section have been reported for the monitoring and detection of several environmental pollutants (Rebollar-Pérez et al. [2016\)](#page-17-5). Some of these pollutants are explained in this section, and the different biosensor detection systems developed to detect these pollutants are also discussed. Table [7.1](#page-10-0) provides a summary of recently developed environmental biosensors based on different principles with their detection limits.

7.3.1 Pesticides

Pesticides are one among the most important environmental pollutants and are significantly present in the environment due to exhaustive utilization in agricultural practices in order to increase the yield and crop productivity (Justino et al. [2017;](#page-16-2) Hashemi Goradel et al. [2018](#page-15-3)). Pesticides can be classified on the basis of their chemical structures into organophosphate (parathion), synthetic pyrethroids, organochlorine (atrazine), carbamate, and inorganic pesticides (Verma and Bhardwaj [2015\)](#page-18-8). These pesticides are dangerous to health as they can cause respiratory and immunological problems, nervous system disorders, and even cancers due to their carcinogenic nature (Sassolas et al. [2012\)](#page-18-9). For highly sensitive detection of these pesticides without the requirement of sample pretreatment, various simple and miniaturized in situ biosensors have been developed (Justino et al. [2017;](#page-16-2) Vogrinc et al. [2015;](#page-18-10) Kim et al. [2015](#page-16-12)), and few of them are summarized in Table [7.1](#page-10-0).

7.3.2 Pathogens

Pathogens are present in environmental matrices, especially in water compartments (Koedrith et al. [2015\)](#page-16-1). These pathogens (like *Escherichia coli* or *E*. *coli*) are dangerous to health and can cause several diseases. Some biosensors have been recently proposed for the environmental monitoring of these pathogens and are described in Table [7.1](#page-10-0) (Justino et al. [2017](#page-16-2); Liong et al. [2013;](#page-16-13) Vidic et al. [2017](#page-18-11); Hashemi Goradel et al. [2018\)](#page-15-3).

Table 7.1 Recently developed biosensors for the detection of various environmental pollutants **Table 7.1** Recently developed biosensors for the detection of various environmental pollutants

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7.3.3 Potentially Toxic Elements or Heavy Metals

Owing to industrialization, the amount of heavy metals (like zinc, mercury, copper, etc.) in the environment has increased. Over the time, these metals accumulate in the environment due to their nondegradable nature, and almost all these metals have toxic effects and produce reactive oxygen species (ROS) (Gutiérrez et al. [2015\)](#page-15-4). Water pollution caused by heavy metals and their respective ions can result in several human health hazards. So, the rapid and highly sensitive on-field analysis of heavy metals at affordable costs is a primary concern worldwide. Different portable biosensors developed (Long et al. [2013](#page-16-20); Kim et al. [2015;](#page-16-12) Hashemi Goradel et al. [2018;](#page-15-3) Justino et al. [2017;](#page-16-2) Vogrinc et al. [2015\)](#page-18-10) for the detection of few of these metals are described in Table [7.1](#page-10-0).

7.3.4 Toxins

Eutrophication of aquatic systems results in the algal blooms of cyanobacteria which produces harmful toxins such as microcystins and brevetoxins, causing the water pollution (Justino et al. [2017;](#page-16-2) Vogrinc et al. [2015](#page-18-10)). So, in order to prevent the life-threatening situations, the early detection of such toxins is much needed. Costeffective and reliable biosensor systems have been developed (Marques et al. [2017;](#page-17-17) McNamee et al. [2013](#page-17-18); Wang et al. [2015](#page-18-18)) in the recent years for the sensitive detection of various toxins as specified in Table [7.1](#page-10-0).

7.3.5 Endocrine-Disrupting Chemicals (EDCs)

EDCs (such as bisphenol A, 4-nonylphenol, etc.) are present in our environment, food, and consumer and personal care products and can interfere with our metabolism, hormone biosynthesis, etc. (Diamanti-Kandarakis et al. [2009\)](#page-15-19). They have been suspected to be associated with the increased breast cancer incidences, immune function variations, altered reproductive functions in females and males, abnormal growth patterns, and neurodevelopmental delays in children (World Health Organization [2012\)](#page-18-21). Different biosensors (Justino et al. [2017;](#page-16-2) Hashemi Goradel et al. [2018;](#page-15-3) Vogrinc et al. [2015;](#page-18-10) Kim et al. [2015\)](#page-16-12) developed for the detection of these deadly EDCs are summarized in Table [7.1.](#page-10-0)

7.3.6 Other Environmental Compounds

Apart from the abovementioned environmental contaminants, pollutants like harmful algal blooms, pharmaceutical wastes, etc. are also present in the environment. Electrochemical genosensors have been developed for the detection of Algal RNA (Orozco et al. [2016;](#page-17-6) McPartlin et al. [2017](#page-17-20)). Pharmaceutical wastes contaminate the water due to the improper excretion of beta-blockers, analgesics/anti-inflammatories, antihypertensive drugs, antibiotics, psychiatric drugs, and so on. Therefore, highly sensitive and specific detection of these compounds is essential in order to prevent life-threating effects on living organisms including humans. For pharmaceutical detections, many enzyme-based biosensors based on the use of tyrosinases, laccases, and peroxidases have been developed (Rebollar-Pérez et al. [2016\)](#page-17-5).

7.4 Summary

In this chapter, different biosensing techniques and their applications in the detection of various environmental pollutants have been summarized. Development of specific, rapid, sensitive, and cost-effective biosensors is crucial for the protection of the ecosystem from life-threatening situations and for its survival in fluctuating and harsh environmental conditions. Conclusively, for the overall environmental management, we need to find and adopt certain ways and techniques that can reduce the side effects of modernization and industrialization. The combined use of such corrective measure techniques and biosensors will help in improving the environment quality and will protect the mankind from deadly situations, eventually making the planet earth a healthy place to live in.

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