

Biosensors for the detection of environmental and urban pollutions[†]

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Abstract

Release of harmful pollutants such as heavy metals, pesticides and pharmaceuticals to the environment is a global concern. Rapid and reproducible detection of these pollutants is thus necessary. Biosensors are the sensitive and high specific tools for detection of environmental pollutants. Broad range various types of biosensors have been fabricated for this purpose. This review focuses on the feature and application of biosensors developed for environmental and urban pollutants detection. This article is protected by copyright. All rights reserved

Key words: heavy metals; pesticides; biosensor; environmental pollutants

Introduction

One of the consequences of rapid human and technological advances is extensive man-made chemicals and by-products made in industries or combustion processes. These deleterious pollutants are increasingly released in the environment and affect the quality of the environment. Environmental pollution is the major source of the mankind health problems and imposes unfavorable socioeconomic sequels (Koedrith et al. 2015). Pesticides, heavy metals, pharmaceuticals and water-borne pathogens are major culprits for environmental and urban pollution. Thus, there is an increasing need for effective tools to detect these toxicants and estimate the related risks associated with the release of large quantities of environmental pollutants. Although traditional approaches have enabled accurate analysis of chemical or physical properties of environmental samples (Nigam and Shukla 2015), there is still need to fast and cost-effective tools for the detection and monitoring of toxic pollutants (Palchetti and Mascini 2008).

Biosensor is known as one of powerful tools for the detection of environmental and urban pollutions. IUPAC definition form a biosensor is “as a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in direct spatial contact with a transduction element “ (Palchetti and Mascini 2008; Vogrinc et al. 2015). Indeed, biosensor is a device which converts a biological or biochemical signal into a measurable electrical signal (Ezeonu et al. 2012) and they are valuable tools for the detection of chemical and harmful substances in health-care products, foods, and the environmental (Rebollar-Pérez et al. 2015). Biosensors can be used for continuous monitoring of a contaminated area. They may also offer advantageous analytical features, such as high specificity and sensitivity. At the same time, biosensors offer the possibility of determining not only specific chemicals, but also their biological effects, such as toxicity, cytotoxicity,

genotoxicity or endocrine disrupting effects (Rodriguez-Mozaz et al. 2006). In comparison with traditional techniques, biosensors have several advantages including portability, miniaturization, capacity of pollutant detection in complex matrices with minimal sample preparation (Palchetti and Mascini 2008; Rodriguez-Mozaz et al. 2006), and rapidity and reliability of detection (Saleem 2013). In addition, the use of conventional methods usually requires skilled personnel to operate as a technician (Saleem 2013) and specialized laboratories (Nigam and Shukla 2015). Here, we present a brief review of the biosensor classes and their applications in the monitoring of environmental pollutions.

Biosensors and their classification

According to the IUPAC definition, there are three basic components in a biosensor: a biological recognition element, a transducer, and a signal processing system (Nigam and Shukla 2015). Recognition of the target analyte is made by a bioreceptor which is a biomolecule, and transducer converts the recognition event into a measurable signal (Koedrith et al. 2015). Figure 1 shows a schematic representation of a biosensor.

On the basis of the bio-recognition principles or signal transduction, biosensors are classified into various categories (Rebollar-Pérez et al. 2015). According to the transducing component, biosensors are designed as electrochemical (amperometric, chronoamperometric, potentiometric, conductometric, capacitive, field-effect transistors), optical (absorbance, reflectance, luminescence, chemiluminescence, bioluminescence, fluorescence, refractive index, light scattering), piezoelectric (mass sensitive quartz crystal microbalance), or thermal (thermistor, pyroelectric) (Ezeonu et al. 2012).

Electrochemical: low cost, ease of use, and construction simplicity are the advantages of electrochemical biosensors (Ronkainen et al. 2010). Electrochemical biosensors measure the

electrical properties of biological systems. Although variety of recognition systems are used in the design of biosensors, electrochemical biosensors predominantly involve enzymes (D'Orazio 2003). In the design of electrochemical biosensors, three electrodes are used: reference electrode (usually Ag/AgCl) for maintaining stable potential, counter or auxiliary electrode for establishing a connection to the electrolytic solution, and working electrode. In the amperometric, potentiometric and conductometric biosensors current, potential or charge accumulation and conductive properties of the medium are measured, respectively (Grieshaber et al. 2008). Impedimetric biosensors measure resistance and reactance, where current measurement as a result of a potentiometric effect at a gate electrode is the field-effect biosensors mechanism (Guiseppi-Elie and Lingerfelt 2005).

Optical: using an optical transducer in order to detect optical properties changes is the design principle of optical biosensors (Eltzov et al. 2011). Adsorption, fluorescence, luminescence, and refractive index are the most commonly used properties of the optical biosensors (Dai and Choi 2013). Optical biosensors employ electromagnetic waves for detecting analyte and could immobilize bio-element interactions which are known by the name of surface plasmon resonance (SPR) (Monošík et al. 2012). SPR employs change in the refractive index of surface metal for detection (Patel et al. 2010).

Piezoelectric: piezoelectric biosensors are sensitive to changes in mass, density, or viscosity of samples in contact with its active surface. The relationship between the resonant frequency of an oscillating piezoelectric crystal and the mass deposited/adsorbed on the crystal surface is the theoretical principle of piezoelectric biosensors (A. Kumar 2000; Marrazza 2014). Quartz crystal microbalance (QCM) is the most well-known piezoelectric biosensor that has a thin plate of quartz, with metallic electrodes (usually gold) attached to each side of the plate (Marrazza 2014). In QCM, the bioreceptor is immobilized on quartz crystal and a constant flow of analyte is applied to the immobilized ligand in order to facilitate the reaction between

bioreceptor and analyte. In the end, sensogram determines viscosity changes as a signal (Jia et al. 2013). Because of bioreceptor immobilization and loosing degree of freedom, true affinities measurement of QCM does not happen (Marrazza 2014).

Thermal: quantification of the release or absorbance of thermal energy during a biochemical reaction is the principle of these biosensors (Monošík et al. 2012). Since thermal activity is one of the essential parts of biochemical reactions, thermal biosensing is a broadly applicable method (L. Wang et al. 2008).

Biosensors can utilize biological molecules (antibody, enzyme, nucleic acid), living biological systems (cell, tissue, or whole organ) or biomimetic materials for detection and recognition (Koedrith et al. 2015). Thus, according to the biorecognition method, biosensors can be classified as:

Enzyme based biosensors: specificity, availability and variety of functions makes enzymes appropriate to be employed as recognition elements. According to the mode of analyte monitoring, enzyme-based biosensors are classified into direct and indirect modes. Monitoring of analyte concentration or formed products during enzymatic reactions is called direct mode and monitoring of enzyme inhibition by the analyte is called indirect mode. In the enzyme-based biosensors, indirect mode is more favorable (Rebollar-Pérez et al. 2015). Immobilization of enzyme on the solid surface is one of the most important aspects of biosensor design. Covalent binding, entrapment, and microencapsulation are the most common immobilization methods. Immobilized enzyme-based biosensors advantages are less sample requirement, rapid analysis and reduction of interference by the differential mode of operation in comparison with free enzyme-based biosensors (Turdean 2011). In most of the biosensors, glucose oxidase (GOx), horseradish peroxidase (HRP) and alkaline phosphatase are used as the enzyme component (Bănică 2012; Cao et al. 2011). As mentioned, enzyme

inhibition (indirect) mode of biosensors is applied for monitoring of a wide range of environmental pollutants. In order to evaluate analyte concentration, percentage of inhibited enzymes after exposure to the inhibitor is measured (Rebollar-Pérez et al. 2015). The pitfall of indirect mode biosensors for the detection of environmental pollutants is that few enzymes are sensitive to heavy metals (Turdean 2011).

DNA based biosensors: binding to a broad range of molecules with high affinity and specificity together with the catalytic activity of DNA molecules make DNA as a valuable option for designing biosensors (Palchetti and Mascini 2008). In biosensors, DNA can be used either in the native (Calf thymus, double-strand DNA as ds-DNA) or modified (single-strand DNA [ss-DNA]) form for the detection of DNA fragments, biological or chemical species (Turdean 2011). Principle of DNA-based biosensing is based on the highly specific hybridization of complementary strands of DNA. DNA-based biosensors are classified into two groups: affinity-based (genosensors and aptasensors) and catalytic-based (DNAzymes and aptazymes). In genosensors, hybridization of a probe (usually a short synthetic and sequence-specific oligonucleotide) and a signal transducer is the principal formation of biosensor. The most important factor for the construction of genosensor is probe selection and immobilization. In aptasensors, selected DNA plays the role of a highly specific receptor of biological or chemical species (Hianik and Wang 2009). Catalytic capability of DNA opens new category of biosensors by the name of DNAzymes. DNAzymes are isolated by *in vitro* selection are not been found in nature. Combination of DNAzymes which are able to make chemical modification on nucleic acids and aptamers which are able to bind to a broad range of molecules, generates aptazymes (Palchetti and Mascini 2008).

Ab-based biosensors (immunosensors): specific interactions between antigen and antibody is the principle in designing immunosensors (Long et al. 2013; North 1985). In electrochemical biosensors, changes in the electrical properties in the gap between two electrodes results from

antigen-antibody reactions (Katz and Willner 2003). Immobilization of antibodies on the surface is one of the most critical steps in designing biosensors which can even cause loss of their function in case of non-oriented immobilization. Non-oriented immobilization results from random immobilization and steric hindrance due to high density of antibodies (Karyakin et al. 2000; Grieshaber et al. 2008).

Whole cell-based biosensors: whole cells have been used as a biorecognition part in biosensors via immobilizing the cells on a transducer electrode (Ziegler 2000). In comparison with isolated enzymes, whole cells provide a better source of enzymatic activity especially for expensive or non-commercially available enzymes (J. Wang 2006). A microbial biosensor is a biosensor in which a microbe is immobilized on a transducer to monitor biochemical processes (Ronkainen et al. 2010). Although using enzymes in the microbes is time- and cost-effective (Dai and Choi 2013), longer recovery times, longer response time and hysteresis effect are disadvantages of microbial biosensors (Ronkainen et al. 2010). Yeast biosensors are another type of whole cell-based biosensors which are highly specific for some targets (Jarque et al. 2016).

Environmental monitoring

As mentioned, environmental pollutants are harmful to human health. Generally environmental pollutants are classified into two groups: organic compounds and inorganic compounds. Pesticides, hormones, polychlorinated biphenyls, dioxins, phenols, bisphenol A, surfactants, linear alkylbenzene sulfonates, alkanes, polycyclic aromatic hydrocarbons, antibiotics, and toxins are organic pollutants. Inorganic pollutants include metals, inorganic phosphates, and nitrate (Marrazza 2014). Table 1 shows some environmental pollutants and their respective biosensor detection systems. In the following sections we discuss three

dangerous types of pollutants. Figure 2 shows a range of Environmental applications of biosensors.

Pesticides: Because of exhaustive use in agriculture to increase productivity and crop yields, pesticides are ubiquitously present in the environment (Verma and Bhardwaj 2015). Pesticides cause nerve system disorders, immunological and respiratory diseases, and are also carcinogenic (Sassolas et al. 2012). Pesticides classification is based on their chemical structure: organochlorine (Atrazine), organophosphate (Parathion), carbamate, synthetic pyrethroids, and inorganic pesticides (Verma and Bhardwaj 2015). Organochlorine pesticides adversely alter reproductive system of fishes and could thus affect ecosystem (Vigneshvar et al. 2016). The most widespread used biosensors for the detection of pesticides are enzyme-based biosensors that are especially based on the inhibition of acetylcholinesterase (AChE) and colin oxidase (Rebollar-Pérez et al. 2015). Inhibition of AChE in nerves result in the accumulation of the neurotransmitter acetylcholine which is life-threatening (Verma and Bhardwaj 2015). Measurement of enzyme inhibition and components involved in the enzymatic reactions are used for the detection of pesticides (Sassolas et al. 2012).

Heavy metals: Heavy metals content is increased by mining and other industrial activities. Heavy metals are not degradable and accumulate in the environment over time. Almost all of heavy metals have toxic effects mediated by producing reactive oxygen species (ROS) (Gutiérrez et al. 2015). Although some metals are needed for different biological processes (Rebollar-Pérez et al. 2015) such as enzymatic reactions (as a cofactor) (Ezeonu et al. 2012), they can induce enzyme inhibition in some cases (Rodriguez-Mozaz et al. 2006). Thus, activation or inhibition of enzymes is used for designing heavy metal biosensors. Zinc and copper can be detected by the activation of alkaline phosphatase and ascorbate oxidase-based

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biosensors, respectively (Sato and Iijima 1995). Glucose oxidase (GO) inhibition can be applied as a biosensor for the detection of heavy metals such as cadmium, cobalt, copper and nickel (Ghica et al. 2013). Optical biosensors for the detection of metals are based on the activity a reporter gene under the control of an inducible promoter (Rodriguez-Mozaz et al. 2006). In this strategy that is called “turn on assay”, the reporter signal level increases according to the pollutant concentration. β -galactosidase (*lacZ*), luciferase (*luc*) and green fluorescent protein (GFP) are the most popular reporter genes used in biosensor systems (Gutiérrez et al. 2015).

Pharmaceuticals: One of the current concerns about water contamination is improper excretion of hospital wastes. Commonly founded pharmaceuticals in the water are likes of antihypertensive drugs, beta-blockers, analgesics/anti-inflammatories, antibiotics and psychiatric drugs. Therefore, detection of these compounds is essential to prevent life-threatening effects on human and other living organisms. Enzyme-based biosensors using peroxidases, laccases, and tyrosinases are main biosensors for the detection of pharmaceuticals (Rebollar-Pérez et al. 2015). Levetiracetam, an antiepileptic drug, can be detected using HRP-based biosensors (Alonso-Lomillo et al. 2009). Another example is the laccase enzyme used for the detection of methyldopa which is an antihypertensive drug (Moccelini et al. 2011).

Conclusion

In this review, we summarized different biosensor types and their application in the environmental pollutant detection. Developing sensitive, specific, and cost-effective biosensors is crucial for the detection of small molecules. Another critical step in designing a biosensor is immobilization of biomolecules on the surface. In recent years, nanotechnology has found more important roles in the design and application of biosensors. Selection of

materials and response to parameters such as pH and temperature are key steps for designing and optimizing sensing systems (Bidmanova et al. 2016). The using of 3D microenvironment could help to encapsulation of whole cell especially transparent materials in the optical methods which will improve biosensor fields (Vigneshvar et al. 2016). Well features of carbon nanotubes and graphene could make them as structures potential candidates for free labeling of biosensors (Ramnani et al. 2016).

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Table 1. Biosensors for detection of organic and inorganic pollutants

Target	Transducer	Bio-element	Reference
Zinc, Cadmium	Optical	Microorganism (E.coli)	(Vogrinc et al. 2015)
Glyphosate	Electrochemical	Enzyme (atemoya peroxidase)	(Oliveira et al. 2012)
Bisphenol A	Optical	Antibody	(Rodriguez-Mozaz et al. 2005)
Copper	Electrochemical	Enzyme (HRP)	(Moyo et al. 2014)
Methyl Parathion	Optical	Microorganism (Flavobacterium)	(Vogrinc et al. 2015)
Aflatoxin	Electrochemical	DNA	(Marrazza et al. 1999)
Atrazine,	Piezoelectric	Antibody	(Jia et al. 2013)

Carbofuran			
Copper	Optical	Microorganism (yeast)	(Vopálenská et al. 2015)
Catechol	Electrochemical	Microorganism (Lactobacillus)	(Vogrinc et al. 2015)
Nonylphenol	Electrochemical	Antibody	(Evtugyn et al. 2006)
Nitrate	Optical	Microorganism (E.coli)	(Taylor et al. 2004)
Parathion	Piezoelectric	Antibody	(Funari et al. 2013)
Mercury	Optical	DNA	(Knecht and Sethi 2009)
p-Nitrophenol	Electrochemical	Microorganism (Pseudomonas)	(Banik et al. 2008)
Arsenite, Selenite	Optical	Microorganism (E.coli)	(Ooi et al. 2015)
Chlorophenol	Electrochemical	Enzyme (HRP)	(Qiu et al. 2013)
Salicylate	Optical	Microorganism (E.coli)	(Shin 2010)
Paraoxon, Carbofuran	Electrochemical	Enzyme (AChE)	(Bachmann and Schmid 1999)
Carbaryl	Piezoelectric	Antibody	(J.-j. Wang et al. 2014)
Serotonin	Optical	Microorganism (yeast)	(Nakamura et al. 2015)

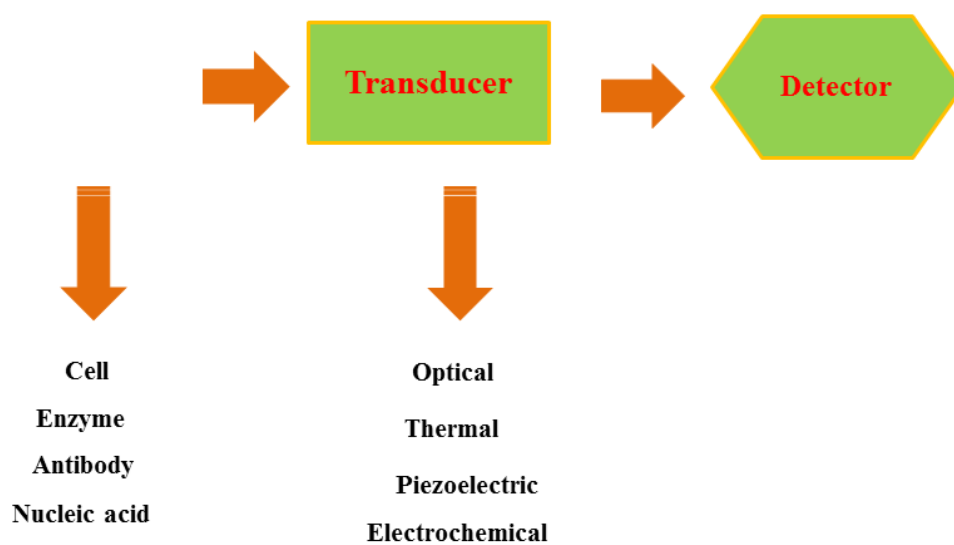


Fig 1: Schematic components of a biosensor

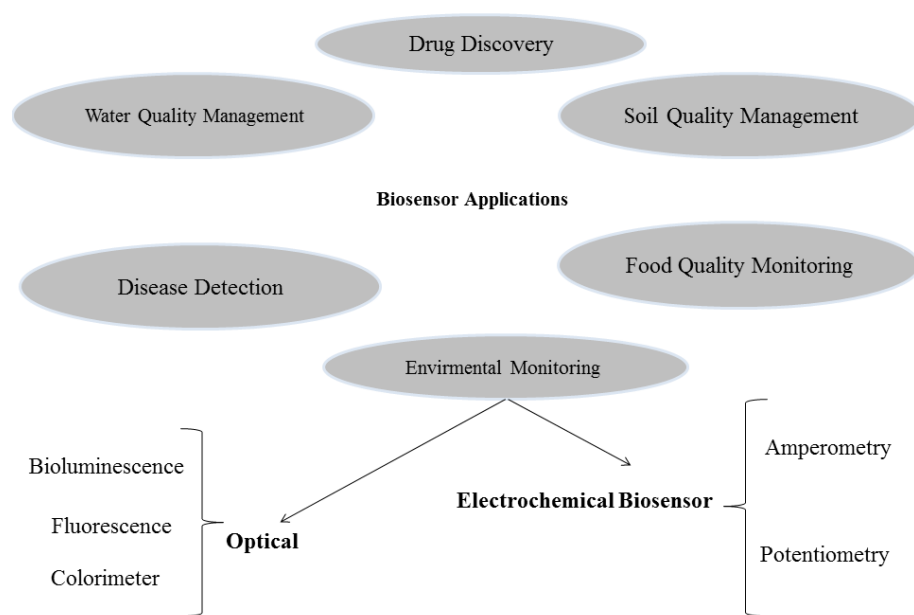


Fig 2: Various applications of biosensors