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Review

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Enzyme Based Biosensors for Detection of Environmental Pollutants-A Review

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Environmental security is one of the major concerns for the safety of living organisms from a number of harmful pollutants in the atmosphere. Different initiatives, legislative actions, as well as scientific and social concerns have been discussed and adopted to control and regulate the threats of environmental pollution, but it still remains a worldwide challenge. Therefore, there is a need for developing certain sensitive, rapid, and selective techniques that can detect and screen the pollutants for effective bioremediation processes. In this perspective, isolated enzymes or biological systems producing enzymes, as whole cells or in immobilized state, can be used as a source for detection, quantification, and degradation or transformation of pollutants to non-polluting compounds to restore the ecological balance. Biosensors are ideal for the detection and measurement of environmental pollution in a reliable, specific, and sensitive way. In this review, the current status of different types of microbial biosensors and mechanisms of detection of various environmental toxicants are discussed.

Keywords: Bioreceptors, biosensors, degradation, enzymes, pollutants, transducers

Introduction

The increase in living standards and higher consumer demands have increased pollution of the air with CO_2 and other greenhouse gases; particulate matter of water along with a variety of chemicals, nutrients, and oil spills; and of the soil owing to the disposal of hazardous wastes, application of herbicides and pesticides, and disposal of non-biodegradable materials, *etc*. Man-made chemicals like pesticides, cosmetics, personal and household care products, and pharmaceuticals, which are in use worldwide and are indispensable for modern society, are adding pollutants to the atmosphere [13]. Furthermore, human activities have resulted in contamination of water resources with biological micropollutants, such as viruses and bacteria. Such agents have generated renewed awareness due to their potential pathogenicity and are referred to as emerging or reemerging pathogens. Biological micropollutants, such as enteric bacteria, mycoplasmas, viruses, and protozoa, are the source of many waterborne diseases and remain a major cause of death worldwide [50]. For the overall safety and security of living systems, there should be a comprehensive analysis of toxicants, as well as their detection and continuous monitoring in environmental samples of soil, water, and air. Two different approaches have been considered for monitoring pollutants in environmental samples. The traditional approach provides accurate analysis of chemical or physical properties of environmental samples but requires costly analytical instruments with specialized laboratories for analysis of pollutants present in the samples. However, these systems of analysis fail to provide information on the bioavailability of pollutants and related effects on biological systems. To overcome shortcomings of the traditional approach, a complementary approach is being evaluated where living systems are used for environmental sample bioassays. Various biological systems such as bacteria, fungi, blue green algae, and enzymes etc are applied for such analyses. Unicellular microorganisms, particularly bacterial systems, are used for such purposes. It is because of their rapid growth rate, large population size, and minimum requirement for growth and maintenance (low cost). In addition, the bacterial

systems can be genetically engineered to analyze the pollutants in their environmental conditions. Furthermore, proteins that are present in cells can also be applied for the detection of a specific analyte [1]. As a result, a simple, specific, sensitive, rapid, and portable method needs to be developed for the analysis and observation of environmental security threats, and hence, biosensors appear as an appropriate choice as an analytical tool for such analysis [37]. Biosensors are devices that use any biological mechanism for the detection of analytes. The biosensor is not necessarily an independent unit, but is regarded as a component of a general designed instrumentation [31].

Biosensors and Their Classification

A biosensor is a self-sufficient integral tool that provides precise, quantitative, and analytical information, using a biochemical receptor that is in direct contact with a transduction element. The biosensor is primarily made up of three constituents; a biological recognition element, a transducer, and a signal processing system [48]. A schematic representation of a biosensor is given in Fig. 1.

The biosensors are further classified on the basis of properties of bioreceptors engaged in the detection procedures (*e.g.*, enzymes, microbial whole cells, plants, animals, antibodies, proteins or DNA fragments) or according to the physicochemical nature of transducers applied for the detection of toxicants (*e.g.*, electrochemical, optical, piezoelectrical, calorimetric, or thermal, *etc.*) [47, 58]. In the creation of biosensors, the main problem occurs during the integration of biological materials with the transducers on some physical surfaces. Direct immobilization without use of a bifunctional agent, immobilization using a bifunctional agent, or use of specialized membranes with or without inclusion of bifunctional agents have been reported [18]. The major tasks during development of a biosensor for detection of an analyte in a wide range of concentrations without other interferences depend on the choice of an appropriate bioreceptor molecule, an appropriate immobilization technique, selection of a precise transducer, and finally the packaging in a portable form. It has been observed that interdisciplinary cooperations among the various streams are essential for the development of a successful biosensor.

Similarly, the key properties during the development of an enzyme-based biosensor include accuracy and precision during the measurement, speed, sensitivity, specificity and range of measurement, reliability to testing, calibration and long-term stability, robustness, size, safety and portability, cost of analysis, and acceptability by users [8]. Biological catalysts (enzymes) can detect the presence of certain analytes by measuring either the consumption or production of certain compounds such as CO_2, NH_3, H_2O_2, H^+, or O_2, and thus transducers identify the pollutants and correlate their existence into the substrates [56].

Biosensors for Detection of Various Pollutants

Various anthropogenic sources are responsible for the release of pollutants in the environment. Although several measures are considered for the monitoring of trace pollutants in the environmental samples by specific techniques, there are still undetected contaminants (pharmaceuticals, endocrine disruptors, hormones, toxins, *etc.*) that need to be identified and quantified [15]. Details about the behavior of pollutants, biochemistry of their action, and threats to ecological and human health are still lacking. The pollutants present in the environmental samples are usually categorized on the basis of their chemical structures or according to their mode of action [42]. Biosensors have been developed or are being studied for the assessment of a variety of pollutants (organic and inorganic) in the environment, with less success for biosensors in air pollution measurement. The key advantages offered by biosensors over conventional investigative methods for environmental applications include their portability, smaller size (compact), work on site, and capacity to analyze contaminants in composite matrices with the least sample preparation. However, most of the biosensors are restricted for a specific toxicant or can be applied for a limited number of pollutants [41, 43, 44]. Recently, a number of initiatives and legislative actions have been made along with growing scientific and social awareness to control environmental pollution, particularly water quality control.

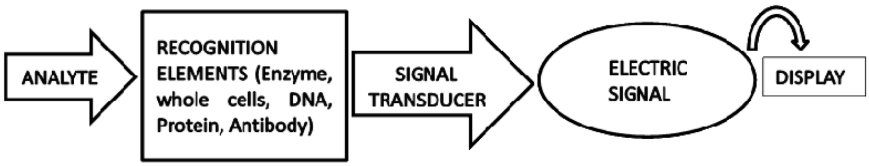

Fig. 1. Different parts of a biosensor.

Measures have been taken for the effective decrease of persistent organic pollutants that pollute the environments. For the effective monitoring of environmental samples and related analysis, several disposable systems or new methodologies are being studied that can analyze the increasing number of pollutants quickly with low cost and with the option of on-site field assessment. Therefore, biosensors have proven effective analytical tools for analyzing environmental pollutants [59]. A recent study showed construction of a new microbial biosensor based on the *ttgR*-regulated promoter, which controls the synthesis of the TtgABC extrusion efflux pump of *Pseudomonas putida*, combined with a *gfp* reporter gene. The designed system was introduced in a strain of *P. putida* DOT-T1E that can survive under high concentrations of various toxic organic compounds. The developed whole-cell biosensor was capable of detecting a wide range of structurally diverse antibiotics, as well as compounds such as toluene or flavonoids [60].

The majority of bacterial biosensors used for the detection of heavy metals in environmental samples employ genes that are resistant against these elements as bioreceptor molecules. A few of the bacterial systems have been evaluated as possible biological receptors for the detection of zinc, copper, silver, tin, mercury, cobalt, *etc*., with the resistance properties towards these metals. Certain biosensors have been constructed by fusing the genes of the resistance mechanism with the genes responsible for expressing bioluminescent proteins like luciferin for the detection of metals present in samples. The detection of metal ions can also be determined by enzyme-catalyzed reactions, as most of the ions specifically inhibit the enzyme at low concentrations.

Phenols and their derivatives are considered as toxic compounds and found in various industrial effluents associated with the production and synthesis of plastics, dyes, polymers, pharmaceuticals, detergents, pesticides, disinfectants, *etc*. Chloro- and nitrophenols are produced as main degradative products from organophosphorus pesticides and chlorinated phenoxyacids. It has been reported that these compounds showed severe toxic effects in animals and plants, causing genotoxicity and mutagenicity, and decreasing other life processes like photosynthesis, respiration, and enzyme-catalyzed reactions at low concentrations. Hence, phenols and particularly substituted ones, owing to their high toxicity, are defined as hazardous pollutants and are listed as hazardous materials and key pollutants of the European Commission and the US Environmental Protection Agency. Some important enzymes like laccase, tyrosinase, and peroxidases are exploited for the development of biosensors for degradation of phenolic compounds. A number of phenol-detecting biosensors are described using various microorganisms either in free-state or in immobilized form [29, 30, 54]. Cyanide is very toxic and inhibits respiration by binding with cytochrome oxidase. *S. cerevisiae* was reported as a microbial sensor for monitoring cyanide concentrations in river water, as the presence of cyanide inhibits the respiration process of yeast [16]. An oxygen electrode has been constructed for monitoring the presence of cyanide, which exploits immobilized bacteria (*P. fluorescens* NCIMB 11764) [5, 25].

Organophosphorus (OP) compounds are chemicals being used as insecticides for regulating a number of insect pests, weeds, and disease-transmitting carriers in agriculture. Because of the toxicity of these pesticides and their presence in environmental samples, the European Commission has set certain concentration limits for their application. The acceptable limit of 0.1 μg/l for individual pesticides and 0.5 μg/l for total pesticides was regarded as safe in potable water by Directive 98/83/EC. Certain enzyme sensors have been constructed for the assessment of OP in different samples on the basis of inhibition of a specific enzyme by these OP compounds. Some of the related examples are development of biosensors for recognition of OP and carbamate pesticides due to their inhibitory properties on acetyl cholinesterase and colin oxidase [2, 3, 12, 22]. Similarly, herbicides (phenyl urea) and triazines that inhibit the process of photosynthesis can be detected by biosensors in which amperometric and optical transducers are used [20]. The construction of an amperometric

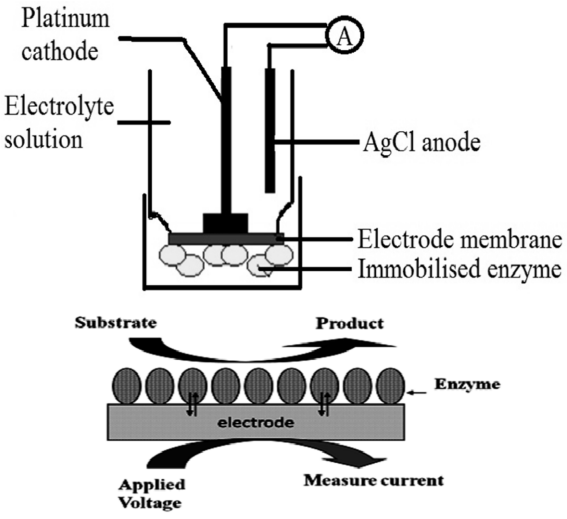

Fig. 2. Schematic representation of an amperometric biosensor.

biosensor is shown in Fig. 2, which is being used for monitoring the detection of phenols. Amperometric microbial biosensors detect the current produced during the oxidation or reduction of a compound in the presence of an enzyme. These amperometric sensors can also be used for the detection of heavy metal ions present in atmospheric samples. A microbial recombinant biosensor of amperometric type has been constructed for the detection of Cu^{2+} by fusion of a Cu^{2+}-inducible promoter with the *lacZ* gene [26].

Different microbial biosensors developed for the determination of various types of pollutant in environmental

Table 1. Important biosensors developed for the detection of certain toxic compounds.

Analytes	Biosensing elements	Transducers	Samples	References
Heavy metals				
Zinc, copper, cadmium, and nickel	*Pseudomonas fluorescens* 10586s pUCD607 with the *lux* insertion on a plasmid	Optical (luminometer)	Soil	Mcgrath *et al*. [32]
Cadmium	DNA	Electrochemical	Standard solutions	Wong *et al.* [62]
Mercury, cadmium, and arsenic	Urease enzyme	Electrochemical	Standard solutions	Pal *et al.* [38]
Zinc, copper, cadmium, nickel, lead, iron, and aluminum	*Chlorella vulgaris* strain CCAP 211/12	Electrochemical	Urban waters	Claude *et al.* [13]
Zinc, cobalt, and copper	*Pseudomonas* sp. B4251, *Bacillus cereus* B4368 and *E. coli* 1257	Electrochemical	Water	Gruzina *et al*. [17]
Mercury(II) and lead(II) ions	DNA	Optical	Water	Knecht *et al*. [23]
Cadmium, copper, and lead	Sol-gel-immobilized urease	Electrochemical	Synthetic effluents	Ilangovan *et al.* [19]
Organophosphates, urea, and ethanol	*Flavobacteium* sp., *Bacillus* sp., and *S. ellipsoideus*	Potentiometric	Effluents and laboratory samples	Gaberlein *et al*. [15] Verma *et al*. [56] Rotariu *et al.* [46]
Phenolic compounds				
Binary mixtures: phenol/chloro-phenol, catechol/phenol, cresol/chloro-cresol and phenol/cresol	Laccase and tyrosinase	Amperometric	Waste water	Yildiz *et al*. [64] Karim and Fakhruddin [24]
m-Cresol or catechol	DNA	Amperometric	Waste water	Claude *et al*. [13]
Phenol	Mushroom tissue (tyrosinase)	Amperometric	Waste water	Silva *et al.* [51]
Phenol, *p*-cresol, *m*-Cresol and catechol	Polyphenol oxidase	Amperometric	Waste water	Karim and Fakhruddin [24]
Pesticides				
Simazina	Peroxidase (Biocatalytic)	Potentiometric	Soil and waste water	Rodriguez Monaz *et al.* [43] Salgado *et al*. [47]
Parathion	Parathion hydrolase (Biocatalytic)	Amperometric	Soil	Mostafa [33]
Paraoxon	Alkaline phosphatase	Optical	Soil and waste water	Mostafa [33]
Carbaril	Acetyl cholinesterase	Amperometric	Soil and waste water	Mostafa [33] Sassolas *et al*. [52]
Herbicides				
2,4-Dichloro-phenoxy acetic acid	Acetyl cholinesterase	Amperometric	Soil	Sassolas *et al*. [52]
Diuron, paraquat	Cyanobacterial	Bioluminescence	Soil	Sassolas *et al*. [52] Akhtar and Marty [7]

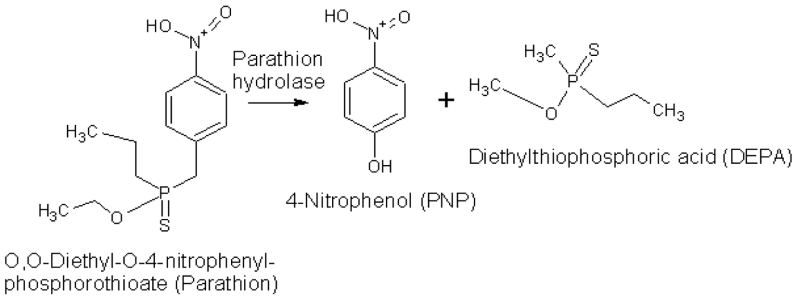

Fig. 3. Biodegradation of parathion by parathion hydrolase.

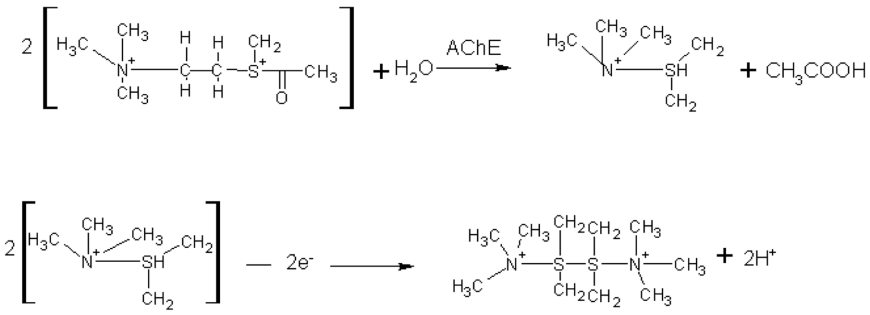

Fig. 4. Mechanism of action of acetylcholine esterase.

samples are summarized in Table 1 [7, 13, 17, 19, 23, 24, 32, 33, 38, 46, 51, 52, 62, 64].

The degradation of the pesticide parathion by parathion hydrolase is given in Fig. 3, where the microbial enzyme was used to detect the presence of pesticide by an amperometric sensor. The same method was also employed for the detection of acetylcholine by biocatalytic degradation in presence of acetylcholine esterase (Fig. 4).

The foodborne diseases due to various pathogens are one of the major problems in food safety. Commonly used methods for the detection of foodborne pathogens rely on conventional culture-based tests, antibody-based assays, and polymerase chain reaction (PCR)-based techniques. These methods are costly, laborious, and time-consuming. A simple and responsive aptamer-based biosensor for the monitoring of *Escherichia coli* O157:H7 was manufactured using two different aptamers specific for the outer membrane of *E. coli* O157:H7. One of the aptamers was used for magnetic bead enrichment, and the other was used as a signal reporter for this pathogen, which was amplified by isothermal strand displacement amplification and further detected by a lateral flow biosensor. The limit of detection of pathogen is as low as ten colony forming units per milliliter. This method could also be applied for the detection of other bacteria by using different bacterium-specific aptamers [61].

For preservation of food and to maintain the fertility of soils, nitrate compounds are being employed, but consumption of these ions in any form leads to severe human health complications, as it reacts with hemoglobin irreversibly, inhibiting oxygen transport (methemoglobinemia, blue baby syndrome in infants, carcinogenicity, and mutagenicity, *etc.*). The high concentration of nitrates in ground and surface waters also harm aquatic environments. A biosensor of amperometric nature was developed to determine nitrite by immobilizing cytochrome *c* nitrate reductase of *Desulfovibrio desulfuricans* and double-layered hydroxide containing anthraquinone-2-sulfonate [10]. The response of the developed sensor was very fast and measured the nitrite concentration in the range of 0.015 to 2.35 µmol with a detection limit of 4 nmol. Fig. 5 shows the degradation of nitrate to nitrile, where change in absorbance of NADPH/NADH is monitored. The conductimetric enzymatic biosensor was also used for measurement of the presence of nitrate in water, where electrodes were modified

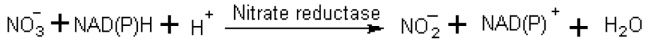

Fig. 5. Mechanism of action of nitrate reductase.

by use of immobilized nitrate reductase from *Aspergillus niger* in the presence of a methyl viologen mediator, bovine serum albumin, and the cation-exchange polymer Nafion [57].

A number of potentiometric biosensors have been described where the pH electrode is combined with recombinant *E. coli*, which expresses intracellular organophosphorus hydrolase at the surface of cells, and a wild-type organophosphorus metabolizing bacterium *Flavobacterium* sp. [14, 28]. A disposable microbial sensor was developed for the detection of urea in milk by combining an ammonium ion selective electrode and urease-enzyme-producing bacteria [55]. Similarly, a potentiometric oxygen electrode was efficiently employed for the measurement of ethanol using immobilized cells of *S. ellipsoideus* [45].

The potentiometric microbial biosensor is constructed using an ion selective electrode enveloped with the immobilized microbial cells on it. The consumption of analyte present in the samples by microbial cells leads to a change in potential, which is measured by the transducers using working and reference electrodes (Fig. 6) [30].

Optical biosensors are also used for the detection of certain pollutants in environmental samples, where the reaction of enzyme with the specific analyte is monitored (Fig. 7) [39]. These biosensors are compact and flexible but measured generated electrical noise. Bioluminescence utilizes the release of illumination by certain microorganisms for real-time process monitoring. The gene responsible for emission of light (*lux* gene) of the bacterial system was engaged as a reporter gene for analysis of samples. Several optical biosensors are created for measuring Hg^{2+} by combining the regulatory portion of the *mer* operon gene (*mer*R) and *lux*CDABE of bacterial systems. The binding of Hg^{2+} to *mer*R activates the *mer* promoter, leading to the synthesis of the *lux* reporter gene followed by light production [9, 40, 49]. The presence of copper in soil is also measured by optical sensors using recombinant *Pseudomonas fluorescens* [34].

A bacterial biosensor (immobilized *Pseudomonas putida* ML2) has been mentioned for analyzing benzene present in air on the basis of flow injection analysis. The biosensor can detect benzene in an air sample in the range of 0.025 to 0.15 μmol with little or no significant interference of other benzene-related compounds (toluene, ethyl benzene and xylene). A recombinant *E. coli* strain coding for dioxygenase and benzene dihydrodiol hydrogenase has been reported for monitoring the level of benzene electrochemically or colorimetrically in the presence of NADH [21].

A biochip-based algal biosensor for the detection of two or more volatile toxic compounds has been described, where immobilized algal cells of genus *Klebsormidium* and *Chlorella* are used. Vapors of formaldehyde (0.05–1 ppm) and methanol (200–1,000 ppm) can be significantly identified by this biosensor [35].

Nanotechnology is an upcoming technology that is being studied for diagnosing various pollutants, and the prevention as well as remediation of toxicants by regulating the shape and size of synthesizing substances to nanoscale. These nanoparticles absorb contaminants or catalyze reactions rapidly because of the increased surface area and high surface energy, reducing energy consumption during the course of analysis and thus preventing the release of contaminants. Because of the nano-size, these particles can reach inaccessible areas and *in situ* bioremediation can be performed. These can be coated with various ligands and their size can be controlled experimentally for designing biosensors for wide applicability [27].

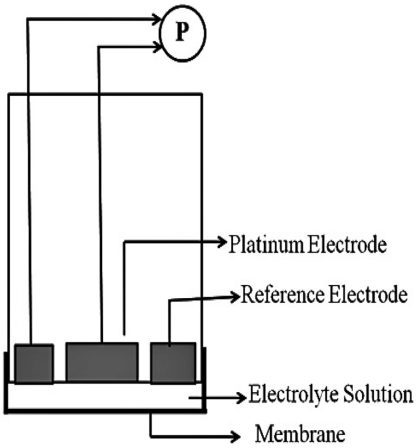

Fig. 6. Schematic representation of a potentiometric sensor.

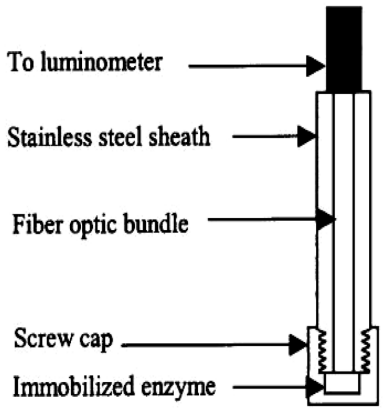

Fig. 7. Construction of an optical biosensor.

Nanoparticles exhibit distinctive properties compared with bulk-sized materials and find extensive applications in various areas, including biomedical, electronic, environmental, pharmaceutical, cosmetic, and energy sectors [6, 11]. The monitoring of the environment and diagnosis of pathogens have been improved by use of nanoparticles in both biotic and abiotic contaminants. The miniature size of these particles corresponds to high surface-to-volume ratios. The surface-modified nanocolloids like gold nanoparticles, magnetic nanoparticles, quantum dots, and carbon nanotubes exhibit precise binding capabilities for analytes. Therefore, the distinct miniature size and nanoscale properties of nanoparticles are considered as the latest development for monitoring toxins, heavy metals and other environmental contaminants [36]. Tian *et al.* [53] discussed the preparation of non-enzymatic glucose sensors from micro or nano materials like noble metals, metal oxides, carbon nanotubes, graphene, polymers, and composites for the detection of Diabetes mellitus. In another study carried out for the detection of glucose using nanostructured tin oxide thin films with immobilized glucose oxidase, it was found that the developed sensor can detect the glucose in a concentration range from 10 to 360 mg/dl [4]. In a recent study, Xu *et al.* [63] successfully developed a biosensor based on glucose oxidase nanoporous gold co-catalysis for the detection of glucose. The amperometric method was employed for the detection of glucose using a GOx/NPG/GCE bioelectrode.

Conclusion

It is understood that the biosensor technologies have been proven to be a rapidly developing research area over the past decades. The market trends showed about 10.4% growth in the development of biosensors for various applications, like in biopharma, food and beverages, biodefense, and environmental analysis. Biosensor devices are ideal tools for environmental monitoring because they are sensitive, selective, easy, and rapid. However, in comparison with biomedical applications, biosensor technologies for environmental applications are still in their infancy and facing many challenges due to intrinsic characteristics of environmental analysis. Low detection limit and specificity for a specific analyte are key issues for the successful development of biosensors in environmental analysis. It has also been noticed that detection in a complex environmental matrix produces interferences, limiting the detection of the target analyte. Various nanomaterials are being evaluated in the development of biosensors for environmental applications. The tailor-made nanocomposites with versatile nanostructures (particles, rods, wires, and tubes) make the modified biosensors more sensitive and flexible for the analysis of analytes in complex environmental samples. A paper-based device is also a promising biosensing platform that is portable, miniaturized, low-cost, and user-friendly, meeting the needs of on-site detection of environmental samples. The integration of nanomaterials within these biosensors brings new strategies for enhancing their analytical performances. Although several biosensors have been developed for a wide range of environmental contaminants at the laboratory scale, only a few biosensors are commercially available at present. It is apparent that more efforts are needed towards bridging innovations that will play a decisive role in the development of automated, continuous, and real-time biosensors with high throughput analysis of environmental samples. Needless to say that such effort for adopting crucial detection technologies for environmental pollutants is the need of the hour.

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