Sustainable Enzyme Technology for Environment: Biosensors for Monitoring of Pollutants and Toxic Compounds

4

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Abstract

The recent developments in enzyme technology have raised the interest of researchers toward developing cost-effective enzyme-based techniques against various pollutants in our environment. Enzyme-based biosensors have diversity of industrial relevance in environmental monitoring. A variety of laboratory sample biosensors have been described recently which moderately measure variety of environmental pollutants. It is envisaged that many reports on biosensor development are directed toward their medical use, so the need of biosensor development for environmental applications and monitoring is growing. The present appraisal highlights recent major research findings on enzyme-based biosensors for determination of environmental pollutants and toxic chemicals.

Keywords

Enzyme technology • Biosensor • Pollutants • Environmental applications • Toxic compounds

Introduction

A biosensor is defined as an analytical device comprising of a biological catalyst or receptor in intimate contact with a suitable transducer such as an electrode and optical fiber (Brooks et al. 1991). The biological component is usually immobilized at or

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near to the surface of the transducer, which converts the particular biochemical event into a quantifiable and readily processable signal (Sethi 1994). According to IUPAC, it is a compact analytical device incorporating a biological or biologically derived sensing element either integrated within or intimately associated with a physicochemical transducer. The aim of a biosensor is to produce either discrete or continuous digital electronic signals, which are proportional to a single analyte.

Biosensor devices may have the capability to provide an analytically powerful and inexpensive alternative to conventional technologies by identifying the target analyte in the presence of interfering species. Reagent-less continuous real-time analysis is also one of the potent advantages offered by these devices. Microbial sensors are devices where microorganisms are used as a sensing element that can specifically recognize the species of interest either intimately connected or integrated within a suitable transducing system (Karube and Nakaniki 1994). Two major types of microbial sensors are being applied, one which involves the respiratory activity, while other uses the metabolites released by the microbes (Reidel et al. 1989). The best example of respiratory activity measurement is with DO probe and metabolite measurement with CO_2 electrode, fuel cell, NH_4 electrode, pH electrode, etc. The advantages of microbial sensors include less sensitivity to inhibition, tolerance to suboptimal pH and temperature of enzyme electrode, longer shelf life, and cheaper in manufacturing, while the disadvantages include specificity due to multienzyme system in cells and longer response time due to mass transfer resistance. Moreover, a noteworthy update about development of a new antibody-based small and sturdy "biosensor" developed to detect marine pollutants like oil much faster and more cheaply than current technologies is also quite impressive toward finding solutions to environmental problems (Spier et al. 2011)

Phenolic compounds are major pollutants in ground and surface water as they are widely used in many industrial processes such as plastic manufacture, resins, wood industry, construction industry, abrasives, plasticizers, cleaning products, pesticide manufacturing, and detergent industries (Lin et al. 2008; Gardziella et al. 2010; Apetrei et al. 2011). Taking into consideration their high toxicity and persistence in the environment, the determination of phenolic compounds becomes an important subject.

Since biosensor technology development demands rapid, inexpensive, and continuous monitoring capabilities, research endeavors to harmonize these issues are important. Biosensors currently developed or in the process of development are for detection of environmental pollutants, viz., phenols, genotoxins, and pesticides such as organophosphates and 2, 4-D.

This chapter highlights the advances in the rapidly developing area of microbial biosensors with particular emphasis to the developments since 2000 as a number of reports on biosensor development have been published during the last decade.

Classification of Biosensor

A biosensor is an analytical device for the detection of an analyte that combines a biological component with a physicochemical detector component with three parts:

(a) Sensitive biological material (e.g., tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids)
(b) Biomimic or biomaterial
(c) Transducer as the detector that can be more easily measured and quantified

There are two main categories of biosensors described in literature (Amine et al. 2006).

Immobilization-Based Biosensors

In this method, the whole cells are used as the biochemical component (Rekha et al. 2000; Durrieu et al. 2004; Chouteau et al. 2005). This category of biosensor can increase the sensor steadiness and provide enzyme regeneration, where the sensing device is attached with matrix of immobilized enzyme (Lee et al. 2002). The lasting activity of the enzyme is estimated by quantifying the product. Nevertheless, these may have problems due to parallel reactions of several enzymes.

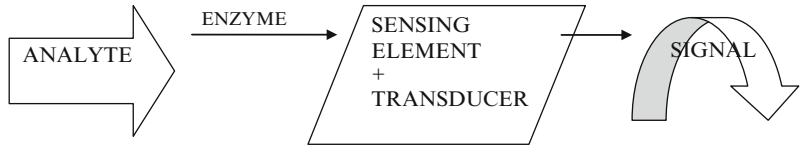

Fig. 4.1 A biosensor setup

Transducer-Based Biosensors

They are based on direct enzyme immobilization on a transducer device. The enzyme and transducer elements are in close contact with each other and incorporated in a single unit. Some biosensors based on enzyme inhibition have been reported in the literature (Tran-Minh 1985; Evtugyn et al. 1999; Luque de Castro and Herrera 2003).

In such biosensors, the enzyme (element) reacts with substrate, and biosensor response is assessed by the product concentration (P) of enzymatic reaction on sensor surface (Guilbault et al. 2004). The reaction is controlled by the rate of two synchronized processes, viz., enzymatic conversion of substrate (S) and diffusion of product. A simple setup is represented in Fig. 4.1 explaining the working principle of a biosensor.

Table 4.1 General requirements for environmental biosensors

Requirement	Specification range
Cost	$1–15 per analysis
Equipment portability	Can be carried by one person; no external power
Assay time	1–60 min
Personnel training	1–2-h training period is sufficient
Format	Reversible, continuous, in situ
Matrix	Minimal preparation for groundwater, soil extract, blood, and urine
Sensitivity	Parts per million (ppm) to parts per billion (ppb) concentration
Dynamic range	At least two orders of magnitude
Specificity	*Enzymes/receptors/nucleic acids:* specific to one or more groups of related compounds
	Antibodies: specific to one compound or closely related group of compounds

Adapted from Rogers and Gerlach (1996)

Parameters for Development of Biosensor

The stability and reproducibility of the biosensor are the most important parameters that depend on the response rate limiting step, substrate concentration, pH, strength of buffer, temperature, organic solvents, addition of additives and dry or wet storage, etc. Although some biosensors have been reported usable under laboratory conditions for periods of more than 1 year, their practical lifetime when incorporated into industrial processes or to biological tissue, such as glucose biosensors implanted in vivo, is either unknown or limited to days or weeks. It is necessary to emphasize that some precise conditions are required to be met for each environmental monitoring field (Table 4.1).

While it is relatively easy to determine the stability of biosensors at the laboratory scale, both during storage and operation in the presence of analyte, procedures for assessing their behavior during several days of introduction into industrial reactors are much more complex to handle. In both cases, it is advisable to specify the storage (shelf) or operational (use) lifetime and the storage and operating conditions in terms of buffer composition, presence of additives and substrate concentration (K_m), initial sensitivity, upper limit of concentration range for calibration, accuracy, and repeatability. The lifetime (LT) of a biosensor is defined as a comparative sensitiveness of different biosensors, developed from the same production batch of homogeneous patterns, after different storage conditions. Alternatively, biosensor stability is also measured as drift rate and useful for biosensors in which sensitivity evolution is either very slow or studied for short periods of time. The various advantages and disadvantageous of enzyme-based biosensor are summarized in Table 4.2.

Table 4.2 Advantages and disadvantages of enzyme-based biosensors

Advantages	Disadvantages
More specific than cell-based sensors	More expensive to produce due to the additional steps involved in isolating the enzyme
Faster response due to shorter diffusion paths (no cell walls)	Enzymes are often unstable when isolated
	Many enzymes need cofactors for their activity and detection of substances

Recent Trends in Biosensor Technology

Various novel developments in biosensor technology and their applications in the field of biotechnology are summarized in this section (Table 4.3). Abdelwahab et al. (2010) have described a nitric oxide nano-composite biosensor immobilizing microperoxidase (MP) toward determination of NO released from rat liver, stomach (AGS), and intestinal (HT-29) cancer cells. A notable work by Apetrei et al. (2011) was based on detection of phenolic compounds using amperometric tyrosinase-based biosensor. Further, for detection of organophosphate pesticides, Crew et al. (2011) developed an amperometric biosensor based on six acetylcholineste-rase enzymes through neural network program. In addition, there are prominent reports on development of tyrosinase-based biosensor for determination of o-diphenols (Daniela et al. 2010), air toxicity monitoring where bioluminescent bacteria were used as bioreporter through bioluminescence assay (Evgeni et al. 2011), algae-based biosensor for determination of environmental impurities of water (Dieter et al. 1998), and DNA biosensor for detection of mercury ions (Long et al. 2011). Recently application of nano-biotechnological tools has been reported for the evaluation of pollutants, e.g., development of a glucose biosensor using covalent cross-linking technique through carbon nanotubes hybrids for glucose estimation (Fu et al. 2011), and a nucleic acid biosensor (NAB) based on horseradish peroxidase (HRP) enzyme for diagnosis of genetic diseases and detection of infectious agents (He et al. 2011). Interestingly, Masojidek et al. (2011) have reported a novel method for measurement of herbicide toxicity and have successfully used this technique for detection of photosynthetic herbicides, e.g., diuron, atrazine, and isoproturon. In this series, other notable biosensors reported are the following: microbial biosensor for detection of methyl parathion (Kumar and D'Souza 2010), determination of hydrogen peroxide (Zhang et al. 2009; Li et al. 2009), amperometric polyphenol biosensor based on covalent immobilization of laccase onto copper nanoparticles for measurement of total polyphenolic content in plant extracts (Chawla et al. 2011), antibody-based KinExA Inline biosensor for estimation of dissolved polycyclic aromatic hydrocarbons (PAHs) (Spier et al. 2011), electrochemical detection of α-ketoglutarate (Poorahong et al. 2011), and amperometric fructose biosensor based upon the D-fructose dehydrogenase (FDH) toward determination of fructose in the real samples of fruit juice, soft drinks, and honey (Trivedi et al. 2009).

Moreover, few diversified remarkable studies like enzyme-amplified electrochemical biosensor through detection of PML–RARα fusion gene for diagnosis of promyelocytic leukemia (Lin et al. 2011), electrochemical DNA biosensor for screening of chlorinated benzene pollutants (Wu et al. 2011), enzyme biosensor based on chemiluminescence system, and using flowerlike ZnO crystals and nano-sized gold particles (Zhang et al. 2009; Yu et al. 2010) are prominent examples of recent knowledge revelation in biosensor development, making significant contribution for emergent biosensor industry for monitoring of pollutants and toxic compounds. Nevertheless, for efficient developments in sustainable enzyme technology, much of the efforts are needed for designing low-cost, ultrasensitive, selective, and quick-response biosensors. Considering above facts and with current advances in enzyme-based biosensor technology, we understand that such biosensors will have strong and momentous role in future development in biotechnology.

Table 4.3 A snapshot review of latest developments (2009–2011)

Type of biosensor technology	Working mechanism	Use	Reference
Nitric oxide nano-composite biosensor	Immobilizing microperoxidase (MP) onto the MWCNT-poly-carboxylic acid (PTTCA) nano-composite	Determination of NO released from rat liver, stomach (AGS), and intestinal (HT-29) cancer cells	Abdelwahab et al. (2010)
Amperometric tyrosinase	Electropolymerized phosphate-doped polypyrrole film as an immobilization support	Detection of phenolic compounds	Apetrei et al. (2011)
Amperometric biosensor	Array incorporated based on six acetylcholinesterase enzymes through neural network program	Determination of organophosphate pesticide	Crew et al. (2011)
Tyrosinase-based biosensor	Optical transparent support immobilized by "layer-by-layer" assembly, alternating the enzyme with the polycation polymer poly (dimethyldial-lylammonium chloride)	Determination of phenolics, e.g., o-diphenols	Daniela et al. (2010)
Algae-based biosensor	Optical immobilized living algae chlorophyll fluorescence depending on the load of water probes	Determination of environmental impurities of water	Dieter et al. (1998)
Fiber optics-based biosensor	Bioluminescent bacteria were used as bioreporter through bioluminescence assay	Air toxicity monitoring	Evgeni et al. (2011)
DNA biosensor	Evanescent wave all-fiber bisensing platform	Detection of mercury ions	Long et al. (2011)
Glucose biosensor	CS-incorporated sol–gel process, covalent cross-linking technique through carbon nanotubes hybrids	Glucose estimation	Fu et al. (2011)
Nucleic acid biosensor (NAB)	Based on horseradish peroxidase (HRP)–gold nanoparticle (Au-NP) dual labels and lateral flow strip biosensor (LFSB)	Detection of nucleic acid samples, diagnosis of genetic diseases, and detection of infectious agents	He et al. (2011)
Electrochemical PS II biosensor	Phytotoxicity index (PI) as a measure of herbicide toxicity	Detection of photosynthetic herbicides diuron, atrazine, and isoproturon	Masojidek et al. (2011)
Optical microbial biosensor	Using *Sphingomonas* sp. immobilized on microplate	Detection of methyl parathion	Kumar and D'Souza (2010)
Enzyme-amplified electrochemical biosensor	Through detection of PML–RARα fusion gene	Diagnosis of promyelocytic leukemia	Lin et al. (2011)
Amperometric enzyme biosensor	Based on in situ electrosynthesized gold/polyaniline core–shell nano-composites on conducting ITO electrode	Determination of hydrogen peroxide	Li et al. (2009)
Amperometric polyphenol biosensor	Based on covalent immobilization of laccase onto copper nanoparticles	Measurement of total polyphenolic content in plant extracts	Chawla et al. (2011)
Antibody-based biosensor	KinExA Inline sensor employed the monoclonal anti-PAH antibody	Assessments of dissolved polycyclic aromatic hydrocarbons (PAHs)	Spier et al. (2011)
α-ketoglutarate biosensor	Co-deposition of Ru and Rh nanoparticles over CFE	Real-time electrochemical detection of α-ketoglutarate	Poorahong et al. (2011)

(continued)

Table 4.3 (continued)

Type of biosensor technology	Working mechanism	Use	Reference
Amperometric fructose biosensor	Based upon the enzyme d-fructose dehydrogenase (FDH)	Determination of fructose in the real samples of fruit juice, soft drinks, and honey	Trivedi et al. (2009)
Electrochemical DNA biosensor	Based on double-stranded DNA modified Au electrode (dsDNA/Au)	Screening of chlorinated benzene pollutants	Wu et al. (2011)
Enzyme biosensor	Based on chemiluminescence system and hollow silica microspheres (HSM) receptor	Determination of glucose in human serum	Yu et al. (2010)
Enzyme-based biosensor	Using flowerlike ZnO crystals and nano-sized gold particles	Determination of hydrogen peroxide	Zhang et al. (2009)

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