Advances in Biological Science Research

A Practical Approach

Edited by
Surya Nandan Meena and Milind Mohan Naik

Advances in Biological Science Research

A Practical Approach

Edited by

Surya Nandan Meena
Biological Oceanography Division, National Institute of Oceanography, Dona Paula, Goa, India

Milind Mohan Naik
Department of Microbiology, Goa University, Goa, India

ACADEMIC PRESS
An imprint of Elsevier

Contents

27. Techniques for the mass production of Arbuscular Mycorrhizal fungal species

James Dsouza

28. Metagenomics: a gateway to drug discovery

Flory Pereira

29. Application of 3D cell culture techniques in cosmeceutical research

Surya Nandan Meena and Chellandi Mohandass

Chapter 8

Recent advances in bioanalytical techniques using enzymatic assay

Kanchanmala Deshpande[1], Geetesh K. Mishra[2]
[1]*Department of Chemistry, Goa University, Taleigao Plateau, Goa, India;* [2]*Multiscale Fluid Mechanics Lab, School of Mechanical Engineering, Sungkyunkwan University, Suwon, South Korea*

8.1 Introduction

Rapid analysis of a large number of samples with good accuracy, precision, and sensitivity has always remained a great challenge. Apart from the above-mentioned analytical parameters, interference due to matrix and subsequent low recoveries increases the difficulty level of analysis. Routinely used techniques that have gained acceptance have shown promise in their use. The standard analytical techniques that are used for the detection of various analytes form environmental or food samples are based on chromatographic techniques such as gas chromatography (GC) and liquid chromatography (LC), coupled with mass spectrometry (MS), such as GC-MS/MS and LC-MS/MS [1]. These methods are highly precise and have the potential of being automated. However, they still suffer from drawbacks such as tedious sample preparation, requirement of large amount of organic solvent, high level of sophistication required, and difficulty for screening a large number of samples within a short time. Several bioanalytical techniques have been developed over the years to minimize the problems stated herein. These techniques can quantify analytes of interest even at trace concentrations with good reliability [2]. Reported bioanalytical techniques can analyze a majority of analytes found in a wide range of matrices [2] but are tremendously challenging sometimes due to the variety of substances in biological samples, the complex molecular structures, and time-dependent concentrations. Most of the bioanalytical protocols require isolation of components from natural environments and separations of complex mixtures. Biosensors, as a discrete bioanalytical tool, are able to measure analytes selectively, often in a natural matrix, without prior separation of multicomponent samples and often producing quantitative data within minutes [3,4].

Advances in Biological Science Research. https://doi.org/10.1016/B978-0-12-817497-5.00008-2

In the past few years, the effects of human activities on environmental quality have been both extensive and diverse in the extent to which they disturb the ecosystem. Work in recent years has observed an emergent need to examine environmental pollutants/food contaminants and to keep a constant watch on them. These contaminants may have mild to harsh effects, which may be visible immediately or only after a long time. Some of the contaminants have lethal effects and could lead to a public health crisis [5]. Therefore, screening of toxic pollutants on a routine basis is gaining more consideration these days. Other than environmental pollutants and food contaminants, emerging areas of concern include reported high amounts of food allergens and pharmaceutical drugs such as antibiotics [6,7]. The presence of various food allergens and antibiotics in food elements is a challenge for food safety management. Thus their monitoring in food is very important for human consumption; even the minute presence of these residues can trigger an allergic reaction in hypersensitive individuals [8]. To address these important issues concerning environmental and food safety, several biosensors have been developed to help in monitoring environment and human health. Herein, we address the significance, basics, and application of biosensor systems for food and environmental monitoring. A practical solution for optimum performance of biosensors is also discussed here.

8.1.1 Why biosensors?

Defining human exposure to various toxic chemicals is an enormous task. Hence, development of novel analytical tools that not only quantify the concentration of toxic chemicals at ultra-trace level but also estimate the biological damage resulting due to their exposure is the need of the hour [9]. These toxic chemicals are reported to be carcinogenic in nature and hinder important enzymatic functions [10]. Considering these points, development of novel tools for predicting concentration and its impact at ultra-trace level is of utmost importance. Over conventional analytical techniques, biosensors excel in various aspects. The significant aspects are high-throughput ability, versatility, capability for speciation studies, minimal use of organic solvents, and suitability for point-of-care testing or field monitoring [11]. These significant advantages have led to the development and commercial success of biosensors [12]. In view of this, over the past few decades, numerous biosensors have been developed for the detection of ions, small molecules of organic or inorganic nature, and various analytes of biological origin such as proteins, deoxyribonucleic acids, and so on [13].

8.1.2 Emergence of biosensors

Biosensors represent new analytical devices that appear to be an analyst's dream. Technically, a biosensor is a probe that integrates a biological

component with an electronic transducer, thereby converting a biochemical signal into a quantifiable electrical response [14]. Biosensors are characterized by a high level of specificity generated by the biocomponent, which specifically reacts with a given analyte or substrate. The combination of this specificity, with a sensitive transducer, gives to biosensors their unique characteristics for the detection of a variety of analytes, even when they occur in complex matrices [2]. It is noteworthy that bioassays having high specificity and capability for analysis of single or multianalyte are often referred as biosensors although most of these systems do not have integrated transducer systems. A schematic representing various components of a biosensor is shown in Fig. 8.1.

A plethora of literature is available that addresses the development and application of biosensors for medical diagnostics [15], food quality assurance [16], environmental monitoring [17,18], industrial process control, and to biological warfare agent detection [19]. In recent years advances in biosensor development has been observed in the area of its miniaturization, its in situ measurement capability, which ultimately leads to its commercialization [15]. By 2022, the global biosensor market will be valued at approximately USD 27.06 billion with home-use health-monitoring devices being dominant.

8.2 Classification of biosensors

Biosensors classification can be performed in different ways. The type of biological signaling mechanism utilized for biosensing can be one of the approaches, whereas the type of signal transduction system employed can also be the basis of its classification. Biological signaling achieved by biosensors

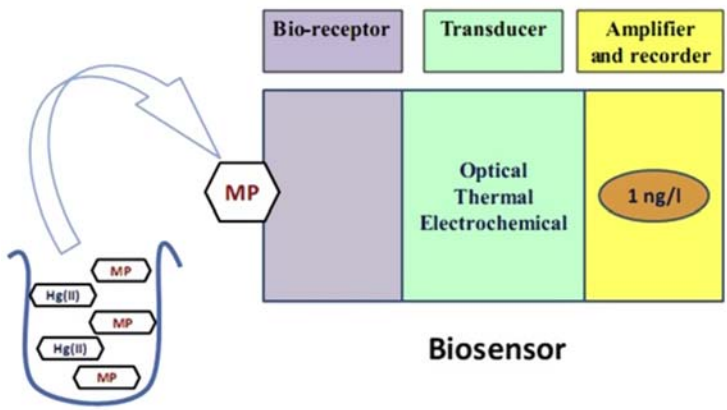

FIGURE 8.1 Schematic representing biosensor components. [Figure represent interaction of MP, i.e., methyl parathion (pollutant) with bioreceptor and subsequent steps in sensing].

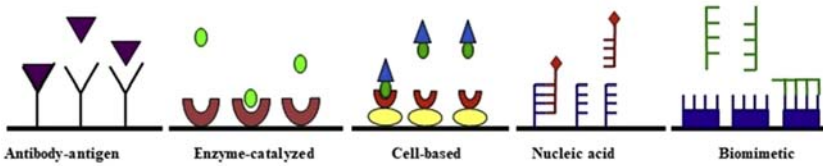

FIGURE 8.2 Type of biocomponent commonly used in biosensors (here the biocomponent is shown in immobilized form for simplicity).

can be due to various biocomponents. Some of the significant biocomponents exploited for biosensor application include antibody/antigen, enzymes, whole cells, nucleic acids, and biomimetic materials (aptamers). Various biocomponents explored in biosensors are compiled in Fig. 8.2.

When biosensors are classified according to their method of signal transduction, i.e., by measuring the change that occurs in the bioreceptor reaction, they are named as optical, thermal, electrochemical, and piezoelectric. Optical transducers respond to an analyte by undergoing a change in their optical properties such as absorption, fluorescence, luminescence, reflectance, emission, or a change in an interferometric pattern. Optical transducers represent the largest and fastest growing area in biosensor technology due to their high amplification capacity [20–24]. Some bioreceptor reactions cause changes in heat, i.e., molar enthalpy. Thermal biosensors measure this change in heat [25–28]. A third class includes electrochemical reactions, which are the most commonly used detectors. These devices measure the current produced from oxidation and reduction reactions. Concentration of the electroactive species present can be predicted form this current [29]. A fourth class includes measurement of changes in mass caused by chemical binding to small piezoelectric crystals. These are popularly known as mass sensitive biosensors.

8.2.1 Enzyme biosensor

Biosensors that utilize enzymes as the recognition elements represent the most extensively studied area. The high specificity of enzyme-substrate interactions, and the usually high turnover rates of biocatalysts, open the way to sensitive and specific enzyme-based biosensor devices development [30,31]. Each enzyme has an active site or sites containing functional groups. The interaction between the active site of enzyme and analyte is highly specific. The products of this interaction can be detected at much lower limits than with other normal interactions. Enzyme-based assay is an extremely broad field that impacts on many major industrial sectors such as the pharmaceutical, health care, food, and agricultural industries, as well as environmental monitoring [30].

Two different approaches can be identified for determining an analyte by use of an enzymatic biosensor: (1) if the enzyme metabolizes the analyte, the analyte can be determined by measuring the enzymatic product, and (2) if the analyte inhibits the enzyme, the decrease of enzymatic product can be measured and correlated to the analyte concentration. In the latter case, the device is designated as a ''biosensor based on enzyme inhibition'' or ''inhibition biosensor.'' Among the reported enzyme-based biosensors, the inhibition based one comprises the major portion.

8.2.1.1 Enzyme inhibition biosensor

A large percentage of environmental pollutants and food toxicants are known to act as enzyme inhibitors, resulting in the development detection methods based on this property. The binding of an inhibitor can stop a substrate from entering the enzyme's active site and/or hinder enzyme catalysis. Inhibitor binding could be either reversible or irreversible. Irreversible inhibitors usually react with the enzyme and change it chemically. These inhibitors modify key amino acid residues required for enzymatic activity; in contrast, reversible inhibitors bind noncovalently and different types of inhibition result depending on whether these inhibitors bind the enzyme, the enzyme-substrate complex, or both [32].

It is well known that the response of a biosensor to the addition of a substrate is determined by the concentration of the product (P) of the enzymatic reaction on the surface of the sensor. The reaction is controlled by the rate of two simultaneous processes, i.e., the enzymatic conversion of the substrate (S) and the diffusion of the product from the enzyme layer. For this conversion, factors such as pH, ionic strength, temperature, enzyme concentration, and substrate concentration play significant roles. Inhibitor (I) selectively inhibits the activity of certain enzymes, thus affecting enzyme activity and the product concentration. Schematic representation of enzyme inhibition is depicted in Fig. 8.3. In the biosensors based on enzyme inhibition, the quantification of the inhibitor concentration is carried out by measuring the enzyme activity before and after exposure of the biocomponent at the target analyte [30,33]. The percentage of inhibition is calculated as follows,

$$I\% = (\mathrm{A0} - \mathrm{AI})/\mathrm{A0} \times 100$$

Where I% = percentage of inhibition; A0 = the enzyme activity before exposure to the analyte; AI = the enzyme activity after exposure to the analyte. Here, the percentage of inhibited enzyme (I%) that results after exposure to the inhibitor is quantitatively related to the inhibitor (i.e., analyte) concentration provided incubation time is constant.

The choice of enzyme/analyte system is based on the specific reaction between functional moieties present on the active site of enzyme and active

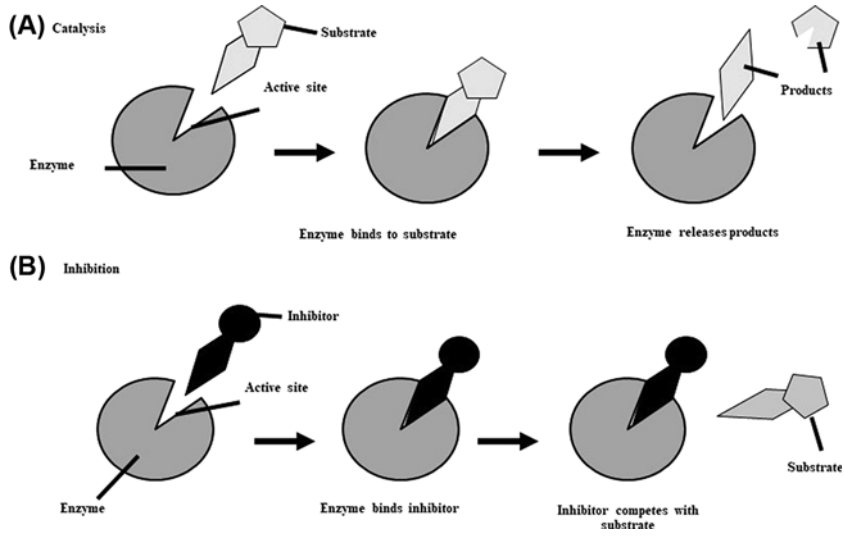

FIGURE 8.3 Schematic representing principles behind enzyme inhibition biosensor.

functional group of analyte. Most of the toxic compounds block the active site, thereby inhibiting normal enzyme function. The enzyme inhibitor reaction is often complex and has been reviewed in the current literature [34–36]. For quantification, a calibration plot is prepared using a range of known concentrations of standard analyte solution in contact with enzyme solution. The difference in the enzyme activity before and after contact is calculated. The steady-state response corrected for a background signal versus the difference in the enzyme activity is plotted against analyte concentration or its logarithm. The analytical figures of merit for assay such as dynamic range, linear range, limit of detection (LOD) and limit of quantification (LOQ) were calculated from the plot. Such calibration plots are used for quantification of unknown concentration [30]. Enzyme inhibition–based biosensors are fascinating to researchers where various enzymes such as glucose oxidase, urease, tyrosinase, cholinesterase, alcohol oxidase, and peroxidase have been utilized as biocomponents [36–38].

8.2.2 Overcoming limitations in enzyme-based biosensors

Although enzyme-catalyzed or enzyme-inhibition-based biosensors show considerable promise, there are a great number of challenges and limitations in exploiting these systems for potential applications. The majority of enzymes are fairly unstable at room temperature in their free form, due to which their application in commercial sensors or kits is often hampered. The impact is visible where lack of sensor stability under storage and operational conditions,

difficulty in reuse of enzyme and recovery of product are observed [34]. Also, a protein's sequence and interactions between residues in the protein core are naturally not fully optimized and only achieve the minimum requirements for proper functioning. This situation leaves plenty of room for improvement.

In order to make enzyme utilization in the most effective way, different methods have been put into practice; immobilization is one of them [30,39]. The term "immobilized enzymes" refers to enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously. Enzyme immobilization offers several advantages over using free enzymes in solution phase. With immobilization, enzyme stability improves. While working with organic solvents, under varied pH range or with different temperature conditions enzymes retain their stability. The added advantage is that with this increased stability, enzyme availability to substrate or analyte increases, which ultimately shows greater turnover over a considerable period of time. The majority of the reported work on enzyme immobilization can be carried out by physical and chemical approaches. Weak interactions between support and enzyme are the characteristics of physical enzyme immobilization, whereas covalent bond formation between enzyme and support are the characteristics of chemical immobilization [27,39,40]. The other significant modes of enzyme immobilization include entrapment and cross-linking.

For effective enzyme immobilization, efficiency of several natural and synthetic supports has been assessed. Inert polymers and inorganic materials are usually used as carrier matrices. Apart from being affordable, an ideal matrix must encompass characteristics like affinity for enzyme. This is assured by the presence of the specific active groups on the carrier, which enable the generation of the enzyme−carrier interactions. However, if absent, the interactions can be tuned by applying intermediate agents (carrier modifiers). Application of enzyme immobilization has been reviewed by several researchers. Combined approaches of proteins/enzymes with nanoparticles have been discussed in detailed by Behera et al. and Pal et al. [41,42]. Significance of enzyme−carrier interactions, especially in terms of adsorption as mode of immobilization has been reviewed by Jesionowski et al. [42]. In this paper he reviewed various carriers utilized for the immobilization with and without the intermediate agents, comparative study of methods of adsorption on different types of the carriers, and a few examples where immobilized enzymes are employed as catalysts in practical applications. Deshpande et al. [3] have demonstrated that zirconia, a white crystalline solid, is also an attractive for enzyme immobilization than the other common materials such as carbon nanotubes, graphite, silica, and magnetic nanoparticles.

Sometimes absence of a functional group required for effective interaction between enzyme and carrier is observed. Surface modification is the best choice in these cases. Here, by chemical modifiers the covalent linkage is possible between enzyme and carrier. The typical common modifying agents

reported for enzyme immobilization include glutaraldehyde [27,28]. Glutaraldehyde is a choice because it possesses high affinity to bacteria, fungi, and proteins. Structurally it contains two reactive aldehyde groups with a five-atom carbon chain which serves as a spacer for enzymes, making their active sites more easily accessible for the substrates. Among the other significant modifiers used for enzyme immobilization are 3-aminopropyltrimethoxysilane and 3-aminopropyltriethoxysilane [43,44]. The latter two compounds interact more strongly with the carrier surface, which is due to the presence of three methoxy or ethoxy groups in their molecules.

Among the different enzyme inhibition assays acetylcholinesterase and butyl cholinesterase share a major role. Reported approaches for stabilization of cholinesterases include AChE with mixture of glucose, trehalose & gelatin, albumin, trehalose & gelatin, BSA, lysozyme & gelatin and combination of sucrose, polygalacturonic acid & dextran sulfate, dextrose in protein standard solution [22,24]. Other significant inhibition-based reported work includes alkaline phosphatase (ALP from calf intestine) immobilized on silica [45].

8.2.3 Application of enzyme biosensor

In recent years the research on the application of biosensors mainly has been focused on four major areas. Fig. 8.4 represents these significant areas in schematics. Among these areas, the potential of enzymatic assays for food and environmental analysis in a high-throughput context has not yet been much assessed. High-throughput analysis can be defined as the implementation of assays in the wells of microplates in combination with liquid handling

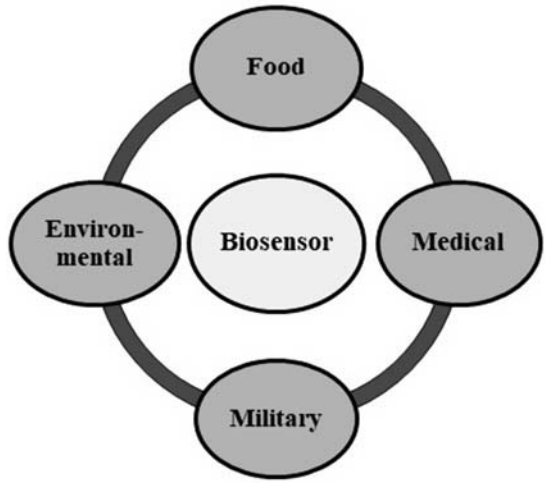

FIGURE 8.4 Current biosensor application areas.

robotics. Such a multianalyte bioanalytical system may result in significant cost reduction with negligible crossover possibilities.

8.3 Enzyme biosensors for environmental monitoring

Enzyme-inhibition-based biosensors show the potential to complement a number of emerging screening and monitoring methods for environmental applications. Potential application areas include: laboratory screening, field screening, or continuous and in situ field monitoring. Analytical tasks associated with site characterization primarily involve the identification of listed contaminants and mapping of the spatial distribution of the compounds of concern. Frequent and repetitive analysis at specific locations for particular compounds of interest is also an important step in remediation. Because enzyme-inhibition-based biosensors show the potential to operate continuously at remote or in situ locations, these devices could be particularly well suited for this task [46]. Portability and rapid analysis makes enzyme-inhibition-based biosensors a promising tool for analyzing various compartments in environment such as soil and water [46].

In the area of water quality monitoring, there is reported enzyme-based biosensor focus on screening of toxic components on a routine basis and their monitoring during remediation phase. Among the reported analytes, toxic components such as pesticides and heavy metals contribute a significant share. Since all these toxic components act as enzyme inhibitors, biosensors based on them are reported to show excellent sensitivity up to ppt level. Considerable number of published works are devoted to biosensors used for the determination of bioavailable metals in soil samples [47]. Here, simple aqueous extract of soils and sediment samples provide information on the bioavailability of the pollutants; this type of information is often more relevant for assessing the potential damage caused by a substance. Air samples have also been analyzed directly with biosensors although the number of devices developed and applied with this objective is limited.

To understand the physiological impact of pesticides in the environment, food safety, and quality control enzyme-based biosensors play a crucial role. Acetylcholinesterase (AChE)/Butyrylcholinesterase (BuCHE) inhibition-based biosensors are reported to determine trace levels of pesticide [3,35,46,51]. Over the last decade or two, AChE/BuChE-inhibition-based biosensors have developed tremendously. Among the different AchE-based sensor Xia and Xiangyou reported multiple organophosphorus compounds such as phoxim, dichlorvos, omethoate, and trichlorfon with detection range 0.01−10.00 ng/L [48]. Multielement analysis and high-throughput analysis have always remained areas of interest for commercial success of biosensors [49]. On the lab scale, the need for higher throughput has driven the shift from microwell plates with 96 wells to those with 384 or 1536 wells. The traditional 96-well format has proved inadequate and is being replaced by microplate with

larger numbers of smaller wells with volumes that range from milliliters to nanoliters. This 4−16-fold increase in the sample throughput with decrease in size means that fewer reagents are needed per assay, and the resulting cost savings has evidently been beneficial. With miniaturized high-throughput assays, highly toxic compounds such as Hg(II) can be analyzed with little μL total assay volume. Microwell plate with automation can analyze thousands of samples within hours or can analyze multiple samples within a very short time. In addition, inhibition studies are performed in closer proximity, which ultimately increases reliability of results. Advancement of high-throughput analysis on various platforms has been successfully demonstrated by various scientists. The wide range of platforms reported for this work includes metal or metal oxide chips, nanoparticles, paper strips, textile sample, glass fiber filters, and even human skin [3,10,19,21−24,46,50]. The interesting fact about disposable strips includes the absence or decrease of the color indicating the AChE inhibition. The biosensor is able to detect organophosphate and carbamate pesticides with good detection limits (methomyl $= 6.16 \times 10^{-4}$ mM and profenofos $= 0.27$ mM) and rapid response times ($\sim$5 min). In this method quantification is done by naked eyes (0.0001 and 0.1 ppm of organophosphorus pesticides) making the method cost effective. On chip platform, effective chemical enzyme mobilization and its utility for sensitive mercury analysis in deep well has also been reported [50].

8.4 Enzyme biosensors for food quality monitoring

The food industry needs appropriate analytical methods for monitoring food quality and food processing. Detection of chemical and biological contaminants in food commodity is of supreme importance because, unlike impurities of a physical nature, they cannot be seen visually. Therefore, it is essential to advance the biosensor development for the analysis of food quality, since they have proven to be an extremely viable alternative to traditional analytical techniques [51]. However, very few biosensors play a prominent role in monitoring food quality or food processing. Considerable efforts have been made to develop enzyme-based biosensors that are inexpensive, reliable, and robust enough to operate under realistic conditions [52]. Advantages of enzyme-based biosensors in food quality monitoring are abundant. Trace level quantification, high precision, low sample volume and analysis time, and reusability of biosensors are some of the advantages [53]. In the area of food quality monitoring, most of the enzyme-based biosensors for food security are largely concentrated on the analysis of food contaminants, allergens, toxins, pathogens, food additives, and adulterants. In recent years, numerous transducers have been explored utilizing several enzymatic assays for the development of biosensing techniques applied for food quality monitoring. Among various biosensors, application of thermal biosensors in food analysis was studied extensively. A large number of enzymatic reactions have been reported

using thermal biosensors, i.e., enzyme thermistor, amid them detection of fructose, choline, and urea were reported in different food stuffs [25]. A highly selective, interference-free biosensor for the measurement of fructose in real syrup samples was developed. The assay was based on the phosphorylation of d(-)fructose to fructose-6-phosphate by hexokinase and subsequent conversion of fructose-6-phosphate to fructose-1,6-biphosphate by fructose-6-phosphate-kinase. Moreover, determination of free and total choline in commercially available milk, milk powder, and dietary supplements was reported using thermal biosensors. The choline biosensor was based on the choline oxidase and catalase enzymes coimmobilized on controlled pore glass (CPG). The heat liberated in the bienzyme reaction was proportional to choline concentration under the optimized condition [27]. Furthermore, a simple, economical, and highly stable thermal biosensor for analysis of urea in adulterated milk was reported by Mishra et al. The biosensor is comprised of immobilized enzyme urease on CPG, which selectively hydrolyzes urea present in the milk sample. The biosensor exhibited lower detection limit and an excellent dynamic range of urea detection in milk [4]. In other reported transducers utilizing enzyme assays, an amperometric biosensor for vitamin C was reported based on the immobilization of ascorbate oxidase into a biocompatible sandwich-type composite film. Content of ascorbic acid in commercial juices was determined by the developed biosensor and characterized by a very good bioelectrocatalytic performance toward the oxidation of vitamin C in solution [54].

As modern-day society requires sensitive, accurate, and express methods of food safety monitoring, the growing field of enzymatic biosensors represents an answer to this demand. Although most of the earlier reported biosensor systems have been tested on buffered solutions, more biosensors that can be applied to real sample analysis have been reported in recent years. In this perspective, biosensors for potential environmental and food applications continue to show improvements in areas such as genetic modification of enzymes and improvements in immobilization of recognition element. Compiled works on recent trends in enzyme-based biosensors are presented in Table 8.1.

8.5 Future prospects and conclusions

The biosensors-based bioanalytical techniques utilizing enzymatic reactions are useful as an alarm or general toxicity indicator for the fast identification of food or environmental contaminants. The market trends showed about 10.4% growth in the development of biosensors for various applications for food and environmental safety, which also leads to improved human health. In contrast with clinical applications, biosensing techniques for food and environmental safety are still in their early stages and facing many difficulties due to inherent features of environmental and food matrices where sensitivity and selectivity toward a specific analyte are the key issues.

TABLE 8.1 Compiled literature on advances in enzyme- based biosensors.

Enzyme	Analyte	Matrix	LOD/Range	Ref.
Acetylcholinesterase	Paraoxon	Milk	1 μg/L	[2]
Urease	Urea	Urine	10 −1000 mM	[4]
Alcohol oxidase	Hg(II)	Water	5−500 ng/L	[10]
Organophosphorus hydrolase	Paraoxon	Gas	90−300 mg/L	[19]
Horseradish peroxidase	Parathion	Fruit	0.001 −500 μg/L	[21]
Butyrylcholinesterase	Paraoxon	Milk	0.005 −50 μg/L	[22]
Hexokinase and fructose-6-phosphate-kinase	Fructose	Syrup	0.5−6.0 mM	[25]
Alkaline phosphatase	Paraoxon	Water	0.01 −500 μg/L	[45]

Sensitivity improvement has always remained the topmost priority of enzyme-based biosensors. In practice, for quantification of toxic components in environmental or food samples highly sensitive enzyme-inhibition-based biosensors are required. To achieve such sensitivity, nanomaterials or nanocomposites tailored with enzymes is a preferred approach. The tailor-made nanocomposites with versatile nanostructures provide more sensitive and flexible analysis in complex matrices with increased analytical performance [41,42]. New strategies for popularizing enzyme-based biosensors for field application or commercial market include disposable paper-based sensors, reusable sensor strips, and microfluidic devices in the form of lab on chip where enzyme immobilization plays a significant role [55]. These different platforms that are very promising are low in cost, miniaturized, user friendly, and meet the needs of on-site detection of environmental and food samples. To increase sensitivity, future work should focus on clarifying the mechanisms of interaction between nanomaterials and enzymes using novel properties to fabricate a new generation of biosensors. Emerging technologies such as lab-on-a-chip microdevices and nanosensors or wearable point-of-use screening tools for environmental and food safety applications offer opportunities for the construction of a new generation of biosensors with much better performance [19,56,57]. The second important issue is high selectivity. Most biosensors reported in the literature work very well in laboratories, however, they may meet serious problems in tests in real samples or in the field. As a result, it is essential to develop novel surface modification approaches in order to avoid

nonspecific adsorption at surfaces [47]. Third, another important issue is multianalysis on a single platform. Multiplexing is critical for saving assay time, which is especially important for assays performed in laboratories. Multiarrays can perform a large number of assays for multiple sample analytes [57,58]. An ideal miniaturized enzyme-based biosensor will be one that is integrated and highly automated. Current lab-on-a-chip technologies (microfluidics) offer a solution toward this goal. We can expect that successful biosensors in the future may incorporate all these features, and can conveniently detect minute targets within a short period. Sensing formats such as lateral flow chambers, disposable electrodes, and colorimetric paper strips are also projected as potential alternatives for facilitating the on-field quantification of potential analytes in a cost-effective manner. Further, development of wireless-networking-equipped biosensors that can be implemented onsite for real-time monitoring of pollutants will also help expand the dimension of research in this field [38]. Overall, for the successful development of biosensors, effective combination of biosensing and biofabrication should be used. For this, different transducers can be utilized [59].

In practice, biosensor development is a contineous process and there is always room for improvement in the desigh and utilizing various components in emerging biosensor. With the addition of newer food toxicants and environmental contaminents that may be threat for humans and the overall ecosystem, the need for fast and accurate biosensor will be of utmost importance. Biosensors today, requires the trust of potential consumer, keeping in mind that the accepance of new efforts is the best indicator of the sucess and achievement for an emerging technology.

References

[1] Grimalt S, Dehouck P. Review of analytical methods for the determination of pesticide residues in grapes. J Chromatogr A 2016;1433:1−23.

[2] Zhang Y, Muench S, Schulze H, Perz R, Yang B, Schmid RD, et al. Disposable biosensor test for organophosphate and carbamate insecticides in milk. J Agric Food Chem 2005;53:5110−5.

[3] Deshpande K, Mishra RK, Bhand S. Determination of methyl parathion in water and its removal on zirconia using optical enzyme assay. Appl Biochem Biotechnol 2011;164(6):906−17.

[4] Mishra GK, Sharma A, Deshpande K, Bhand S. Flow injection analysis biosensor for urea analysis in urine using enzyme thermistor. Appl Biochem Biotechnol 2014;174:998−1009.

[5] Hayat A, Marty JL. Aptamer based electrochemical sensors for emerging environmental pollutants. Front Chem 2014;2:41.

[6] Mishra GK, Sharma V, Mishra RK. Electrochemical aptasensors for food and environmental safeguarding: a review. Biosensors 2018;8:28.

[7] Wang W, Han J, Wu Y, Yuan F, Chen Y, Ge Y. Simultaneous detection of eight food allergens using optical thin-film biosensor chips. J Agric Food Chem 2011;59(13):6889−94.

[8] Mishra GK, Sharma A, Bhand S. Ultrasensitive detection of streptomycin using flow injection analysis-electrochemical quartz crystal nanobalance (FIA-EQCN) biosensor. Biosens Bioelectron 2015;67:532−9.
[9] Ramanathan K, Gary R, Apostol A, Rogers K. A Fluorescence based assay for DNA damage induced by radiation chemical mutagens and enzymes. Curr Appl Phys 2003;3:99−106.
[10] Deshpande K, Mishra RK, Bhand S. A High sensitivity microformat chemiluminescence enzyme inhibition assay for determination of Hg(II). Sensors 2010;10:6377−94.
[11] Prieto-Simón B, Campa's M, Andreescu S, Marty JL. Trends in flow-based biosensing systems for pesticide assessment. Sensors 2006;6:1161−86.
[12] Kim B-M, Abd El-Aty AM, Hwang T-E, Jin L-T, Kim Y-S, Shim J-H. Development of an Acetyl cholinesterase-based detection kit for the determination of organophosphorus and carbamate pesticide residues in agricultural samples. Bull Korean Chem Soc 2007;28:929−35.
[13] Dorst B, Mehta J, Bekaert K, Rouah-Martin E, Coen W, Dubruel P, et al. Recent advances in recognition elements of food and environmental biosensors: a review. Biosens Bioelectron 2010;26:1178−94.
[14] Scheller F, Schubert F. Biosensors: techniques and instrumentation in analytical chemistry, vol. 11. Netherland: Elsevier Science Publishers B.V.; 1992. p. 1−7.
[15] Newman JD, Turner AP. Home blood glucose biosensors: a commercial perspective. Biosens Bioelectron 2005;20:2435−53.
[16] Neethirajan S, Ragavan V, Weng X, Chand R. Review biosensors for sustainable food engineering: challenges and perspectives. Biosensors 2018;8:23.
[17] Mozaz S, Marco M, Alda M, Barcelo D. Biosensors for environmental applications: future development trends. Pure Appl Chem 2004;76:723−52.
[18] Selid P, Xu H, Collins E, Collins M, Zhao J. Sensing mercury for biomedical and environmental monitoring. Sensors 2009;9:5446−59.
[19] Mishra RK, Martín A, Nakagawa T, Barfidokht A, Lu X, Sempionatto JR, et al. Detection of vapor-phase organophosphate threats using wearable conformable integrated epidermal and textile wireless biosensor systems. Biosens Bioelectron 2018;101:227−34.
[20] Borisov M, Wolfbeis O. Optical biosensors. Chem Rev 2008;8:423−61.
[21] Chouhan RS, Vinayaka AC, Thakur MS. Chemiluminescence based technique for the detection of methyl parathion in water and fruit beverages. Anal methods 2010;2:924−8.
[22] Mishra RK, Deshpande K, Bhand S. A high-throughput enzyme assay for organophosphate residues in milk. Sensors 2010;10:11274−86.
[23] Verma N, Singh M. Biosensors for heavy metals. Biometals 2005;18:121−9.
[24] Weetall HH, Mishra NN, Mahfouz A, Rogers KR. An approach for screening cholinesterase inhibitors in drinking water using an immobilized enzyme assay. Anal Lett 2004;37:1297−305.
[25] Bhand S, Soundararajan S, Wärnmark IS, Milea JS, Dey ES, Yakovleva M, et al. Fructose-selective calorimetric biosensor in flow injection analysis. Anal Chim Acta 2010;668:13−8.
[26] Danielsson B, Mattiasson B, Mosbach K. Enzyme thermistor devices and their analytical applications. Appl Biochem Bioeng 1981;3:97−143.
[27] Deshpande K, Danielsson B, Bhand S. Flow injection analysis of choline in milk and dietary supplements using an enzyme thermistor. Chemical Sensors 2011a;1(1):16.
[28] Mishra GK, Mishra RK, Bhand S. Flow injection analysis biosensor for urea analysis in adulterated milk using enzyme thermistor. Biosens Bioelectron 2010;26(4):1560−4.

[29] Kanungo L, Bacher G, Bhand S. Flow-based impedimetric immunosensor for aflatoxin analysis in milk products. Appl Biochem Biotechnol 2014;174:1157–65.
[30] Amine A, Mohammadi H, Bourais I, Palleschi G. Enzyme inhibition-based biosensors for food safety and environmental monitoring. Biosens Bioelectron 2006;21:1405–23.
[31] Harrad L, Bourais I, Mohammadi H, Amine A. Recent advances in electrochemical biosensors based on enzyme inhibition for clinical and pharmaceutical applications. Sensors 2018;18:164.
[32] Fukuto RT. Mechanism of action of organophosphorus and carbamate insecticides. Environ Health Perspect 1990;87:245–54.
[33] Arduini F, Amine A, Moscone D, Palleschi G. Reversible enzyme inhibition-based biosensors: applications and analytical improvement through diagnostic inhibition. Anal Lett 2009;42:1258–93.
[34] Luque de Castro MD, Herrera MC. Enzyme inhibition-based biosensors and biosensing systems: questionable analytical devices. Biosens Bioelectron 2003;18:279–94.
[35] Pundir CS, Chauhan N. Acetylcholinesterase inhibition-based biosensors for pesticide determination: a review. Anal Biochem 2012;429:19–31.
[36] Upadhyay L, Verma N. Enzyme inhibition based biosensors: a review. Anal Lett 2013;46(2):225–41.
[37] Bucur B, Munteanu FD, Marty JL, Vasilescu A. Advances in enzyme-based biosensors for pesticide detection. Biosensors 2018;8:27.
[38] Mehta J, Bhardwaj SK, Bhardwaj N, Paul AK, Kumar P, Kim K-H, et al. Progress in the biosensing techniques for trace-level heavy metals. Biotechnol Adv 2016;34:47–60.
[39] Datta S, Christena LR, Rani Y, Rajaram S. Enzyme immobilization: an overview on techniques and support materials. Biotech 2013;3:1–9.
[40] Ahmad R, Sardar M. Enzyme immobilization: an overview on nanoparticles as immobilization matrix. Biochem Anal Biochem 2015;4:2.
[41] Pal S, Sharma MK, Danielsson B, Willander M, Chatterjee R, Bhand S. A miniaturized nanobiosensor for choline analysis. Biosens Bioelectron 2014;54:558–64.
[42] Jesionowski T, Zdarta J, Krajewska B. Enzyme immobilization by adsorption: a review. Adsorption 2014;20:801–21.
[43] Zhou G, Fung KK, Wong LW, Chen Y, Renneberg R, Yang S. Immobilization of glucose oxidase on rod-like and vesicle like mesoporous silica for enhancing current responses of glucose biosensors. Talanta 2011;84:659–65.
[44] Falahti M, Saboury AA, Ma'mani L, Shafiee A, Rafieepour A. The effect of functionalization of mesoporous silica nanoparticles on the interaction and stability of confined enzyme. Int J Biol Macromol 2012;50:1048–54.
[45] Mishra RK, Mishra GK, Dharma Teja V, Danielsson B, Bhand S. Visual colorimetric dual readout bioassay for determination of pesticide residues in drinking water. Chemical Sensors 2013;3:12.
[46] Kumar J, Jha SK, D'Souza SF. Optical microbial biosensor for detection of methyl parathion pesticide using *flavobacterium Sp*. whole cells adsorbed on glass fiber filters as disposable biocomponent. Biosens Bioelectron 2006;21:2100–5.
[47] Rodriguez-Mozaz S, Lopez A, Barceló DMD. Biosensors as useful tools for environmental analysis and monitoring. Anal Bioanal Chem 2006;386:1025–41.
[48] Xia S, Xiangyou W. Acetylcholinesterase biosensor based on prussian-blue-modified electrode for detecting organophosphorus pesticide. Biosens Bioelectron 2010;25:2611–4.

[49] Mishra A, Kumar J, Melo JS. An optical microplate biosensor for the detection of methyl parathion pesticide using a biohybrid of *Sphingomonas* sp. cells-silica nanoparticles. Biosens Bioelectron 2017;87:332−8.
[50] Deshpande K, Mishra RK, pal S, Danielsson B, Williander M, Bhand S. A novel on chip analysis of dissolved Hg(II) in drinking water nanotech, vol. 3; 2010. p. 133−6.
[51] Da Costa Silva LM, Santos VPS, Salgado AM, Pereira KS. Biosensors for contaminants monitoring in food and environment for human and environmental health. In: State of the art in biosensors − environ med Appl; 2013. p. 152−68.
[52] Luong JHT, Groom CA, Male KB. The potential role of biosensors in the food and drink industries. Biosens Bioelectron 1991;6:547−54.
[53] Velasco-garcía MN, Mottram T. Biosensor technology addressing agricultural problems. Biosyst Eng 2003;84:1−12.
[54] Wen Y, Xu J, Liu M, Li D, He H. Amperometric vitamin C biosensor based on the immobilization of ascorbate oxidase into the biocompatible sandwich-type composite film. Appl Biochem Biotechnol 2012;167:2023−38.
[55] D'Souza SF. Immobilization and stabilization of biomaterials for biosensor applications. Appl Biochem Biotechnol 2001;96:225−38.
[56] Lee TMH. Over-the-counter biosensors: past, present, and future. Sensors 2008;8:5535−59.
[57] Mishra RK, Hubble LJ, Martín A, Kumar R, Barfidokht A, Kim J, et al. Wearable flexible and stretchable glove biosensor for on-site detection of organophosphorus chemical threats. ACS Sens 2017;2(4):553−61.
[58] Bhand S, Surugiu I, Dzgoev A, Ramanathan K, Sundaram PV, Danielsson B. Immunoarrays for multianalyte analysis of chlorotriazines. Talanta 2005;65(2):331−6.
[59] Vigneshvar S, Sudhakumari VS, Senthilkumaran B, Prakash H. Recent advances in biosensor technology for potential applications − an overview. Front. Bioeng. Biotechnol 2016;4:11.

Further reading

[1] Andreescu S, Marty JL. Twenty years research in cholinesterase biosensors: from basic research to practical applications. Biomol Eng 2006;23:1−15.
[2] Nigam VK, Shukla P. Enzyme based biosensors for detection of environmental pollutants–a review. J Microbiol Biotechnol 2015;25(11):1773−81.
[3] Ovalle M, Stoytcheva M, Zlatev R, Valdez B. Electrochemical study of rat brain acetylcholinesterase inhibition by chlorofos: kinetic aspects and analytical applications. Electrochim Acta 2009;55:516−20.
[4] Badawy ME, El-Aswad AF. Bioactive paper sensor on the acetylcholinesterase for the rapid detection of organophosphate and carbamate pesticides. Int J Anal Chem 2014;10:1−8.