Biosensors

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Abstract

Biosensors appear as suitable, fast responsive, and cost-effective analytical tools that are extensively being used in monitoring programs including food quality control, agriculture, bioprocess control, environmental monitoring, military, and medical diagnostics. Biosensor is a self-contained integrated device which uses biological mechanism and provides specific quantitative and semiquantitative information about the analyte of interest. Biosensing systems and methods are being developed as environmental quality monitoring tools in the assessment of ecological/biological quality to determine the potentially harmful pollutants (organic and inorganic) and also provide information about their toxic effects. Detection of small amount of biological samples, requirement of minimal tissue damage for *in vivo* screening, on-site monitoring of clinical metabolites, and increased specificity and sensitivity in the order of ng/ml or pg/ml are some of the major concerns for the increasing need to develop biosensors as fast and economic methods for analysis in medical diagnostics. Mass production of molecular recognition elements with improved selectivity, affinity, and stability, immobilization techniques, miniaturization, multisensor array determinations, and operating conditions are some of the major potential areas of development that are expected to have an impact in biosensor technology. The future research for sustainable application of biosensors should rely on more efficient structure and function specificity of the biological components, noninvasive interfacing with the target molecule through mini-reactors, and improved digitization of the generated signal. Real-time parallel monitoring of multiple species is yet another driving force toward the development and commercialization of multichannel biosensors which are required for direct analysis in high-throughput screening systems.

Keywords

Biosensors • Environmental and clinical monitoring • Biorecognition element • Analyte • Transducer

11.1 Introduction

Over a period of recent years, analytes have been increased in number, and therefore, for better environmental control, more suitable analytical methods are required. They have been routinely used by regulatory authorities and industries for screening and testing large number of samples/analytes and provide enough information. Biosensor technology has gained enormous attention over the recent years in food and beverages; agricultural, environmental, and bioprocess control; clinical diagnostics; pharmaceutical industries; and many more.

Over the past many decades, discoveries at the frontiers of basic sciences (physics, chemistry, biology), engineering sciences (biotechnology, electronics and communication, biomedical engineering), and medical sciences (pathology, anatomy,

and physiology) led to the development of new biosensors for various applications (Fig. 11.1). Clark in 1956 initiated research in the field of biosensors by publishing work on oxygen electrode (Clark 1956). Since then, biosensor research is one of the fast growing fields where billions of dollars are invested to develop them as potential analytical tools, many of them aimed at on-site analysis. Biosensors can provide fast, sensitive, reliable measurements at low cost as compared to conventional methods of detection which are time-consuming, are expensive, and require the use of highly trained personnel. Moreover, off-site analysis is possible with conventional methods where the samples are sent to laboratory. Nevertheless, field monitoring is more preferred which has driven the development of biosensors as new analytical tools which are easy to use.

The definition of biosensors has been appropriately chosen by Newman et al. (2004). Biosensors are compact analytical devices that incorporate recognition element of biological origin. Recognition element is either closely connected to or integrated with the second important component of biosensors called transducer. The property of selective interaction of biomolecules, utilized as biosensors with other molecules, is the basis for the designing of biosensors. This specific interaction of analyte of interest to the complementary biorecognition element immobilized on support matrix results in change in physicochemical properties including heat transfer, pH change, mass change, electron transfer, and uptake or release of

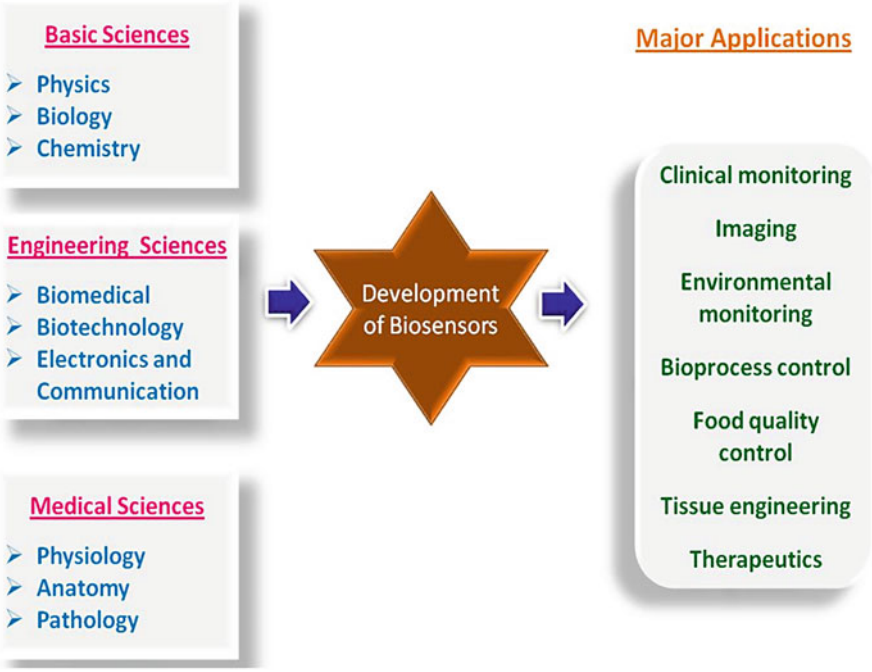

Fig. 11.1 Inventions at the frontiers of scientific fields contribute to the development of biosensors for wide range of applicants

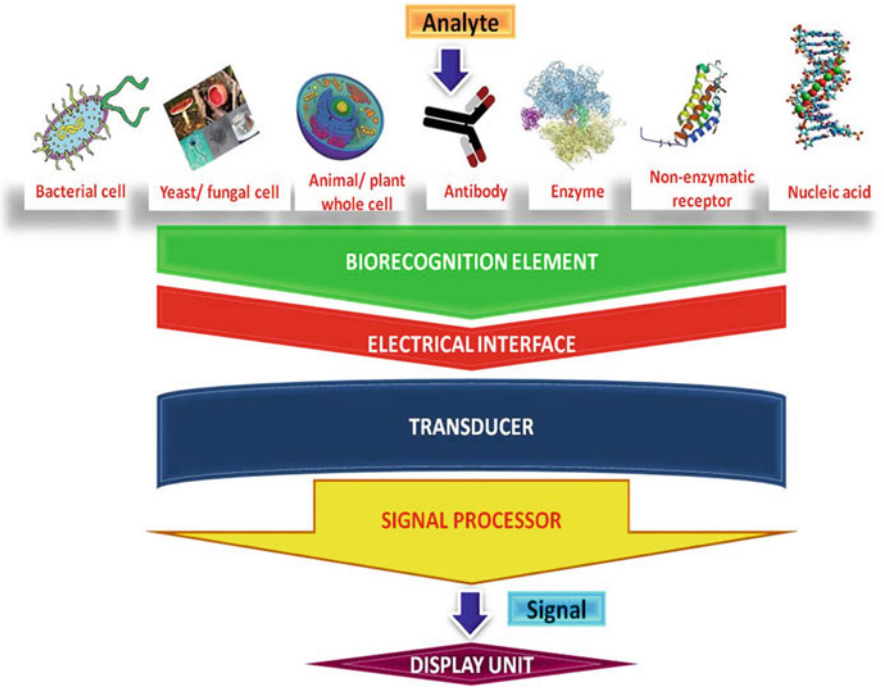

Fig. 11.2 Working principle of biosensor

gases or specific ions. These changes are detected and measured by transducer in the form of electronic signal which is proportional to concentration of bound analytes. Measuring biological outputs in the form of signals generated and monitoring the process is being utilized in biosensor technology. Figure 11.2 describes the general working principle of biosensors.

Most of the times, biosensors are case specific in its approach and use a specific bioactive component/inherent property of cells or tissues for the desired reaction/conversion to generate a signal which can be monitored. Sometimes enzyme-based biosensors generate the signal by catalytic conversion of substrate to form the product, disappearance of substrate, or coenzyme conversion. The biochemical reaction can either be measured to monitor the process or can be superimposed with other biochemical events like the use of kinetics or coupling with other reactions.

With the advent of genetic engineering technologies, more flexible and efficient biosensors can be constructed. Molecular tools make it possible to improve the analysis by:

1. Constructing microorganisms that produce surfactants to increase the bioavailability of pollutants
2. Constructing more efficient microorganisms in degrading pollutant

3. Constructing microorganisms which are capable of utilizing multiple types of compounds
4. Constructing microorganisms which can survive harsh environmental conditions like very high and very low temperature, high salinity, or areas where oxygen is in limited amounts
5. Selection of microorganisms with new enzymatic capabilities

Biosensors provide fast and accurate detection of contaminated sites and clinical metabolites for environmental and clinical monitoring, respectively. The other advantages that are being offered over other analytical tools include portability, working on-site, and ability to measure contaminants/metabolites in complex matrices with minimal sample preparation. Lots of other important information on samples including biological effects (toxicity, endocrine-disrupting effects, etc.) of the chemicals can also be accurately derived. This chapter presents a comprehensive overview of the fundamental principles for biosensor design, operating mechanisms, summarizes important recent applications of biosensors in environmental and clinical monitoring, and addresses the need for fundamental and continued research for further development of biosensor technology.

11.1.1 Components of Biosensor

Description on functions of a typical biosensor at five different levels is shown in Fig. 11.3. The five different components and their functions in a biosensor system include:

1. Biorecognition element – bioreceptors bind specifically to the analyte.
2. Electrical interface – specific biological processes occur at this interface which give rise to a signal.
3. Transducer element – specific biochemical reaction is being converted into electrical signal.
4. Signal processor – electronic signal is converted into a physical parameter.
5. Display unit – an interface to display the results to the operator.

Biosensor thus becomes an excellent analytical device to analyze, detect, and record the biological data for monitoring a number of biological materials and their conversion, verification of product content, and early detection of contaminants or hazardous chemicals or biological materials.

11.1.2 Biosensor Configurations

The general classification of biosensors based on recognition elements and transducers is described in Fig. 11.4. The type of recognition elements forms the basis of classification of biosensor. Enzymes, receptors [natural (proteins of non-catalytic,

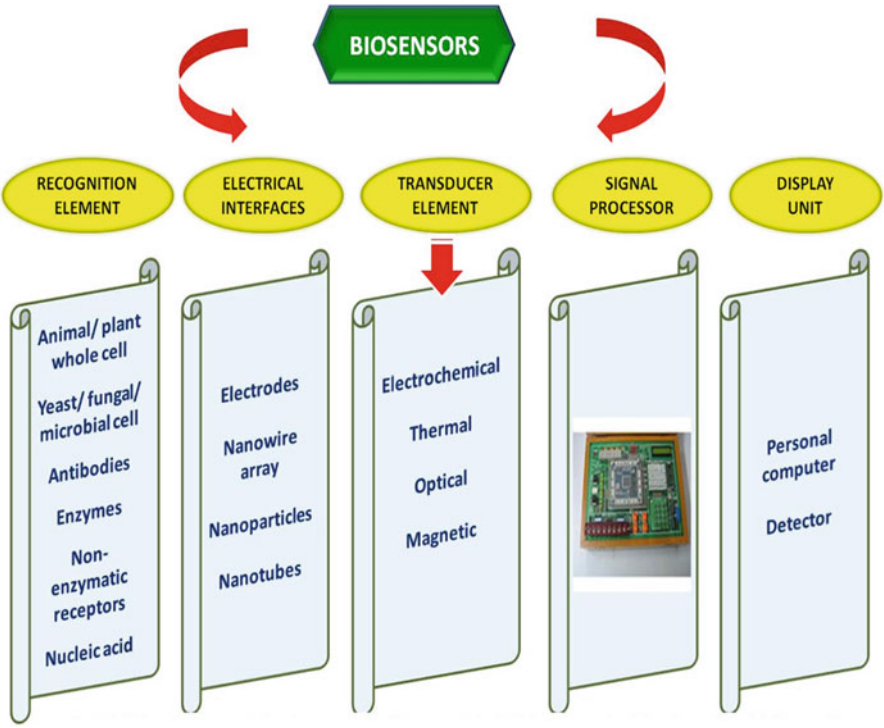

Fig. 11.3 Schematic representative of components of biosensor: a typical biosensor works at five different levels. (**a**) Recognition element; (**b**) electrical surfaces; (**c**) transducer element; (**d**) signal processor; and (**e**) display unit

or non-immunogenic origin) or synthetic], antigens/antibodies, nucleic acid/complementary sequences, animal or plant whole-cell organisms, yeast, fungi, or microbial cells and tissue slices are the examples of biological material that can be incorporated in a biosensing system. Hence, according to the biorecognition principle and the type of specific interaction between the analyte and biological element, biosensors are classified as immunosensors and enzymatic, nonenzymatic, whole-cell, and DNA biosensors. The support or matrix used to immobilize the type of biorecognition element and this immobilized molecule decides the specificity of a biosensor system. Immobilization imparts stability to the biomaterial and ensures proximity between the biomaterial and the transducer. Cross-linking between the molecules; physical adsorption at solid surface; entrapment within a membrane, polymer, or microcapsule and surfactant matrix; covalent binding to a surface; gel entrapment; self-assembled biomembranes; electropolymerization; and bulk modification are some of the popular methods of immobilization.

Depending on the type of transducing element and the method of signal transduction, biosensors are categorized as piezoelectric, electrochemical (amperometric and potentiometric), thermal, optical, mechanical, and magnetic biosensors.

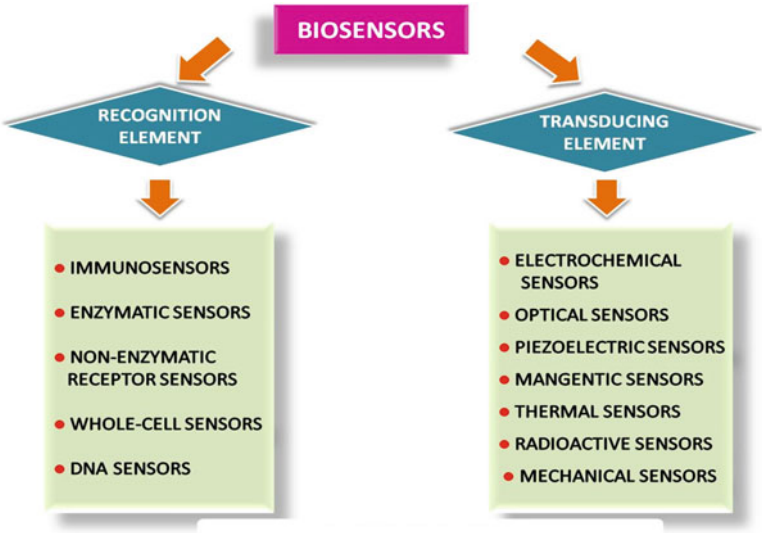

Fig. 11.4 Classification of biosensor

Light absorption, fluorescence/phosphorescence, reflectance, refractive index, bioluminescence, and chemiluminescence are the properties for detection that are being exploited by optical transducers. The characteristic property of bending of silicon cantilevers caused by the adsorption of target molecules onto the cantilever surface (where receptor molecules are immobilized) is being employed in cantilever biosensors.

A large amount of research has taken place recently to develop biosensors capable of efficiently determining several analytes alone or simultaneously and, therefore, represent an interesting tool in environmental monitoring and clinical screening (Fig. 11.5).

11.2 Biosensors for Environmental Monitoring

One of the first environmental biosensors was based on the enzyme acetylcholinesterase to detect nerve gas and developed for military in the late 1970s. This enzyme is involved in transmitting messages in the nervous system and produces an electrochemical reactive product. Presence of nerve gas inhibits the production of an enzyme and hence the electrochemical signal. Since then many biosensors are being developed to detect wide range of potentially harmful pollutants of environmental concern on the basis of specific recognition of biomolecule (Table 11.1). In the following section, the biosensors reported for different environmental applications are being described.

Fig. 11.5 Applications of biosensor in environmental monitoring and clinical screening

11.2.1 Toxicity

Bioluminescence assays, where genetic manipulation of lux gene in various organisms is done to allow controlled emission of light in response to metabolism of certain material, are used in toxicity sensors to determine toxicity in water, food samples, etc. Microtox® (Azure, Bucks, UK), or ToxAlert® (Merck, Darmstadt, Germany), and Cellsense are some of the reliable sensors for rapid ecotoxicity analysis. Among them, Cellsense gains wide popularity owing to multiple applications which include investigation of the toxicity of 3,5-dichlorophenol and other phenols in wastewater, analysis of wastewater treatment works (WWTW), influent and effluent evaluation of concentration of nonionic surfactants and benzene sulfonate compounds, and toxicity testing of wastewaters and sewage sludge (Farre et al. 2001; Aracic et al. 2015). This biosensor is an amperometric type which incorporates whole microbial cell, *Escherichia coli*. This sensor uses ferricyanide, a soluble electron mediator that allows the electrons to divert from the respiratory system of the immobilized bacteria which, therefore, generates current. The resultant current produced measures bacterial respiratory activity, and the change in the magnitude of current can be detected due to presence of pollutants.

11.2.2 Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand (BOD) sensor is one of the commercially most successful environmental biosensors which uses immobilized microorganisms to measure assimilated carbons (amount of biodegradable organic matter) in wastewater.

Table 11.1 Biosensors designed and developed for various environmental applications

Type of biosensor	Analyte	Biorecognition element	Transducer element	Matrix	References
Toxicity					
Bacterial biosensor	Toxicity	*Escherichia coli*	Electrochemical (amperometric)	Wastewater	Farre et al. (2001)
Bacterial biosensor	Toxicity	Genetically engineered bioluminescent bacteria	Optical (bioluminescence)	No real sample	Choi and Gu (2002)
Biochemical oxygen demand (BOD)					
Bacterial biosensor	Low BOD	*Pseudomonas putida*	Optical (fiber optic)	River water	Chee et al. (2000)
Whole-cell biosensor	BOD	Multispecies culture	Electrochemical (amperometric)	Municipal and industrial water	Tan and Wu (1999)
Hormones and endocrine disruptors					
Immunosensor	Estradiol	Antibodies	Electrochemical (amperometric)	No real sample	Tiefenauer et al. (1997)
Immunosensor	Estrone	Antibodies	Optical	River water	Rodriguez-Mozaz et al. (2004)
Phenolic compounds					
Enzyme based biosensor	Phenols	Enzyme (tyrosinase)	Electrochemical/amperometric	Soil, sludge, and water after extraction	Parellada et al. (1998)
Enzyme-based biosensor	Phenols, m-cresol, p-cresol, catechols	Polyphenol oxidase	Amperometric	Wastewater	Chen et al. (2007)
DNA biosensor	m-cresol or catechol	DNA	Amperometric	Wastewater	Chen et al. (2007)
Heavy metals					
Bacterial biosensor	Zinc, copper, cadmium, nickel	*Pseudomonas fluorescens*	Optical	Soil	Mcgrath et al. (1999)

(continued)

Table 11.1 (continued)

Type of biosensor	Analyte	Biorecognition element	Transducer element	Matrix	References
Microalgae-based biosensors	Zinc, copper, cadmium, nickel, lead, iron, aluminum	*Chlorella vulgaris*	Electrochemical	Urban waters	Claude et al. (2007)
Bacterial biosensor	Nickel ions	*Bacillus sphaericus* strain MTCC 5100	Electrochemical	Industrial effluents and foods	Verma and Singh (2006)
DNA biosensor	Mercury (II) and lead (II) ions	DNA	Optical	Water	Knecht and Sethi (2009)
Pesticides and herbicides					
Enzyme-based biosensor	Organophosphorus compounds	Enzyme (acetylcholine esterase)	Optical	Water	Choi et al. (2001)
Immunosensor	Pesticides	Antibodies	Optical	River water	Rodriguez-Mozaz et al. (2004)
Enzyme-based biosensor	Paraoxon and carbofuran	Enzyme (acetylcholine esterase)	Electrochemical (amperometric)	Wastewater	Bachmann and Schmid (1999)
Microalgae-based biosensors	Atrazine and endrine (herbicides)	*Scenedesmus subspicatus* (*algal cells*)	Optical (fluorescence)	No real sample	Frense et al. 1998
Microbial organisms					
Immunosensor	*Salmonella enteritidis*, *Listeria monocytogenes*	Antibodies	Optical (SPR)	No real sample	Koubova et al. (2001)
Immunosensor	*Escherichia coli*	Antibodies	Electrochemical (potentiometric)	Drinking water	Ercole et al. (2002)
DNA biosensor	*Aeromonas hydrophila*	DNA (hybridization)	Piezoelectric	Mineral and drinking water	Tombelli et al. (2000)

BOD sensor was invented in Japan in the late 1970s, and the first commercial BOD sensor was produced by the Japanese company Nisshin Electric in 1983 (Rodriguez-Mozaz et al. 2004). Since then it had become a very popular and reliable device as an early warning system to detect possible contamination of waterways, and a number of other commercial BOD biosensors based on microbial cells have been marketed. Optical biosensor has been developed, for real-time analysis of BOD in multiple wastewater samples.

11.2.3 Hormones and Endocrine Disruptors

Endocrine-disrupting compounds (EDCs) are chemically ill-defined environmental contaminants that interfere with endogenous hormone homeostasis and impose adverse effects which include decrease of human sperm numbers and increased incidence of testicular, breast, and thyroid cancers. Hormones like estradiol, estrone and ethinyl estradiol, progesterone, and testosterone are discharged in the environment as a result of human/animal excretion or intensive farming and have been found to have endocrine-disrupting effects in aquatic or terrestrial organisms even at low concentrations (ng/l). An electrochemical biosensor to determine progesterone levels in cow's milk has been developed. The operating principle of this sensor is based on competitive binding between analyte and conjugate (alkaline-phosphatase-labeled progesterone) for the immobilized anti-progesterone monoclonal antibody (mAb) sites. The amperometric signal generated in the presence of *p*-nitrophenyl phosphate using either colorimetric assays or cyclic voltammetry measures the concentration of analyte. Surface plasmon resonance (SPR) biosensor BIAcore and piezoelectric biosensors utilize human estrogen receptor to determine the levels of estrogens and xenoestrogens.

11.2.4 Heavy Metals

Toxic heavy metals like chromium, zinc, mercury, cadmium, and copper are non-biodegradable and have been observed to be accumulated in the environment as contaminants and pose great threat to the environment and human health even in low concentrations. Need for trained personnel and high cost associated with traditional analytical methods, biosensors have become more popular to measure heavy metal concentration in environmental samples. Bacterial biosensors are developed which contain specific genes responsible for bacterial resistance to heavy metals fused with gene codes for bioluminescent proteins. Based on the inhibitory action of metal ions on urease activity, enzyme-based biosensors are employed to detect concentration of various heavy metal ions like Hg(II), Ag(I), Cu(II), Ni(II), Zn(II), Co(II), and Pb(II). An optical biosensor has been developed to determine lead and cadmium ions. These ions were shown to inhibit activity of alkaline phosphatase present on external membrane of *Chlorella vulgaris* microalgae as biological element (Durrieu and Tran-Minhw 2002).

11.2.5 Pesticides and Herbicides

An evitable use of toxic chemical pesticides for agricultural purposes has raised concerns for their persistence in atmosphere, food, water, soil, and plants. The limit of 0.1 ug/l for individual pesticide and 0.5 ug/l for total pesticides to check the quality of water for human consumption has been set by the European Community (Directive 98/83/EC) (Rodriguez-Mozaz et al. 2004).

Enzymatic sensors for faster on-site analysis of concentration of pesticides in samples have been developed which are based on inhibition of selected enzymes by the analyte. Choi et al. (2001) described the biosensors based on the inhibition of acetyl cholinesterase (AChE) and colin oxidase for examining the concentration of organophosphorus (paraoxon, parathion) and carbamate pesticides. Tyrosinase-based oxygen sensors can detect diazinon and dichlorvos at limits of 5 uM and 75 uM, respectively. Inhibition of the enzyme aldehyde dehydrogenase by dithiocarbamate fungicides can help in its detection. The lack of specificity of the enzymes to identify individual or class of pesticides can be overcome by genetic engineering of the existing enzymatic systems to produce new specific enzymes for desired analysis. Production of recombinant AChEs for various biosensor applications has been extensively reviewed.

The toxic effect of inhibition of photosynthetic electron flow by blocking the photosystem II (PSII) quinone-binding site and thus modification of chlorophyll fluorescence has been shown by the 30 % of herbicides including phenylurea, triazine, and phenolic herbicides. Biosensors are being developed that utilize membrane receptors of thylakoid and chloroplasts, photosystems and reaction centers, or complete cells like unicellular alga as biorecognition element and amperometric and optical transducers.

11.2.6 Nitrogen Compounds

Nitrites can react irreversibly with hemoglobin, and hence continuous consumption of these ions can cause serious health problems. Increased accumulation of nitrates in groundwater and surface water is of serious concern as they can harm the aquatic environment. The working principle of the biosensor designed to determine the nitrate/nitrite levels is based on the diffusion of nitrate/nitrite through a tip membrane into a dense mass of immobilized denitrifying bacteria which convert these ions into nitrous oxide (N_2O) followed by their electrochemical detection. These biosensors are commonly used for on-site determination in activated sludge systems, sewage treatment plants, and industry.

11.2.7 Inorganic Phosphorous Compounds

The degree of eutrophism, which is recognized as water pollution, depends upon the presence of inorganic phosphate mainly which enters in surface waters through

detergents, fertilizers, or sewage. Increased biomass of toxic or inedible phytoplankton species, increased water turbidity, change in quality of water like its color and smell, depletion of dissolved oxygen, and increased incidence of fish kills and other aquatic animals are some of the major environmental pollution problems associated with eutrophication. Traditional methods for quantitative and qualitative determination of phosphate ions are available which include chromatographic methods, spectrophotometric analysis, and volumetric titration. However, these methods are time-consuming, labor-intensive, and not cost-effective; hence, there is a need to develop faster and cheaper ways to determine inorganic phosphate concentration in water samples.

Reagent less enzymatic phosphate sensors that are based on sequential action of enzymes have been recently developed and represent an effective alternative to conventional methods (Parellada et al. 1998). The sensitive enzyme electrode of the biosensor is constructed by co-immobilizing four enzymes, namely, maltose phosphorylase, phosphatase, mutarotase, and glucose oxidase. The concentration of co-substrate "maltose" is kept constant while measuring inorganic phosphate. Maltose phosphorylase in the presence of inorganic phosphate hydrolyzes maltose to glucose-1-phosphate and α-D-glucose. Mutarotase brings about the mutarotation of α-D-glucose to β-D-glucose followed by its conversion by glucose oxidase. The product of this catalytic reaction influences the sensor signal.

11.2.8 Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls are considered as important environmental pollutants and are widely used in industries as dielectric fluids in electrical transformers and capacitors. In spite of strict ban on the production of PCBs in many countries since past many years, these pollutants are still present in the environment and pose threat to public health. Determining the presence of PCBs in the environment quantitatively can be possible with the use of various biosensor configurations. Few of them which are commonly employed in PCB detection include immunosensors with SPR, electrochemical or fluorescence detection principles, and DNA biosensor with chronopotentiometric detection principle.

11.2.9 Phenols

Paper and pulp industries, industries based on the production of drugs, dyes, and antioxidants, are the major contributors of the release of phenols especially chlorophenols as toxic pollutants which accumulate in the environment. Vasoconstriction, renal tube degeneration, decrease in liver function, cancer, and neurodegenerative diseases are some of the health issues observed when catechol, a phenolic derivative, is absorbed by the gastrointestinal tract. Determination of phenol index in environmental samples can be done using amperometric biosensor which consists of tyrosinase (polyphenol oxidase having wide selectivity for phenolic compounds) as

a biological component, immobilized in a hidrogel on a graphite electrode. Chlorophenols or other substituted phenols can be detected using flow injection chemiluminescence fiber optic biosensors which exploit the ability of analytes to enhance the chemiluminescence reaction of luminol, catalyzed by horseradish peroxidases.

11.2.10 Surfactants

Surfactants can be anionic surfactants which are widely used and can be cationic surfactants which represent only 5 % of the total.

Pseudomonas rathonis T which bears a plasmid for surfactant degradation acts a biological component in an amperometric biosensor for detection of anionic surfactants. Biosensors based on *Achromobacter* have also been constructed for their ability to degrade anionic surfactants. Decrease in dissolved oxygen concentration and hence change in the oxygen electrode current due to degradation of surfactants by the bacteria can be monitored. Oxygen consumption not only acts as an indicator of cell metabolism but also provides information on the surfactant content in the sample. It is possible to achieve high sensitivity, selectivity, and reproducibility with microbial sensors. One of the examples of whole-cell biosensors consists of immobilized linear alkylbenzene sulfonates (LASs) degrading bacteria which are based on the detection of consumed dissolved oxygen concentration in the degradation of LASs.

Alkylphenol ethoxylates (APEs) are group of nonionic surfactants and are shown to be estrogenic both *in vitro* and *in vivo*. APEs are used in wide variety of industries including paper and pulp, textiles, paints, metals, rubber, resins, adhesives, plastics, and latex, but due to their endocrine-disrupting properties, their detection gains prominent importance. Degradation of APEs into alkylphenols (APs) pose more risk as they show grater estrogenic activity. Biosensors have been developed which utilize capillary-based immunoassay (CIA) where glucose dehydrogenase is used as label for the detection of APEs and APs.

11.2.11 Antibiotics

Release of antibiotics in the environment has recently been considered as a matter of great concern as they promote antibiotic resistance. Genetic selection of more harmful bacteria is one of the major limitations of the tremendous use of antibiotics for therapeutic purposes or as growth promoters in dairy cattle or as feed additives in fish farms or in livestock during the past few years. Due to widespread administration of antibiotics, antibiotic resistance can be transferred to humans via ingesting affected meat and milk products. This raises serious food safety issues, and hence biosensors are developed to determine their presence in biological or food samples. Detection of penicillin G or tetracyclines in milk, sulfamethazines (cause

allergic reactions) using an optical immunosensor in animal urine and studying the cross-reactivity between two sulfonamides (sulfamethazine and furosemide) using a commercial biosensor, BIACORE 3000, are some of the potential applications of biosensors.

11.2.12 Microbial Organisms

Bacteria, viruses, and other microorganisms in polluted, treated, and untreated water pose great threat to public health worldwide and sometimes can also be used as biological warfare agents. Pathogens may enter rivers and streams through agricultural runoff and via domestic wastewater. These pathogens/pathogenic compounds may reach humans if such water is being consumed for recreation or sport, for irrigation of fruits and vegetables, and as drinking water. It is important to monitor water supply for the presence of pathogenic compounds or organisms and to prevent the transmission of diseases from these sources.

Current conventional analytical methods for detection of microorganisms are based on colony-forming unit (CFU) count; require selective culture, biochemical, and serological characterization; and thus are expensive and time-consuming (Ercole et al. 2002). Methods employed in biosensors to detect microbial content of the sample rely on identification of consumption of oxygen or the appearance/disappearance of electrochemically active metabolite, analysis of nucleic acid by polymerase chain reaction methods, and immunological techniques. One of the examples of commonly employed biosensors include determination of *Escherichia coli* (*E. coli*) in water samples by an immunochemical potentiometric biosensor by monitoring change in redox potential due to production of ammonia by urease-*E. coli* antibody conjugate linked with *E. coli* cells in wastewater. Another example of sensor capable of detecting 10^3–10^4 *E. coli* cells/ml after an enrichment step is based on tyrosinase-catalyzed oxidation of polyphenolic compounds, which are produced from salicylic acid microbiologically. SPR sensor based on antibodies immobilized on gold sensor surface has been designed to detect *Salmonella enteritidis* and *Listeria monocytogenes* (Koubova et al. 2001).

11.2.13 Bioremediation

Bioremediation is one of the important technologies which involve microorganisms for treating environmental pollution. It is useful in treating oil pollution and brings out an efficient mineralization of hydrocarbons into water and carbon dioxide. Microorganisms are also shown to curb heavy metal pollution by binding with heavy metals and removing them from the environment or change the valency of the metals like chromium or mercury and reduce their toxicity. This process is effective but it is time-consuming, and sometimes removal of pollutants is not possible by indigenous population of bacteria.

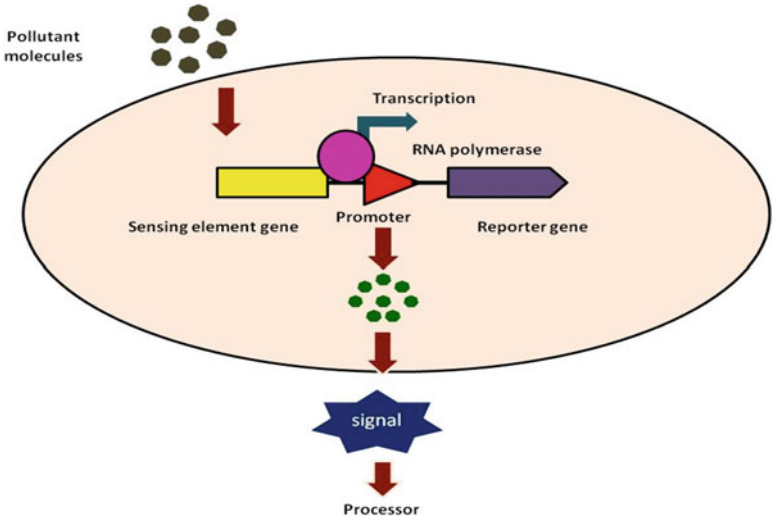

Fig. 11.6 Whole cell microbial bionsensor. Promoter selected from a generic operon and fused with a reporter system, can be made turn on or off based on the specific interaction of the biological component with the target molecule and generate signal

Biosensors are available molecular tools for monitoring pollution on site, *in situ*, and in a cost-effective manner. Biosensors consist of a biological component which is based on either a recombinant plasmid or a whole cell. It has got a reporter, a sensing element whose expression is sensitive to a target molecule (analyte), and a promoter which can be turned on or off in the presence or absence of target molecule. The reporter system is required to generate the signal where its intensity is directly proportional to the expression of promoter (Fig. 11.6).

Reporter systems code for specific gene is a part of expression vector and catalyzes biochemical reactions to generate a signal. Some of the commonly employed reporter systems include bacterial luciferase and green fluorescent protein (GFP) reporter system. The activity of bacterial luciferase reporter system depends upon the emission of light in the form of bioluminescence which is an enzymatic response of luciferase (coded by lux operon and fused with promoter and sensing element gene) activity. Emitted light can be received by a photomultiplier tube for signal analysis. GFP has got an internal chromophore, which confers its fluorescent property and, hence, emits bright green light when excited with ultraviolet or blue light.

Recent attempts have been made to construct biosensors/whole-cell biosensors to monitor the level of environmental pollutants such as toluene, octane, m-Xylene, and other aromatic hydrocarbons, heavy metals, etc. The efficacy of bioremediation can be determined by measuring the rate of elimination of pollutants from its site.

11.3 Biosensors for Clinical Monitoring

The complexity and diversity of human diseases have posed many challenges in the medical field, but owing to high selectivity and specificity toward the target analytes, inexpensive, integrated, and ready-to-use biosensor devices have been developed to improve the ability to detect pathogens or perform genetic analysis in hospitals and clinics or for point-of-care analysis.

More than 80 % of commercial devices based on biosensors are used in clinical monitoring. Yellow Spring Instruments produced first commercial biosensor device meant for glucose determination gained lot of popularity. Over the last decade, immunosensors and more recently DNA-based sensors have been extensively used to detect the presence of pathogenic species and to identify genetic polymorphisms and point mutations. Subsequent section focuses on applications of biosensors in clinical diagnostics for the appropriate interpretation of the identified and quantified biomarkers. Commercialization of biosensor technology in the field of clinical diagnostics can be explained by citing examples of its potential applications. Some of such examples are described in Table 11.2.

11.3.1 Cancer

Tumor biomarkers being the analytes can be detected by biosensors. Measuring the expression or secretion of proteins by tumor cells can help detect the presence or absence of tumor and its condition whether benign or cancerous or whether the treatment can be effective in eliminating or reducing cancerous cells. Detection of multiple tumor biomarkers by biosensors helps in diagnosis with improved sensitivity, specificity, and reproducibility.

No single oncogene or tumor suppressor gene has been found to be altered in all the cancers; hence, in the plethora of molecular biomarkers to decipher genome-related changes, protein under-/overexpression can be analyzed for tumor classification, diagnosis, monitoring treatment, and disease recurrence. Potential biomarkers can be of various molecular origins which include DNA (specific mutation, translocation, amplification, loss of heterozygosity), RNA, and protein (hormone, antibody, oncogene, tumor suppressor) and are typically detected in the serum, blood, cerebral spinal fluid, urine, and tumor tissues/cells.

Multi-analyte immunosensor has been constructed for the detection of seven tumor markers including α-fetoprotein (AFP), ferritin, β-human chorionic gonadotropin (β-hCG), carcinoembryonic antigen, cancer antigen 125 (CA 125), cancer antigen 15-3 (CA 15-3), and cancer antigen 19-9 (CA 19-9) by a competitive immunoassay with the detection limit of <2 ng/ml for all the markers. Capture antibodies are immobilized on electrodes which can capture specific target antigen. The detection is accomplished when signal transduction is realized via secondary antibody tagged with redox molecules or enzymes and oxidation current is generated simultaneously for all the electrodes.

Table 11.2 Potential clinical applications of biosensors

Type of diseases	Type of biosensor	Target/biomarker/analyte	Biorecognition element	Type of assay	Transducer element
Various cancers					
Cancer	Immunosensor	α-Fetoprotein (AFP)	Antibody	Antibody competitive assay	Electrochemical
Cancer	Immunosensor	Carcinoembryonic antigen (CEA)	Antibody	Antibody competitive assay	Electrochemical
Cancer	Immunosensor	Human chorionic gonadotropin (hCG)	Antibody	Antibody competitive assay	Electrochemical
Cancer	Immunosensor	Interleukin 6 (IL-6)	Antibody	Antibody direct assay	Acoustic (SAW)
Breast cancer	Immunosensor	Cancer antigen 15-3 (CA 15-3)	Antibody	Antibody competitive assay	Electrochemical
Breast cancer	DNA sensor	BRCA1 gene	Nucleic acid	Nucleic acid based assay	Electrochemical
Breast cancer	Immunosensor	Epidermal growth factor receptor 2 (HER-2)	Antibody	Antibody direct assay	Optical (SPR)
Ovarian cancer	Immunosensor	Cancer antigen 125 (CA 125)	Antibody	Antibody competitive assay	Electrochemical
Gastrointestinal tract carcinoma	Immunosensor	Cancer antigen 19-9 (CA 19-9)	Antibody	Antibody competitive assay	Electrochemical
Anemia cancer	Immunosensor	Ferritin	Antibody	Antibody competitive assay	Electrochemical
Oral cancer	Immunosensor	Interleukin 8 (IL-8)	Antibody	Antibody sandwich assay	Optical (fluorescence)
Prostate cancer	Immunosensor	Prostate-specific antigen (PSA)	Antibody	Antibody sandwich assay	Optical (SPR)

Chronic myelogenous leukemia	DNA sensor	BCR/ABL gene	Nucleic acid	Nucleic acid based assay	Electrochemical
Acute myocardial infarction					
	Immunosensor	Cardiac troponin T (cTnT)	Antibody	Antibody direct assay	Optical (SPR)
	Immunosensor	Cardiac troponin I (cTnI)	Antibody	Antibody sandwich assay	Electrochemical
				Antibody direct assay	Optical (FRET)
Cardiovascular diseases					
	Immunosensor	C-reactive protein (CRP)	Antibody	Antibody sandwich assay	Magnetic
	DNA sensor	Thrombin	Aptamer	Aptamer sandwich assay	Electrochemical
Inflammation					
	Immunosensor	C-reactive protein (CRP)	Antibody	Antibody sandwich assay	Magnetic
General physical stress					
	Immunosensor	Cortisol	Antibody	Antibody competitive assay	Optical (SPR)

Abbreviations: *SPR* surface plasmon resonance, *FRET* fluorescence resonance energy transfer, *SAW* surface acoustic wave, *BCR/ABL gene* breakpoint cluster region/Abelson gene

Prostate-specific antigen (PSA) is one of the first identified, reliable tumor marker for screening cancer at early stage and monitoring the recurrence of the disease after treatment. Conventional analytical methods to detect PSA have been replaced by innovative biosensors which are based on different transduction techniques, from electrochemical, piezoelectrico optical methods. Recently attention has been diverted to the use of electrochemical immunosensors based on carbon nanotubes with a detection limit of 4 pg/ml in low volumes of human serum and tissue samples. The design of biosensor is composed of 20–30 nm-long terminally carboxylated single-wallet carbon nanotubes (SWNTs) self-assembled on Nafion-iron oxide decorated conductive surfaces. The working principle of electrochemical immunosensor is based on specific interaction of primary antibodies attached to the SWNT forest with target antigen. The detection is achieved by monitoring the response of secondary antibody labeled with horseradish peroxidase (HRP) to hydrogen peroxide substrate. High sensitivity can be achieved by using high HRP/antibody ratio and linking secondary antibodies to wallet carbon nanotubes (CNT).

11.3.2 Hormones

Sex steroids regulate immune response and play important functions by modulating some inflammatory and autoimmune disorders. Radioimmunoassay (RIA) kits are commonly employed to determine progesterone, C21 (carbon 21) steroid levels in the serum or saliva. However, owning to problems associated with radioactivity, the use of immunosensor is an alternative approach. Immunosensors utilizes screen-printed electrodes as solid phase for a competitive immunoassay with estimated limit of detection of progesterone as 32 pg/ml.

Cortisol, another steroid hormone, is important for cardiovascular function and metabolic activities and considered as an indicator marker of stress and disease state. Detection of cortisol in saliva or serum using cortisol-specific monoclonal antibody is based on a competitive immunoassay with a six-channel portable SPR biosensor (Stevens et al. 2008). Human chorionic gonadotropin (hCG), an important diagnostic marker of pregnancy, has been considered as a target of electrochemical immunosensors which are based on the use of gold nanoparticles and ormosil sol-gel membranes.

11.3.3 Cardiovascular Diseases

Cardiac troponin I or T (cTnI/T), myoglobin, and natriuretic peptide (ANP), particularly of B type (B NP), are considered as potential biomarkers to diagnose heart infarction. Owning to slow response time and expensive nature of the conventional analytical methods including ELISA (enzyme-linked immunosorbent immunoassay), RIA, immune-chromatographic assays, and the use of several biosensors based on electrochemical and optical transduction are recommended. Immediate release of troponin T (TnT) in the bloodstream during heart infarction and its monitoring in

short time by biosensors could improve patient care by allowing definitive diagnosis of myocardial infarction in real time (Cody Stringer et al. 2008).

C-reactive protein (CRP) has been recently used for conventional inflammation diagnosis. In routine clinical analysis, ELISA helps to determine CRP levels in the bloodstream (normal range in humans, 1–5 mg/l; protein levels, > 5 mg/l) with detection limits down to 0.2 mg/l as an indicator of inflammatory processes. It also serves as an important diagnostic marker to assist low-grade inflammation and risk in the patients for cardiovascular diseases. New methods in clinical diagnosis of CRP in cardiovascular diseases require its rapid quantification in native matrices such as saliva, urine and human serum. This is possible with the use of magnetic biosensors which utilize two CRP antibodies where one of them can be immobilized on polyethylene-sintered filters in ABICAP® plastic columns and acts as capturing antibody, while the other acts as secondary antibody, biotinylated and attached to streptavidin-coated magnetic beads. Interaction of this antibody-magnetic complex with the captured CRP on the primary antibody can be quantified by a magnetic reader. This highly sensitive system helps to determine CRP in native matrices with a very low detection limit of 0.025 mg/l (Meyer et al. 2007).

11.4 Future Perspectives

The primary aim of global research activities is to improve the quality of life and work for the welfare of the society. The better quality of life is closely related to medical diagnostics facilities, better control of diseases, drug and food quality control and safety, environmental monitoring, and pollution control. Sensitive, fast, continuous, and reliable monitoring is required to control important parameters, which is possible with the use of biosensors. Biosensors are the promising analytical tools which are simple to use, specific, cost-effective, and reliable and provide reproducible results.

Detailed study of biological processes in a biosensor for clinical and environmental analysis is required. Rendering artificial environment to the biomolecules in a biosensor can result in rapid loss of their activity, reduced stability, and low reproducibility of the response. The biosensor performance depends upon the nature and stability of biological element, method for immobilization of biomolecules, analyte specificity, type of transducer used, physiochemical properties of analyte, and operating conditions.

Detection of key substrate without prior separation, high selectivity, sensitivity in the order of ng/ml or pg/ml, short response times, quickness of data collection, and low cost-benefit ratio are some of the major advantages over traditional analytical tools which lead to their pronounced use in biomedical and environmental monitoring.

Although the concept of biosensor is simple and many of them with innovative working applications are at scientific stage. However, only few of them are commercialized and reached marketplace. Extensive research efforts need to be undertaken to produce new sensing elements with the capability to broaden the spectra

of selectivities. Miniaturization of portable biosensors emerges as an important aspect of future bioelectronics as it allows on-field screening, handling of low-volume samples, reduction in reagent consumption and waste generation, and high-density information storage and increases sample throughput. The use of miniaturized flow cell and microsensor will have an impact not only on environment but also on economy and can become key technology of future times. Moreover, the vision of the future of biosensors is enormous and can be imagined to include chip-scale devices which when placed on human body, monitor vital signs for the disease, correcting abnormalities or even signaling a call for help after sensing an emergency situation.

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Suggested Further Readings

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