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BIOSENSOR FOR ENVIRONMENTAL MONITORING

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ABSTRACT

Biosensors show the potential to complement laboratory-based analytical methods for environmental applications. Although biosensors for potential environmental-monitoring applications have been reported for a wide range of environmental pollutants, from a regulatory perspective the decision to develop a biosensor method for an environmental application should consider several interrelated issues. These issues are discussed in terms of the needs, policies, and mechanisms associated with the identification and selection of appropriate monitoring methods.

KEY WORDS: sensors, sensing elements, bio recognizing elements, transducer etc.

INTRODUCTION

In recent years, a growing number of initiatives and legislative actions for environmental pollution control, with particular emphasis on water quality control, have been adopted in parallel with increasing scientific and social concern in this area. The need for disposable systems or tools for environmental monitoring has encouraged the development of new technologies and more suitable methodologies, the ability to monitor the increasing number of analyses of environmental relevance as quickly and as cheaply as possible, and even the possibility of allowing on-site field monitoring. In this respect, biosensors have demonstrated a great potential in recent years and thus arise as proposed analytical tools for effective monitoring in these programs. A biosensor is defined by IUPAC as a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in direct spatial contact with a transduction element. A biosensor should be clearly distinguished from a bio analytical system, which requires additional processing steps, such as reagent addition. A device that is both disposable after one measurement, i.e., is single use, and unable to monitor the analytic concentration continuously or after rapid and reproducible regeneration should be designated a single-use biosensor.

The main advantages offered by biosensors over conventional analytical techniques are the possibility of portability, of miniaturisation and working on-site, and the ability to measure pollutants in complex matrices with minimal sample preparation. Although many of the systems developed cannot compete with conventional analytical methods in terms of accuracy and reproducibility, they can be used by regulatory authorities and by industry to provide enough information for routine testing and screening of samples. For the time being, the monitoring of water quality has generally relied on the collection of spot water samples followed by extraction and laboratory-based instrumental analysis. However, this provides only a snapshot of the situation at the sampling time and fails to provide more realistic information due to spatio-temporal variations in water characteristics. Biosensors can be useful, for example, for the continuous monitoring of a contaminated area. They may also present advantageous analytical features, such as high specificity and sensitivity (inherent in the particular biological recognition bioassay). At the same time, biosensors offer the possibility of determining not only specific chemicals but also their biological effects, such as toxicity, cytotoxicity, genotoxicity or endocrine disrupting effects, i.e., relevant information that in some occasions is more meaningful than the chemical composition itself. They can provide, finally, total and bio available/bio accesible pollutant concentrations. Despite these advantages, the application of biosensors in the environmental field is still limited in comparison to medical or pharmaceutical applications, where most research and development has converged. Nevertheless, the majority of the systems developed are prototypes that still need to be validated before being used extensively or before their commercialization. Biosensors can be used as environmental quality monitoring tools in the assessment of biological/ecological quality or for the chemical monitoring of both inorganic and organic priority pollutants. In this review article we provide an overview of biosensor systems for environmental applications, and in the following sections we describe the various biosensors that have been developed for environmental monitoring, considering the pollutants and analysis that are usually mentioned in the literature.

IMPORTANCE

As a result of human and technological development, a wide range of man-made chemicals and by-products formed in industrial or combustion processes have been, and still are, released in the environment. Some of these substances, such as pesticides, heavy metals or PCBs, are well-recognized contaminants known to affect the quality of the environment. As a consequence, a variety of biosensors have already been developed and applied to their environmental determination. For organ phosphorous and carbamate pesticides, for example, various enzymatic biosensors based on the activity of the choline oxidase and on the inhibition of acetyl cholinesterase and butyrylcholinesterase (BChE) have been developed. For environmental pollution risk assessment, the integration of both chemical and effect-related analyses (toxicity, endocrine disruption activity, etc.) is essential. Many efforts have been made during the last years to develop different bioassays and biosensors for toxicity evaluation of water samples In the case of endocrine disrupting compounds (EDCs), there is a need to develop integrated analytical chemistry/toxicity identification evaluation procedures. At present, apart from biosensors for chemical analysis of some specific EDCs there are other sensors, based on estrogen receptors (ER), conceived for evaluation of their biological effects. The natural sensing element most commonly used is the human estrogen receptor. The binding ability of the chemicals toward the ER is measured in these biosensors as an indicator of their estrogenic activity. Examples of ER-based biosensor are the surface plasmon resonance (SPR) biosensors developed by Usamietal., Hock et al. and Seifert et al. . Even though the number of chemicals amenable to analysis by biosensors continuously increases, there is still a lack of systems suitable for determination of emerging contaminants, such as bisphenol A, phtalates and polybrominated compounds, many of which act as EDCs.

PRINCIPLE OF BIOSENSOR

Ecology tells us that each organism grows in specific set of conditions (niche) that can be defined in terms of food, temperature, moisture, pH, etc. One can thus use each organism as a biosensor for a set of conditions. After we learn to read these biosensors, pollution monitoring becomes a simple and quick job. We give below, a few guiding principles that one can use while learning this technique:

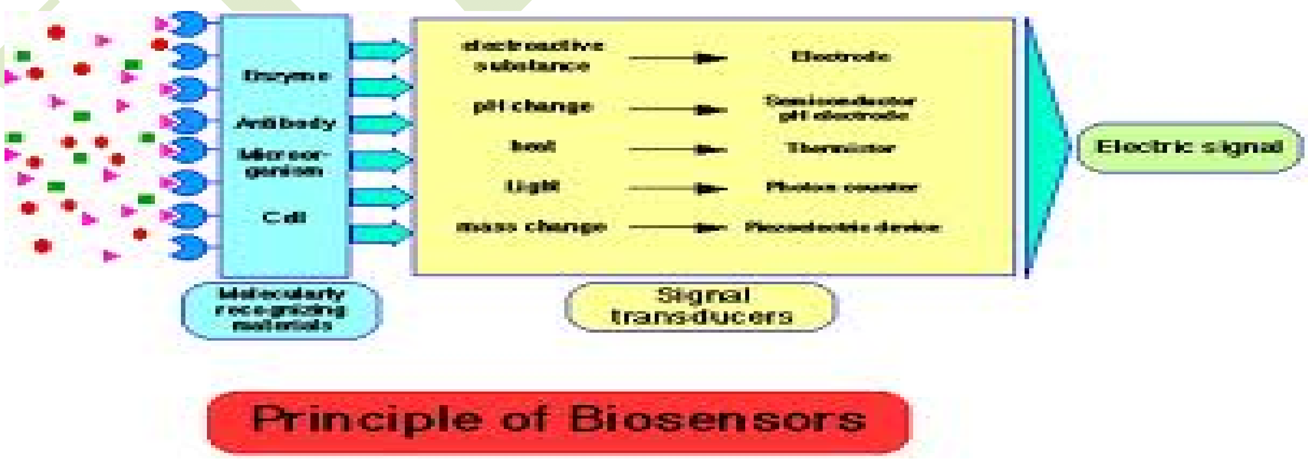
1. Pollution is a result of waste of resources, or in other words, waste is a misplaced resource.

2. Signs of pollution are visible and unpleasant, of varying degree, and only serve as warning signals. It is necessary to read this message and not fight with these signs of pollution (such as odor, pathogens, pests and several other unpleasant natural phenomena).

3.Biosensors inform us of the band or degree of pollution. This is quite enough to guide us towards an appropriate action.

4. Appropriate action not only stops the signs of pollution, but display signs of prosperity. These are clean air, clean water stream, flourishing vegetation, singing birds, etc. and absence of visible nuisance-causing organisms (pests).

A biosensor is an analytical device composed of a biological sensing element (enzyme, receptor antibody or DNA) in intimate contact with a physical transducer (optical, mass or electrochemical) which together relate the concentration of an analyte to a measurable electrical signal. In theory, and verified to a certain extent in the literature, any biological sensing element may be paired with any physical transducer. The majority of reported biosensor research has been directed toward development of devices for clinical markets; however, driven by a need for better methods for environmental surveillance, research into this technology is also expanding to encompass environmental applications.



TYPES OF BIOSENSOR

Biosensors can be grouped according to their biological element or their transduction element. Biological elements include enzymes, antibodies, micro-organisms, biological tissue, and organelles. Antibody-based biosensors are also called immune sensors. When the binding of the sensing element and the analyte is the detected event, the instrument is described as an affinity sensor. When the interaction between the biological element and the analyte is accompanied or followed by a chemical change in which the concentration of one of the substrates or products is measured the instrument is described as a metabolism sensor.

The method of transduction depends on the type of physicochemical change resulting from the sensing event. Often, an important ancillary part of a biosensor is a membrane that covers the biological sensing element and has the main functions of selective permeation and diffusion control of analyte, protection against mechanical stresses, and support for the biological element.

On the basis of the transducing element, biosensors can be categorised as

Electrochemical. :Amperometric and potentiometric transducers are the most commonly used electrochemical transducers. In amperometric transducers, the potential between the two electrodes is set and the current produced by the oxidation or reduction of electro active species is measured and correlated to the concentration of the analyte of interest. Most electrodes are S0044made of metals like platinum, gold, sliver, and stainless steel, or carbon-based materials that are inert at the potentials at which the electrochemical reaction takes place.

Optical: Fiber optic probes on the tip of which enzymes and dyes (often fluorescent) have been co-immobilized are used. These probes consist of at least two fibers. One is connected to a light source of a given wave length range that produces the excitation wave. The other, connected to a photodiode, detects the change in optical density at the appropriate wavelength .

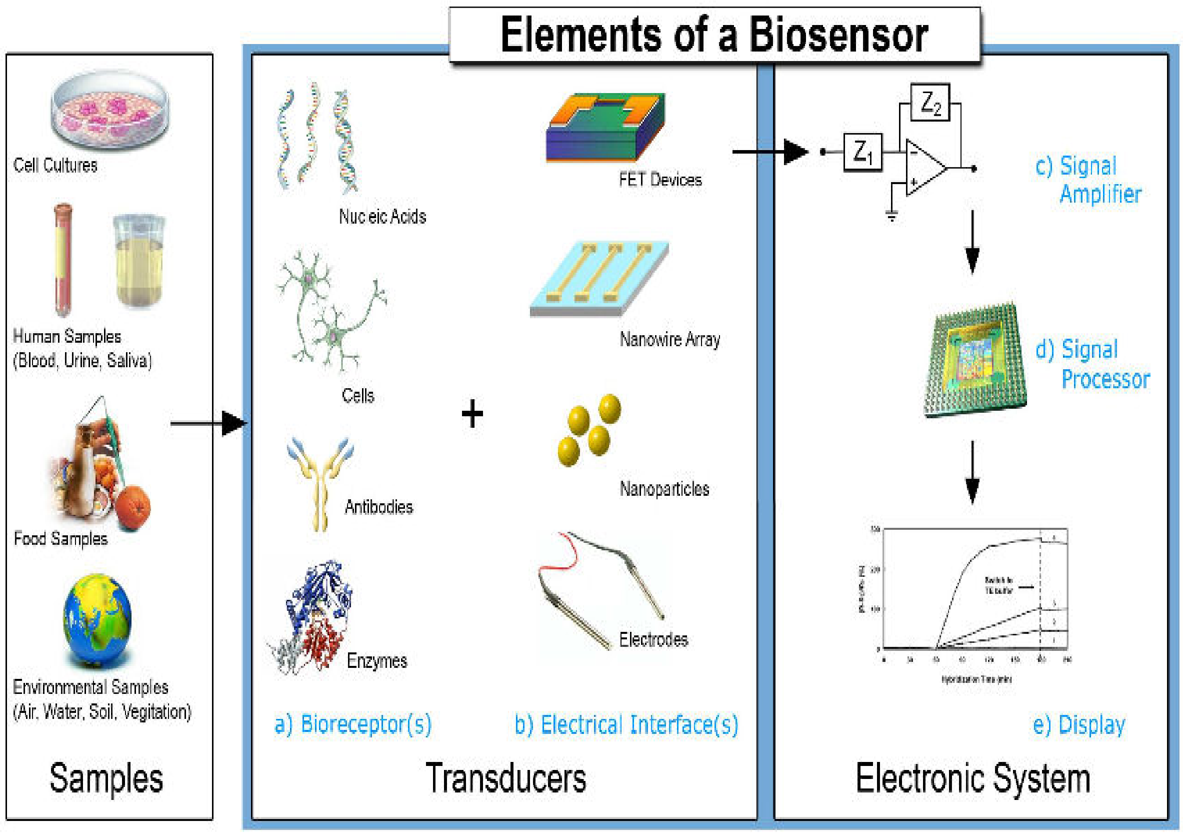
Calorimetric: Calorimetric transducers measure the heat of a biochemical reaction at the sensing element. These devices can be classified according to the way heat is transferred. Isothermal calorimeters maintain the reaction cell at constant temperature using Joule heating or Peltier cooling and the amount of energy required is measured. Heat conduction calorimeters measure the temperature difference between the reaction vessel and an isothermal heat sink surrounding it.

According to the biorecognition principle, biosensors are classified into :

Enzymes: Enzymes are proteins with high catalytic activity and selectivity towards substrates (see the article Enzyme Kinetics). They have been used for decades to assay the concentration of diverse analytes. Their commercial availability at high purity levels makes them very attractive for mass production of enzyme sensors. Their main limitations are that pH, ionic strength, chemical inhibitors, and temperature affect their activity.

Antibodies: Antibodies are proteins that show outstanding selectivity. They are produced by b-lymphocytes in response to antigenic structures, that is, substances foreign to the organism. Molecules larger than about 10 kDa can stimulate an immune response. Smaller molecules like vitamins or steroids can be antigenic (also called haptens) but they do not cause an immune response unless they are conjugated to larger ones like bovine serum albumin. Many antibodies are commercially available and commonly used in immunoassays

Microbes: The use micro-organisms as biological elements in biosensors is based on the measurement of their metabolism, in many cases accompanied by the consumption of oxygen or carbon dioxide, and is, in most cases, measured electrochemically .



Application of Biosensor:

BOD Measurement : Biochemical oxygen demand (BOD or BOD5) is a parameter widely used to indicate the amount of biodegradable organic material in water. Its determination is time consuming, and consequently it is not suitable for online process monitoring. Fast determination of BOD could be achieved with biosensor-based methods. Most BOD sensors rely on the measurement of the bacterial respiration rate in close proximity to a transducer, commonly of the Clark type. With this system the real time analysis of multiple samples was possible. These handy devices have been marketed primarily for food and pharmaceutical industries. Moreover, an optical biosensor for parallel multi-sample determination of biochemical oxygen demand in wastewater samples has been developed by Kwok *et al*. The biosensor monitors the dissolved oxygen concentration in artificial wastewater through an oxygen sensing film immobilized on the bottom of glass sample vials. Then, the microbial samples were immobilized on this film and the BOD value was determined from the rate of oxygen consumption by the microorganisms in the first 20 minutes n Escherichia coli Electrochemical Wastewater Zinc, cobalt and copper.

Heavy Metal Measurement: Heavy metals are currently the cause of some of the most serious pollution problems. Even in small concentrations, they are a threat to the environment and human health because they are non-biodegradable. People are constantly been exposed to heavy metals in the environment. The dangers associated with heavy metals are due to the ubiquitous presence of these elements in the biosphere, their bioavailability from both natural and anthropogenic sources, and their high toxicity. Thus, there are several cases described in the literature where exposure of populations to these pollutants has resulted in severe damage to their health, including a significant amount of deaths. Many of the bacterial biosensors developed for analysis of heavy metals in environmental samples, make use of specific genes responsible for bacterial resistance to these elements, such as biological receptors. Bacterial strains resistant to a number of metals such as zinc, copper, tin, silver, mercury and cobalt have been isolated as possible biological receptors

Nitrogen compound measurement: Nitrites are widely used for food preservation and for fertilization of soils. However, continuous consumption of these ions can cause serious implications on human health, particularly because it can react irreversibly with hemoglobin . The increasing levels of nitrate found in groundwater and surface water are of concern because they can harm the aquatic environment. Developed a biosensor for amperometric determination of nitrite using cytochrome c nitrite reductase (ccNiR) from Desulfovibrio desulfuricans immobilized and electrically connected on a glassy carbon electrode by entrapment into redox active [ZnCr- AQS] double layered hydroxide containing anthraquinone-2-sulfonate (AQS).

The instrument showed a fast response to nitrite (5 seconds) with a linear range between concentrations of nitrite 0.015 and 2.35 µM and a detection limit of 4nM. A highly sensitive, fast and stable conduct metric enzymatic biosensor for the determination of nitrate in water.

PCBs Measurement: The level of PCBs in the environment depends on the matrix where it originated. There are 209 polychlorinated biphenyl congeners that persist worldwide in the environment and food chain. These congeners are divided into three classes based upon the orientation of the chlorine moieties, *i.e.*, coplanar, mono-*ortho* coplanar, and non-coplanar. Conventional techniques used for the analysis of PCBs are generally based on gas chromatography coupled with mass spectrometry (GC.MS) Moreover, immunoassays are simple, sensitive, reliable, and relatively selective for PCBs testing. Among several immunoassay techniques, the enzyme-linked immunosorbent assay (ELISA) combined with colorimetric end-point detection are the most popular. Another interesting approach is the use of immunosensor technology . Imunosensors are a class of biosensors that use as biological recognition elements, antibodies or antigens . Pribyl *et al*. developed a novel piezoelectric immunosensor for determination of PCB congeners in the range of concentrations usually found in real matrices (soil).)

Multi-analyte determination: Sensors capable of determining several analytes simultaneously allow a reduction in time and sample volume and other reagents required and thus constitute a valuable tool for environmental monitoring. Large-scale biosensor arrays, composed of highly miniaturised signal transducer elements, enable the real-time parallel monitoring of multiple species and are an important driving force in biosensor research . In recent years, several examples of multi-analyte determinations have appeared in the literature, such as a portable SPR immunosensor designed for on-site analysis, which was applied to the simultaneous determination of benzopyrene and 2-hydroxybiphenyl , and another SPR biosensor that enabled the division of wavelengths on serial sensing channels by means of a specially designed SPR prism element . A planar array immunosensor, equipped with a charge-coupled device (CCD) as a detector and a diode laser as light source, has been also developed and applied to either the determination of multiple compounds, such as viruses, toxins and bacterial spores, in a single sample analysis or a single analyte in multiple samples simultaneously.

CURRENT RESEARCH AND TRENDS:

Because in many cases the transduction technology is well established, most of the research is focused on improving immobilization techniques of the biological element to increase sensitivity, selectivity, and stability. While critical, the latter has received relatively little attention probably in part because there is a tendency to design disposable devices that are most useful in quality assurance laboratories but do not allow on-line implementation for process control. Another dynamic area of research is miniaturization of sensors and flow systems. Development of these technologies is mainly driven by the need for in vivo applications for medical diagnosis and may not find immediate use in the agricultural and food industries. After almost 40 yr of research in biosensors, a wide gap between research and application is evident. The lack of validation, standardization, and certification of biosensors has resulted in a very slow transfer of technology. With faster computers and automated systems this process should accelerate in the future.

CONCLUSION

Most biosensor systems have been tested only on distilled water or buffered solutions, but more biosensors that can be applied to real samples have appeared in recent years. In this context, biosensors for potential environmental applications continue to show advances inareas such as genetic modification of enzymes and microorganisms, improvement of recognition element immobilization and sensor interfaces.

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