

## Environmental Biosensors



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### Introduction

Stricter regulations and a greater public awareness of environmental issues have necessitated the need to monitor wider range of analytes in air, water and soil, and to do so with greater frequency and accuracy. Analysts currently have a range of

portable analytical techniques at their disposal for monitoring across a variety of environmental analytes. More recently, biosensors have emerged as another promising technology in the analyst's armory, especially for applications requiring continuous monitoring. The term biosensor is

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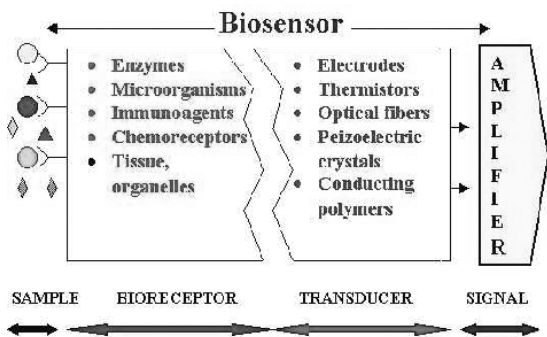


Fig. 1 Components of biosensor: Schematic diagram

defined as a sensor incorporating biological elements such as enzymes, antibodies, receptors proteins, nucleic acids, cells, or tissue sections - as the recognition element, coupled to a transducer. Specific interactions between the analyte and the biorecognition element produce a physico-chemical change, which is detected and measured by the transducer (Fig. 1). The amount of signal generated is proportional to the concentration of the analyte, allowing for both quantitative and qualitative measurements in time [1].

### Biosensor Technology

The two main elements in a biosensor are a biological recognition element or bioreceptor and a signal transducer. The bioreceptor is a biomolecule that recognizes the target analyte and can be divided into three distinct groups: biocatalytic, bioaffinity, and microbe-based systems. Biocatalysis-based biosensors depend on the use of pure or crude enzymes to moderate a biochemical reaction. For environmental applications, enzyme-based reactions involve enzymatic transformation of a pollutant or inhibition of enzyme activity by the pollutant. Enzyme inhibition approaches tend to cater for a larger number of environmental pollutants, usually of a particular chemical class such as pesticides and heavy metals. However, such methods require the use of substrates and in some cases the biosensor may need to be reactivated due to the inhibition.

Bioaffinity-based biosensors rely on the use of proteins or DNA to recognize and bind a particular

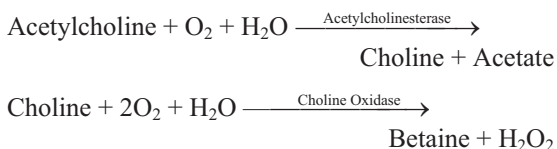
target. For environmental applications such systems depend primarily on the use of antibodies. This is due to the ready availability of monoclonal and polyclonal antibodies directed toward a wide range of environmental pollutants, as well as the relative affinity and selectivity of these recognition proteins for a specific compound or closely related groups of compounds. Nucleic acid-based affinity and electrochemical biosensors for potential environmental applications have recently been reported. Application areas for these include the detection of chemically induced DNA damage and the detection of microorganisms through the hybridization of species-specific sequences of DNA [2].

Microbial biosensors use microorganisms as the biological recognition element. These generally involve the measurement of microbial respiration, or its inhibition, by the analyte of interest. Compared to enzyme-based approaches, microorganism-based biosensors are relatively inexpensive to construct and can operate over a wide range of pH and temperature. The broad specificity of microbial biosensors to environmental toxins make them particularly applicable for general toxicity screening like biological oxygen demand (BOD) or in situations where the toxic compounds are well defined, or where there is a desire to measure total toxicity through a common mode of action. Biosensors have also been developed using genetically modified microorganisms (GMOs) that recognize and report the presence of specific environmental pollutants [1].

A signal transducer is the second essential component of a biosensor. It converts the recognition event into a measurable signal. The transducer can take many forms depending upon the parameters being measured. The most well developed classes of transducers are potentiometric, amperometric, conductometric, optical, acoustic or piezoelectric etc. These utilize various electrochemical responses to measure changes in the electrical properties of the biological recognition element. Most of the reported potentiometric biosensors for detection of environmental pollutants have used enzymes that catalyze the consumption or production of protons. Phosphoric and carbamate pesticides can be evaluated through the use of a pH

electrode that measures the activity of acetylcholinesterase [1]. The activity of the enzyme is affected by the presence of pesticides. In another application, heavy metals can be measured using the inhibition of enzyme urease, coupled to an ammonia selective electrode [3].

Amperometric biosensors are based on monitoring the current associated with oxidation or reduction of an electroactive species involved in the recognition process. The current produced is linearly proportional to the concentration of the electroactive product, which in turn is proportional to the non-electroactive enzyme substrate. An example of this configuration would be acetylcholinesterase coupled to an amperometric sensor used to detect hydrogen peroxide as described in the following reaction:



Compounds of environmental concern, measured using amperometric and electrochemical electrodes include 2,4-Toluene diamine (2,4-T) [4] polychlorinated biphenyls (PCBs), triazines and various toxins such as serin and soman [5].

Enzyme reactions, which produce or consume ionic species, can be monitored conductometrically depending on the total ionic strength of the media. Thin film interdigitated planar conductometric electrodes have been used to measure heavy metals [6]. Glucose oxidase, alcohol oxidase, butyryl oxidase and urease have been immobilized on transducer surfaces and used as bioactive elements for detection of  $\text{Ag}^+$ ,  $\text{Hg}^{+2}$  and  $\text{Pb}^{2+}$  [7].

Transducers based on optical detection techniques have also been used in the field of biosensors. These may employ linear optical phenomenon, including fluorescence, phosphorescence, polarization, rotation, interference, surface plasmon resonance (SPR), total internal reflection fluorescence (TIRF), etc. or non-linear phenomena, such as second harmonic generation [8]. Advantages of optical techniques involve the speed and reproducibility of the measurement. Optical transducers have been used

for affinity-based biosensors, and for a few enzyme- as well as microbial biosensors for environmental applications. A microbial-based optical biosensor for the detection of organophosphate pesticide is being developed in our laboratory. Fiber optic immunosensor for 2,4,6-trinitrotoluene (TNT) and RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) have also been developed [9].

Piezoelectric crystals have also been used in biosensor applications. The vibration of piezoelectric crystals produces an oscillating electric field in which the resonant frequency of the crystal depends on its chemical nature, size, shape and mass. By placing the crystal in an oscillating circuit, the frequency can be measured as a function of the mass. The detection limit of mass bound to the electrode surface is about  $10^{-10}$  to  $10^{-11}$  g. These transducers have been coupled with various biomolecules especially antibodies to detect analytes, including microbial load, formaldehyde, cocaine and parathion. Direct as well as indirect antibody-based piezoelectric sensors have been reported for the atrazine and 2,4-D [2].

The basic requirement of a biosensor is that the biological material should bring the physico-chemical changes in close proximity of a transducer. In this direction immobilization technology has played a major role [1]. Immobilization not only helps in forming the required close proximity between the biomaterial and the transducer, but also helps in stabilizing it for reuse. The biological material is immobilized directly on the transducer or in most cases, in membranes, which can subsequently be mounted on the transducer. Selection of a technique and/or support would depend on the nature of the biomaterial and the substrate and configuration of the transducer used [10,11].

Some of the widely used immobilization techniques include adsorption, entrapment, covalent binding and cross-linking [12,13]. Immobilization of enzymes and whole cells through adsorption perhaps is the simplest of all the techniques. Enzymes have been immobilized through adsorption on a variety of ion exchange, hydrophobic and affinity surfaces [12]. Most of these techniques have the drawbacks of weak adhesion as well as complexity of the process. Novel techniques have

**Table 1 - Biosensors applied for the determination of pollutants in real samples**

Analyte	Sample source	Transducer, recognition element
Pesticides	River water	Optical, immunochemical
Phenols	Wastewater	Electrochemical, enzymatic
Linear alkyl benzene sulphonate (LAS)	River water	Electrochemical, bacteria
Toxicity	Wastewater	Electrochemical, bacteria
Toxicity	Wastewater	Optical, bacteria
Alkanes	Groundwater	Optical, bacteria
Estrogens and xenoestrogens	Real water samples (lake and a sewage plant)	Optical, human estrogen receptor (EC)
BOD	River water	Optical, Pseudomonas sp.
Zinc dichromate chromate	Soil (extract)	Optical, bacteria
Mercury arsenite	Soil (extract)	Optical, Pseudomonas sp.
Daunomycin, PCBs, aflatoxin	River water (preconcentrated)	Electrochemical, DNA
Chlamydia trachomatis (DNA)	River water (preconcentrated)	Electrochemical, DNA

been developed in our laboratory for immobilizing viable or non-viable cells through adhesion on a variety of polymeric surfaces including glass, cotton fabric and synthetic polymeric membranes using polyethylenimine (PEI) [14]. This technique is gaining importance in the introduction of enzymes and microbes on transducer surfaces [15].

Synthetic polymers are also used for the entrapment of the biological materials in a membranous form for biosensor applications. Some of these include polyacrylamide, polyurethane-based hydrogels, photo cross-linkable resins and polyvinyl alcohol (PVA). Natural polymers used for the entrapment of the biomolecules include alginate, carrageenan, low-melting agarose, chitosan, etc. [1, 10,11]. These polymers are known to be very useful in obtaining viable cell-immobilized systems. Apart from these, a number of polymeric membranes can be prepared using radiation polymerization under frozen conditions. This helps in not only controlling the shape, size and porosity of the membranes but also minimizes the damage to the enzymes by thermal denaturation encountered in the chemical polymerization approach [16].

#### Commercial Biosensors

Although most biosensors systems have been tested only on non-real samples (such as in distilled water or buffer solutions), a few biosensors applied to real samples have appeared in recent years. Some representative examples of their application to the determination of different classes of key pollutants and environmental quality parameters, such as biological oxygen demand (BOD), toxicity or endocrine effects, in a variety of matrices are listed in Table 1 [17]. The application of biosensors to real samples must be a necessary step before their commercialization, which is, in general, the aim of the device development. Results must also be validated by comparison with those obtained with standard protocols in order to get the acceptance of end users.

Most commercial biosensors developed are focused in clinical applications, such as for glucose and lactate. Prospective biosensor market for food, pharmaceutical, agriculture, military, veterinary and environment are still to be explored. A brief list of commercially available biosensors for environmental applications is listed in Table 2.

**Table 2 - Commercially available biosensors for environmental applications**

Company	Instrument	Analyte	Transducer & recognition elements	Web page
Texas Instruments Inc. USA	Spreeta	Toxicity assay, liquid quality & concentration.	Optical SPR, biofilm as recognition element	www.ti.com
XanTec Bioanalytics, Germany	Ibis	Toxicity assay from wastestream	SPR Based immunosensors	www.xantec.com
Remedios, Scotland	Remedios	Volatile hydrocarbon, non-volatile hydrocarbon, heavy metals	Optical Bioluminescence inhibition, whole cell	Www.remedios.uk.com
Aclara Bioscience, USA	eTag Assay System	Whole cells, pathogens detection,	Optical eTag fluorescent reporters linked to antibodies and peptides	www.aclara.com
Lincoln Ventures Ltd, New Zealand	Micredox	Environmental monitoring based on mediated cellular Respiration, For BOD and Toxicity (Cu, Cr, and As)	Amperometric, Whole cell based using microelectrodes	www.lincolntechnology.co.nz

**Future Prospects**

Some of the obstacles common to biosensor technology include: the diversity of compounds and the complexity of environmental samples. These hurdles also include: relatively high development costs for single analyte systems, limited shelf and operational life times for pre-manufactured biorecognition components and complexity in devising potentially portable biosensor systems. Nevertheless, there are a number of areas where the unique capabilities of biosensors might be exploited to meet the requirements of environmental monitoring. Advances in areas such as multi-pollutant-screening could allow these techniques to be more competitive. The present scenario demands for increased range of detectable analytes with portable device structure. Solving the resulting integration issues will require further convergence with associated technologies such as biochemistry, polymer chemistry, electronics, micro-fluidics and separation technology.

Micro-Electro-Mechanical Systems or MEMS technology is one of the promising areas that may be going to fulfill these demands in future. The technology is an integration of mechanical elements, sensors, actuators, and electronics on a common silicon substrate through micro fabrication technology. Biochips and sensor arrays for detection of a wide range of hazardous chemical and biological agents can be made out of these MEMS based devices, making it feasible for simultaneous detection of multiple analytes. This also brings the lab-on-chip concept. However, Immobilization and stabilization of biomolecules on these nanodevices may be a greater challenge. Some of the works in these areas have already been initiated. Utilization of molecular recognition ability of biomolecules like avidin-biotin or streptavidin-biotin in conjunction with a lithographic technique is being investigated for the micro immobilization of enzymes on silicon wafers for biosensor applications [18]. Immobilization of enzymes on silicon supports has attracted attention in biosensor chip technology and a variety of classical techniques have been proposed [2].

There are interesting possibilities within the field of biosensors. Given the existing advances in biological sciences, coupled with advances in various other scientific and engineering disciplines, it is imminent that many analytical applications will be replaced by biosensors. A fruitful fusion between biological sciences and other disciplines will help to realize the full potential of this technology in the future.

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