

Biosensors for environmental monitoring A global perspective

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Abstract

The intention of this article is to reflect the advances and describe the trends on biosensors for environmental applications. Biosensors are useful analytical tools for environmental monitoring, capable of providing results in real time, simple to use, portable and cost-effective. Some examples of biosensors in advanced stage of development, which have been applied to real samples, as well as of commercial devices, are given. Biosensors designed for measurement of either specific chemicals or their biological effects, such as toxicity biosensors and endocrine effect biosensors, are discussed. This overview also addresses the support provided by public institutions for biosensor research in the USA, Japan and, especially, in Europe. Future prospects of biosensor technology, with special emphasis in the development of new sensing elements, are foreseen.

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1. Introduction

The increasing number of potentially harmful pollutants in the environment calls for fast and cost-effective analytical techniques to be used in extensive monitoring programs. The requirements, both in terms of time and costs, of most traditional analytical methods (e.g. chromatographic methods) often constitute an important impediment for their application on regular basis. In this context, biosensors appear as suitable alternative or complementary analytical tools.

A biosensor is a self-contained integrated device, consisting of a biological recognition element in direct contact with a transduction element, which converts the biological recognition event into a useable output signal. Biosensors should thus be distinguished from bioassays where the transducer is not an integral part of the analytical system. Biosensors are usually classified into various basic groups according either

to the method of signal transduction or to the biorecognition principle. Accordingly, biosensors can be categorized as electrochemical, optical, piezoelectric and thermal sensors on the basis of the transducing element, and as immunochemical, enzymatic, non-enzymatic receptor, whole-cell and DNA biosensors on the basis of the biorecognition principle. Reviews on these different groups of biosensors, including optical- [1,2], electrochemical- [3] thermal- [4] microcantilevers- [5], immuno- [6,7], whole-cell- [8] and DNA- [9,10] based biosensors can be found in the literature.

One key step in the development of biosensors is the immobilization of the biological component at the transducer surface. The immobilization procures both the stabilization of the biomaterial and the proximity between the biomaterial and the transducer. The immobilization methods most generally employed are: physical adsorption at a solid surface, cross-linking between molecules, covalent binding to a surface, and entrapment within a membrane, surfactant matrix, polymer or microcapsule [11]. In addition to these conventional methods, sol–gel entrapment,

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Langmuir–Blodgett (LB) deposition, electropolymerization, self-assembled biomembranes and bulk modification have been recently used.

The main advantages of the biosensors over other kinds of sensors are their specificity of response and, in some cases, their ability to work in very dirty environments. However, much research is needed in order to improve their performance in a variety of ways [12]. Biosensor technology has reached maturity for determination of the biological oxygen demand (BOD), phenols, heavy metals, and many pesticides (specially those included in the list of 33 priority substances established in the EU Council Directive 2000/60/EC). The analysis of new relevant and emerging pollutants, such as surfactants, hormones and antibiotics, by biosensors is progressively being approached. Biosensors offer the possibility of determining not only specific chemicals but also biological effects, such as toxicity or endocrine disrupting effects, which are becoming more demanded information about the sample.

2. Environmental applications

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 organophosphorous and carbamate pesticides, for example, various enzymatic biosensors based on the activity of the choline oxidase and on the inhibition of acetyl cholinesterase (AChE) and butyrylcholinesterase (BChE) have been developed [13–16].

For the determination of heavy metals, whole-cell biosensors, able to react only to the available fraction of metal ions, have been recently developed [17], such as the recombinant luminescent bacterial sensor used by Ivask et al. [18]. Other well-known contaminants can be nowadays detected by biosensors, such as PCBs and dioxins [19], phenols [20,21], surfactants [22] and polycyclic aromatic hydrocarbons (PAHs) [23,24]. In addition to specific chemical analysis, fast determination of general quality parameters, such as the BOD, and assessment of biological contamination by pathogenic organisms [25,26], is currently possible by biosensor-based methods. BOD commercial biosensors for wastewater are reported, for example, by Liu and Mattiasson [27].

Nonetheless, a great variety of non-regulated chemicals are also continuously released in the environment with unforeseen consequences. This is the case of the so-called emerging contaminants, which include compounds used in everyday life, such as surfactants, pharmaceuticals, gasoline additives, flame retardants, steroids and hormones. Among these, alkylphenolic surfactants, steroid sex hormones, and

pharmaceuticals are of particular concern both because of the volume of these substances used and because of their activity as endocrine disruptors or as causative agents of bacterial resistance, as is the case of antibiotics [28]. In response to this concern, new biosensors have been developed lately for the determination of some of these compounds as, for instance, estrone [29] and alkylphenol ethoxylates [30]. A review on biosensors developed for antibiotics has been recently published by Patel [31]. Most of them were applied to biological or food samples but could be amenable for use in the environmental field. Other reviews have covered the application of biosensors to the determination of toxicity [32], BOD [27], heavy metals [17,33], dioxins [34], biological threat agents [35], pesticides [36,37] and endocrine disruptors in general [38].

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 [39]. As a result, various bioassays based on whole organisms, such as Microtox[®] (Azure, Bucks, UK), ToxAlert[®] (Merck Darmstadt, Germany) and Lumistox (Dr. Lange, Duesseldorf, Germany), which are based on the luminescent bacteria *Vibrio fischeri*, and biosensors, such as Cellsense[®] (an amperometric biosensor, which incorporates *Escherichia coli* bacterial cells for rapid ecotoxicity analysis [32]), have been commercialized. DNA sensors, on the other hand, have been proposed as a general indicator of pollution of environmental samples. These biosensors monitor the interaction of small pollutants with affinities with the immobilised DNA layer [40]. 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 [41]. 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 Usami et al. [42], Hock et al. [43] and Seifert et al. [44].

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, phthalates and polybrominated compounds, many of which act as EDCs. In an effort to produce new biosensors for EDCs, different European collaborative projects are supported by the EU [45] (see Table 1). In the frame of the EU project SANDRINE (“Biosensor tracing of endocrine disrupting compounds in surface water and sludge for water quality assessment”), for instance, different biosensors, bioassays and receptor assays were developed for detection of EDCs.

Table 1
Selected research projects with the presence of biosensors supported by European Union fundings, 1992–2003

Title acronym	Contract no.	Duration
Optical biosensing techniques for monitoring organic pollutants in the aquatic environment, BIOPTICAS	EV5V-CT92-0067	1993–1996
Development of new multisensing biosensors for the detection of bioavailable heavy metals in solid matrices, BIOMETSENSOR	ENV4-CT95-0141	1996–1999
River analyzer: an analytical system for measuring multiple analytes in river water, RIANA	ENV4-CT95-0066	1996–1999
Integrated immuno extraction sampling and portable biosensor prototype for in-field monitoring, INExS-PORT	ENV4-CT97-0476	1997–2000
Prediction of the behaviour of potential endocrine disruptors in soils using vitellogenin ELISA as biosensors, PRENDISENSOR	ENV4-CT97-0473	1997–2000
Biosensor tracing of endocrine disrupting compounds in waste water and sludge, SANDRINE	ENV4-CT98-0801	1999–2002
The application of integrated biosensors with antibody and macrocyclic receptor libraries in the measurement of algal cells and toxins in water, ALGAETOX	ENV4-CT98-0784	1999–2002
Development of improved detection systems for monitoring of toxic heavy metals in contaminated groundwaters and soils, DIMDESMOTOM	EVK1-CT-1999-00002	2000–2003
Protection of groundwater resources at industrially contaminated sites, PURE	EVK1-CT-1999-00030	2000–2003
Automated water analyzer computer supported system, AWACSS	EVK1-CT-2000-00045	2001–2004
Water catchment areas: tools for management and control of hazardous compounds, WATCH	EVK1-CT-2000-00059	2001–2004
Artificial research demonstration project, ARTDEMO	EVK1-CT-2002-00114	2002–2005
Concerted actions		
Biosensors for environmental monitoring/environmental technology, BIOSET	ENV4-CT97-0482	1997–2000
Thematic network: sensors for monitoring water pollution from contaminated land, landfills and sediments, SENSPOLE	EVK1-CT-1999-20001	2000–2003
Evaluation/validation of novel biosensors in real environmental and food samples, VALIDATION of BIOSENSORS	QLK3-2000-01311	2000–2003

3. Application to real-world samples

Although most biosensors systems have been tested only on non-real samples (such as in distilled water or buffer solutions), more 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 BOD, toxicity or endocrine effects, in a variety of matrices are listed in Table 2. 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.

For field applications, an ideal detection method should be able to handle crude samples or at least to require minimum

sample preparation [35]. Classical analytical methods usually involve a laborious sample pre-treatment step. The biosensor technology offers the possibility of identifying and quantifying specific compounds directly in air or water, although in some cases previous sample preparation is also needed, as in the analysis of pollutants in soil samples. Also, for nucleic acid-based detection, the DNA sometimes needs to be previously released from the samples and to be denaturated to open the doubled-stranded conformation.

On the other hand, classical analytical methods of detection usually provide a measurement of the total concentration of potentially harmful substances but not a true assessment of bioavailability or impact [46]. Biosensors can complement classical analytical methods because they are able to distinguish between bioavailable and unavailable forms of contaminants.

Table 2
Biosensors applied to the determination of pollutants in real samples

Analyte	Matrix	Transducing and recognition element	Reference
Pesticides and estrone	River water	Optical immunochemical	[29,63–65]
Phenols	Wastewater	Electrochemical enzymatic	[66]
Linear alkyl benzene sulphonate (LAS)	River water	Electrochemical bacteria	[22]
Toxicity	Wastewater	Electrochemical bacteria	[67]
Toxicity	Wastewater	Optical bacteria	[68]
Alkanes	Groundwater	Optical bacteria	[69]
Estrogens and xenoestrogens	Real water samples (lake and a sewage plant)	Optical human estrogen receptor (EC)	[44]
BOD	River water	Optical pseudomona	[70]
Zinc dichromate chromate	Soil (extract)	Optical bacteria	[18]
Mercury arsenite	Soil (extract)	Optical pseudomonas	[71]
Daunomicyn PCBs, aflatoxin	River water (preconcentrated)	Electrochemical DNA	[72]
<i>Chlamydia trachomatis</i> (DNA)	River water (preconcentrated)	Electrochemical DNA	[72]

4. Research in Europe, the USA and Japan

Europe (and within Europe, Germany, Great Britain and Sweden), the USA and Japan are the trend leaders in biosensor research and manufacture [47], being health applications the greatest area of development. However, extensive research is also being carried out in Europe in the field of biosensors for environmental applications. The European research funding on the area of biosensors for environmental monitoring as a specific topic started in 1992 and the first workshop was held in Berlin in 1993. At present, EU funding has accomplished 10 years. A selection of projects developed in the past years under different framework programs is listed in Table 1. Some of them have already finished and others are still in progress. The first years of the EU funding were devoted to support research projects related to the determination of pesticides in water, for which maximum levels of $0.1 \mu\text{g l}^{-1}$ and $0.5 \mu\text{g l}^{-1}$ for individual pesticides and total pesticides, respectively, are established in the European Union Drinking Water Directive (2000/60/EC). A good example of such projects was BIOPTICAS, which was followed up by RIANA and AWACSS, being the most successful projects at the EU level in that area. In the frame of these projects, a multianalyte application with an optical immunosensor (Fig. 1) was performed, for example, for the simultaneous determination of two pesticides (atrazine and isoproturon) and a natural estrogen (estrone) in natural water samples [29]. It is important to highlight BIOSET and SENSPOL concerted actions, which have been organising various

annual workshops and technical meetings on different areas of biosensors for environmental monitoring in order to stimulate research in that topic in Europe and to demonstrate the applicability of biosensors under real-world conditions. Validation of the biosensor technology has been emphasized also by another concerted action: “Evaluation/validation of novel biosensors in real environmental and food samples”. To summarize public research in EU, it is worth to mention that the total budget spent at the EU reaches 25 millions of euros, being the number of projects supported during the last 10.

In the USA, “biodefense” and research with potential contribution to national security is nowadays an issue of particular concern, and biosensors detecting biohazards have been lately developed [48,49]. International congresses taken place recently, such as “Biosensors-2000” in San Diego (USA), “The Biosensor and Biological Techniques Workshop” held in Ithaca (USA) in 2002, and the “Workshop on International R&D in Biosensing” organised by the World Technology Evaluation Center (WTEC), gave an idea of the main trends in biosensor research and technology, which covered, besides medical diagnosis, environmental monitoring. The National Science Foundation (NSF), on the other hand, has recently offered funding opportunities for biosensing and biosensing network proposals (proposals are nowadays under review), aimed at the design of new sensors and sensing systems as well as sensor networking. Another funding resource is the “The National Center For Environmental Research” (NCER), which funds research grants and graduate fellowships in numerous environmental science and engineering disciplines. The Office of Naval Research also promotes the science and technology programs through government laboratories, such as the “Naval Research Laboratory”, which is one of the leading groups in biosensors research in the USA. They have developed several single and multianalyte biosensors for toxins, bacteria and virus detection [49] in clinical and environmental samples. Other remarkable research group, the biosensor group at UC Davis undertakes research into the development of miniaturized, fast and sensitive biosensors for use in environmental research and monitoring.

Regarding biosensor research in Japan, it is important to mention that biocomputing and nanotechnology are areas of increasing interest. One of the main research groups devoted to biosensor development is the Karube’s group in the “Research Center for Advanced Science and Technology”, at the University of Tokyo. Japan Fund for Global Environment (JFGE) provides grants for environmental conservation activities. For example, support is provided for activities, such as conserving air and water quality, and organizing symposiums, seminars and workshops.

5. Available commercial systems.

There are many biosensors under development and also extensive literature on this area. However, a small part of



Fig. 1. Prototype set-up of the optical immunosensor RIANA (river analyzer). A FIA system delivers sample, buffer and regeneration solutions to the flow-cell, where a transducer is mounted. Laser light excites the fluorescently labeled antibodies involved in the immunoreaction taking place over the transducer. Fluorescent light is detected by photodiodes and acquired by lock-in detection.

Table 3
Commercial biosensors for environmental applications

Instrument	Company	Transducing and recognition element	Web page
BIACORE	Biacore AB (Uppsala, Sweden)	Optical BI	http://www.biacore.com
IBIS	Windsor Scientific Ltd. (Berks, UK)	Optical BI	http://www.windsor-ltd.co.uk
SPR-CELLIA	Nippon Laser and Electronics Lab (Japan)	Optical whole cells or macromolecules	http://www.riken.com
Spreeta	Texas Instruments Inc. (Dallas, USA)	Optical BI	http://www.ti.com
BIOS-1	Artificial Sensing Instruments (Zurich, Switzerland)	Optical BI	
	Amersham International	Optical immunoreagent	http://www.amersham.com
	XanTec Bioanalytics GmbH (Münster, Germany)	Optical BI	http://www.xantec.com
Kinomics Plasmoon™	BioTul AG (Munich, Germany)	Optical BI	http://www.biotul.com
IASys plus™	Affinity Sensors, (UK)	Optical Antibody	http://www.affinity-sensors.com
REMEDIOS	Remedios (Aberdeen, Scotland)	Optical whole cell	http://www.remedios.uk.com
Cellsense	Euroclon Ltd. (Yorkshire, UK)	Electrochemical <i>Escherichia coli</i>	http://www.euroclone.net/envirom/ env_cellsns.htm
PZ 106 Immunobiosensor System	Universal Sensors, (Kinsale, IR)	Piezoelectric antibody	http://intel.ucc.ie/sensors/universal/
ARAS BOD	Dr. Bruno Lange GmbH (Duesseldorf, Germany)	Electrochemical whole cell	http://www.drlange.com
ToxSen™	Abtech Scientific Inc., (Yardley, USA)	Electrochemical BI	http://www.abtechsci.com
	Universal Sensors Inc., (New Orleans, USA)	Electrochemical enzymes	http://intel.ucc.ie/sensors/universal/
NECi's Nitrate Biosensor	Nitrate Elimination Co. Inc., (Michigan, USA)	Amperometric enzyme	http://www.nitrate.com/
eTag Assay System	ACLARA Bioscience (Mountain View, USA)	Optical eTag reporters	http://www.aclara.com/

BI: biomolecular interaction. These affinity-based biosensors monitor interactions between the analyte and the selected biological element, which is usually the element immobilised in the surface of the sensor chip.

all biosensors developed are commercially available, being the commercialization, the best indicator of the success of a biosensor. Additionally, most commercial biosensors are focused in medical applications, such as glucose-detecting biosensors. Food, agriculture, military, veterinary and environment are potential markets still to be established.

A list of some commercially available biosensors for environmental applications is shown in Table 3. Some of these biosensors are based on SPR detection, which allows a configuration for environmental, but also for industrial medical and biological applications. That is the case of BIACORE, one of the biosensors most frequently cited in the literature, which is used both in the industry and in research. Like BIACORE, many of the other listed commercial biosensors are very versatile since they can be used with different biological and non-biological recognition elements (depending on the kind of recognition element they are classified as either biosensors or sensors). Some references report the use of these commercial biosensors for detection of different environmental pollutants, such as sulfonamides [50], PCBs [51], pathogens [52] and nitrate [53], as well as for toxicity determination [32].

6. Future trends

The advances observed in the areas of biochemistry, chemistry, electronics and bioelectronics will markedly influence future biosensor production. Progresses in biosensors technology focus on two main aspects: transducer technology development and sensing element development. New improved detection systems developed under the areas of microelec-

tronics or even nanoelectronics can be used in biosensors. The increasing market of telecommunications, for example, supports the development of new optical materials. Miniaturized diode lasers, optical multiplexes, optical frequency tuners and monolithic detection systems are state of the art [54]. The cost of sophisticated instrumentation is, in principle, expensive but there is a continuous decrease of component costs as the technology progresses. Thus, miniaturizations of devices as well as multi-sensor arrays are expected to have a marked impact in biosensors technology. However, since biosensor sensitivity and selectivity depend basically on the properties of the biorecognition elements, a crucial aspect in future biosensors is the development of improved molecular recognition elements. In this respect, biotechnology and genetic engineering offer the possibility of tailor binding molecules with predefined properties. For biosensor applications gene engineering focuses on both genetically engineered receptor molecules and genetically transformed cells. Through combinatorial screening or design of the amino acid sequence of the binding region of the antibody, recombinant antibodies are produced. In addition, phage display libraries can be applied to select and isolate specific suitable antibodies or peptides with binding affinities similar to, if not higher than, monoclonal antibodies for any imaginable antigen [35]. Different authors, such as Hock et al. [43], are now investigating the potential to use phage-displayed peptides as reagents for biosensor applications. Synthetic nucleic acids (aptamers) are novel recognition elements able to bind, in a manner conceptually similar to antibodies, to a wide array of target molecules with high affinity and specificity. They can be used in biosensors (aptasensors) and allosteric ribozymes (aptazymes) [55].

Peptide nucleic acid (PNA) has demonstrated remarkable hybridization properties towards complementary oligonucleotides. These synthetic DNA analogs can also be used as recognition elements in PNA-based biosensors [56]. Modified cell biosensors, on the other hand, are also being designed based on a cell property, such as bioluminescence, which can be induced after a genetic modification, by the presence of the target compound. An example of these recombinant bacterial sensors is that developed by Ivask et al. [18] for the determination of cadmium, zinc, mercury and chromium.

Even though molecularly imprinted polymers (MIPs) cannot be considered biorecognition elements, they represent another class of advanced recognition elements that mimic the biological activity of antibodies and are suitable for use in sensors [57]. They combine highly selective molecular recognition, comparable to biological systems, with typical properties of polymers, such as thermal, mechanical and chemical stability. MIPs-based sensors have already been developed for pesticides [58] and PAHs [59].

Another current trend is the combination of physics and biology in the creation of new nanostructures. Nanotechnology comprises a group of emerging techniques from physics, chemistry, biology, engineering and microelectronics that are capable of manipulating matter at nanoscale. This novel technology bridges the gap between materials science, coming from the micrometer range, and biochemistry/chemistry, where individual molecules are of major interest [60]. Inspired by nature, molecular self-assembly has been proposed for the synthesis of nanostructures capable to perform unique functions. For example, Lazarides et al. [61] have developed gold colloidal nanoparticle aggregates that are linked by short pieces of DNA. These materials exhibit a color change from red to blue after DNA hybridization and allow the detection of the target DNA.

Finally, another important aspect is the establishment of networked analytical stations in different natural environments. The emergence of these networked instruments requires compact, robust and fully automated analytical instrumentation, and biosensors are in this aspect an ideal option. An example of a networked system based on biosensors is that presented by Gu et al. [62].

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