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REVIEW

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Biosensors as useful tools for environmental analysis and monitoring

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Abstract Recent advances in the development and application of biosensors for environmental analysis and monitoring are reviewed in this article. Several examples of biosensors developed for relevant environmental pollutants and parameters are briefly overviewed. Special attention is paid to the application of biosensors to real environmental samples, taking into consideration aspects such as sample pretreatment, matrix effects and validation of biosensor measurements. Current trends in biosensor development are also considered and commented on in this work. In this context, nanotechnology, miniaturisation, multi-sensor array development and, especially, biotechnology arise as fast-growing areas that will have a marked influence on the development of new biosensing strategies in the near future.

Keywords Biosensors · Environmental analysis · Review · Trends

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 international agreement, "The Stockholm Convention on Persistent Organic Pollutants" (http://www.pops.int/) establishes, for example, an initial assessment of the effective reduction of persistent organic pollutants (POPs) in 2008, and then at subsequent intervals. The first 12 POPs included within the agreement comprise classical organochlorine pollutants (polychlorinated biphenyls, pesticides, dioxins and furans), although there has been a suggestion that the brominated diphenylether flame retardants should be added [1].

At the same time, the European Union Water Framework Directive (WFD), one of the most important pieces of environmental legislation, is likely to transform the way that determination of water quality is undertaken [2]. Within the next few years, the implementation of the WFD (the deadline for all monitoring programs to be operational is December 2006) will require a considerable additional monitoring effort to be undertaken and a wide range of substances of different chemical groups to be identified. The WFD does not mandate the use of a particular set of monitoring methods, but aims at ensuring the establishment of an adequate monitoring program [2]. The Expert Group on the Analysis and Monitoring of Priority Substances (AMPS) is currently examining existing analytical methodologies for their use in the implementation of the WFD, and finding a number of instances in which either suitably robust and validated methods do not exist, or the methods available are insufficiently sensitive [3].

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 analytes 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 bioanalytical 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 analyte concentration continuously or after rapid and reproducible regen-

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eration should be designated a single-use biosensor [4]. 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 can not 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 [5].

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 [2]. 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, both total and bioavailable/bioaccesible 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 commercialisation.

Biosensors can be classified into various groups, according either to signal transduction or biorecognition principle. On the basis of the transducing element, biosensors can be categorised as electrochemical, optical, piezoelectric or thermal sensors. Most catalytic biosensors are based on electrochemical methods, whereas affinity biosensors have generally proved more amenable to optical detection methods [6]. Optical transducers exploit properties such as simple light absorption, fluorescence/phosphorescence, bio/chemiluminescence, reflectance, Raman scattering and refractive index [7]. Cantilever biosensors constitute an emerging group of biosensors and are based on the bending of silicon cantilevers caused by the adsorption of target molecules onto the cantilever surface where receptor molecules are immobilized. Finally, thermometric biosensors monitor the absorption or evolution of heat in biological reactions.

According to the biorecognition principle, biosensors are classified into immunochemical, enzymatic, non-enzymatic receptor, whole-cell and DNA biosensors. Immunosensors are very sensitive, selective and versatile since antibodies can be generated to bind a wide range of compounds that are structurally different. In general, enzymatic biosensors are based on the selective inhibition of specific enzymes by different classes of compounds, with the decrease in activity of the immobilized enzyme in the presence of the target analyte as the parameter that is frequently used for quantification. There is a wide range of enzymes suitable for acting as recognition elements and very often their catalytic properties or substrate specificity can be modified by means of genetic engineering. Biosensors based on natural receptors can be built by integrating the specific receptor within a membrane and by coupling it to a transducing device. These natural receptors are proteins of non-catalytic or non-immunogenic origin, which span cell membranes and can specifically bind certain compounds. Whole cells of living organisms, such as bacteria, yeast, fungi, plant and animal cells, or even tissue slices, have been used as the recognition component by measuring their general metabolic status. These biosensors are useful in the determination of the toxicity of certain compounds to the cells of choice.

Another application of whole-cell biosensors is the determination of the "biological oxygen demand" (BOD). As was discussed above, genetically engineered bacteria are often used in cell-based biosensors. In the case of DNA biosensors, two strategies are applied to detect pollutants: one is the hybridisation detection of nucleic acid sequences from infectious microorganisms, and the other is the monitoring of small pollutants interacting with the immobilized DNA layer (drugs, mutagenic pollutants, etc.) [8]. More comprehensive information about each of the groups of biosensors mentioned can be found in several more-specific reviews [4, 9–17].

Biosensors for environmental monitoring

Biosensors can be used as environmental quality monitoring tools in the assessment of biological/ecological quality elements or for the chemical monitoring of both inorganic and organic priority pollutants. Pollutants are usually classified into groups according to their chemical structure but can also be divided into groups according to their mode of action, such as endocrine disruption, cytotoxicity, carcinogenicity, mutagenicity, or genotoxicity. The following sections describe the biosensors that have been developed for environmental monitoring, considering first those that measure effects, such as toxicity or endocrine activity, and second, biosensors that detect a compound or a group of compounds based on the specific biorecognition of a molecule. Within the latter, a wide variety of compounds of environmental concern or under suspicion are considered. Table 1 lists the most recent reports on the use of biosensors for different environmental applications.

Toxicity and genotoxicity

Although chemical-physical methods are currently employed for the monitoring of environmental pollutants, the effects of substances in the environment, including syner-

Table 1 Biosensors for determination of organic and inorganic compounds and relevant parameters in the environment

Environmently relevant compounds or parameters	Transducing element	Biorecognition element	Features	Reference
Toxicity	Optical (bioluminescence)	Recombinant bioluminescent bacteria	Cell array to classify toxicity	[18]
Toxicity	Optical (bioluminescence)	Genetically engineered bioluminescent bacteria	Portable	[219]
Genotoxicity	Optical (bioluminescence)	Recombinant *Escherichia coli*		[27]
EDCs	Optical (fluorescence microscopy)	Recombinant yeast		[32]
EDCs	Optical (SPR)	Estrogen receptor	LOD: 0.1 μg/L (estradiol)	[30]
EDCs	Electrochemical (amperometric)	Enzymatic (Tyrosinase)		[34]
BOD	Electrochemical (amperometric)	Multispecies culture	Minimum measurable BOD=0.088 mg/L O_2; (biosensor BOD/BOD_5) ratio=0.80	[223]
Low BOD	Optical (optic fibre)	*Pseudomonas putida*	Minimum measurable BOD=0.5 mg/L O_2; comparison with BOD_5: R^2=0.971	[41]
Chlamydia trachomatis (DNA)	Electrochemical (chronopotentiometric)	DNA (hybridisation)	Previous PCR amplification LOD: 0.2 mg/L	[94]
Salmonella enteriditis, Lysteria monocytogenes	Optical (SPR)	Antibodies	10^6 cell/mL	[47]
Salmonella Typhymurium	Acoustic	Antibodies	100 cells/mL	[48]
Escherichia Coli	Electrochemical (potentiometric)	Antibodies	10 cells/mL	[49]
Organic compounds				
Isoproturon, diuron simazine	Optical (fluorescence)	*Chlorella vulgaris* (Algae cells)	0.025 μg/L 0.5 μg/L	[69]
Propanil	Optical (fluorescence)	Antibodies	0.6 ng/L	[224]
Carbaryl	Optical (SPR)	Antibodies	Self-assembled monolayers LOD: 1.38 μg/L	[77]
Organophosphorous compounds	Electrochemical (amperometrical)	Enzyme (organophosphorous hydrolase)	Modified carbon nanotube	[225]
Dichlorvos, parathion, anzinphos (organophosphorous pesticides)	Electrochemical, sonochemical	Enzyme (AChE)	Array of microelectrodes	[57]
Paraoxon and carbofuran (pesticides)	Electrochemical (amperometric)	Enzyme (AChE)	Discrimination between different AChE inhibitors by neuronal networks LOD: 0.2 μg/L	[194]
Pesticides and estrone	Optical (fluorescence)	Antibodies	Natural water samples	[74–76, 90]
Hormones (estrone, progesterone, testosterone)	Optical (fluorescence)	Antibodies	LOD: sub-ng/L	[91–93]
PCBs	Optical (fluorescence)	Antibodies	LOD: 0.1 ng/L	[96]
Dioxine and dioxine-like chemicals	Optical (luminescence)	Recombinant hepatome cells	LOD: 10 pM	[101]
Phenols	Electrochemical (amperometric)	Enzymatic (cellobiose dehydrogenase and quinoprotein-dependent glucose dehydrogenase)	In-field measurements LOD: 0.8 μg/L	[226]

Table 1 (continued)

Environmently relevant compounds or parameters	Transducing element	Biorecognition element	Features	Reference
Chlorophenols	Optical chemiluminescence (fibre-optic)	Enzymatic (horseradish peroxidase)	LOD: 1.4–1975 μg/L	[102]
Bisphenol A	Optical (fluorescence)	Antibodies	Monitoring in a waterworks	[106]
Bisphenol A	Optical (SPR)	Antibodies	SPE prior to analysis to improve sensitivity	[105]
Surfactants	Electrochemical (amperometric)	*Pseudomonas* and *Achromobacter* (plasmid for anionic surfactant degradation)	LOD: 0.25 mg/L (SDS)	[108, 109]
LAS	Electrochemical (amperometric) (O_2 consumption measurement)	*Trichosporon cutaneum* (LAS-degrading bacteria)	In-situ measurement LOD: 0.2 mg/L	[111]
Alkylphenols and their ethoxylates	Electrochemical	Antibodies	LOD: μg/L range	[112]
Nonylphenol	Electrochemical (amperometric)	Antibodies	LOD: 10 μg/L range	[113]
Benzene and its derivatives	Optical (luminescence)	Recombinant *Escherichia coli*	Air determination LOD: 0.5 mg/L LOD: 0.5 mg/L	[117]
Naphthalene and phenanthrene	Optical (fluorescence)	DNA	Sol–gel array	[118]
Daunomicyn PCBs, Aflatoxin	Electrochemical (chronopotentiometry)	DNA	LOD: 0.3 mg/L LOD: 0.2 mg/L LOD: 10 mg/L	[94]
Antibiotics	Optical (fluorescence)	Antibodies		[197]
Inorganic compounds				
Mercury arsenite	Optical (bioluminescence)	*Pseudomonas fluorescens* (with sensors plasmids)	0.003 μg/kg 0.7 μg/kg	[143]
Cadmium, copper and mercury	Electrochemical, optical and mass signal	Enzyme (AChE and urease)	Array biosensor for the measurement of pH, urea, heavy metals and ACh	[227]
Heavy metals	Optical (bioluminescence)	Recombinant *Escherichia coli*	Bioavailable fraction in soils	[145]
Inorganic phosphate	Electrochemical (amperometric)	Enzymatic (trienzymatic configuration)	LOD: 0.57 mg/L	[5]
Nitrate	Optical (fluorescence)	Recombinant *Escherichia coli*	Without interference of phosphate, chloride or nitrite	[149]

gistic and antagonistic effects, as well as the potential effects of unknown substances can not be adequately estimated on the basis of the analysed substances′ concentrations. The effects of substances can be assessed, however, through the use of biological components [18]. The determination of toxicity provides an integrated picture of the overall impact on the environment that can be produced by an effluent, sediment or soil from a contaminated site [19]. In the European Union, along with more stringent demands for water treatment (Council Directive 91/271/EEC), industrial and urban wastewater effluents must reach certain limits of non-toxicity before the effluent can be discharged into the environment. Thus, much effort has been made during recent years to develop and use different bioassays and biosensors for toxicity evaluation of water samples [20].

Whole organisms are used to measure the potential biological impact (toxicity) of a water or soil sample. For example, Cellsense, an amperometric sensor that incorporates *Escherichia coli* bacterial cells for rapid ecotoxicity analysis, has been proposed as one of the newer, rapid toxicity assessment methods within the direct toxicity assessment (DTA) demonstration program of the UK Environmental Agency [21]. A multi-channel two-stage mini-bioreactor system using a genetically engineered bioluminescent bacteria has been applied to determine the toxicity of some endocrine-disruptor compounds (EDCs) [22] and compounds responsive to superoxide damage

[23]. Other recombinant microorganisms that respond sensitively to a broad variety of chemicals have been developed to serve as microbial toxicity biosensors [24]. In a recent work, Lee et al. presented a cell-based array technology that uses 20 recombinant bioluminescent bacteria to detect and classify environmental toxicity [18]. Most environmental biosensors have focused on bacterial systems; eukariotic biosensors are rare. The mammalian cell, which is more complex than bacteria, can give a more sensitive response than bacteria, as is the case with the recombinant fluorescent Chinese hamster ovary cell line that, utilising a fluorescent reporter system, was used to monitor various toxicants, especially EDCs in diverse aqueous environments [25].

Sensors for other areas of ecotoxicology, such as genotoxicity and mutagenicity, have also been developed and have been described as "biosensors for environmental stresses" [1]. They are often based on the interaction of compounds with nucleic acids or genetically engineered microorganisms, which are designed to respond to stresses such as toxicity [24, 26]. Genotoxicity is associated with different compounds, such as phenols, chlorophenols, PCBs and PAHs, and constitutes an early warning screening parameter for possible cancer-inducing pollution activity [19]. Over the last decade, recombinant technology has created new luminescent bacteria for application in diverse toxicity and genotoxicity tests. An example of this is the green fluorescent protein-based biosensor employed for detecting the activity of genotoxic compounds using recombinant *Escherichia coli* strains [27].

Endocrine-effect biosensors

EDCs are a class of substances not defined by their chemical nature but by their biological effect. EDCs interfere with endogenous hormone systems, and many of them can bind to the natural estrogen receptor (ER) as agonists or antagonists. The binding ability of the chemicals toward the ER can be measured in biosensors based on these natural receptors in order to screen or test their potential environmental impact. Using the commonly used human estrogen receptor, the SPR biosensor BIAcore has been applied in the determination of estrogens and xenoestrogens and in binding studies of target compounds [28–30]. Other optical biosensors based on recombinant cells to co-express human ER have recently been developed for the determination of estrogenic activity in water samples [31, 32]. In addition to optical biosensors, electrochemical [33, 34] and piezoelectric [35] biosensors, also based on estrogen receptors, have been developed.

The sensors reported above are designed to detect the interaction of a range of different chemicals with an estrogen receptor whereas other sensors measure the endocrine-disrupting effect by measuring vitellogenin, an egg yolk precursor protein, which is a biomarker of this kind of biological effect in fish [36].

Biochemical oxygen demand

Biochemical oxygen demand (BOD or BOD_5) is a parameter widely used to indicate the amount of biodegradable organic material in water [37]. Its determination is time consuming, and consequently it is not suitable for on-line 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 the Clark type (an amperometric sensor developed by Clark in 1956 for measuring dissolved oxygen [38]). Some BOD sensors have been developed and marketed by various manufacturers in both biofilm and bioreactor-type configurations. Instrument information about many BOD commercial biosensors was provided by Liu and Mattiason [38]. BOD biosensor systems still present a series of limitations that restrict their applications: the lack of standardisation and legislation in most countries, complicated maintenance requirements, and insufficient resistance to various toxic compounds. It is possible to eliminate the toxic effects of heavy metal ions by using a chelating agent that complexes the ions, e.g. ethylene diamine tetra-acetate (EDTA) or sodium diethyl dithiocarbamate (DDTC) [39, 40]. Prevention of contamination by other microbes is also important for a reliable biofilm-type BOD sensor [38].

Many BOD biosensors have been developed for the determination of high BOD values such as those typical of industrial wastewaters. In contrast, Chee et al. have developed an optical fibre biosensor for the evaluation of low BOD values in river waters with a time response of 15 min [41]. Other BOD biosensors recently reported are those based on the photocatalysis of the sample [42] and on a novel microbial membrane [43].

Microorganisms

Surface waters may play an important role in the transmission of pathogens. Bacteria, viruses, and other microorganisms are widely found in polluted, untreated and treated waters, which implies a worldwide public health problem. Suitable monitoring of the water supply for the presence of pathogens can assist in preventing diseases from these sources [44], and thus, new technologies such as biosensors have been developed to provide fast identification of contamination by microorganisms at source and in real time, whereas with conventional analytical methods, days or weeks are required to get a result.

DNA biosensors can be more specific than immunologically based detection systems, and the sensitivity can be improved by combination with polymerase chain reaction (PCR) methods. Gene probes are already finding application in the detection of disease-causing microorganisms in water supplies, food, or in plants, animal or human tissues [45].

Immunological detection, on the other hand, is faster and more robust than DNA detection. Moreover, it has the ability to detect not only contaminating organisms but also their biotoxins [46]. Koubaka et al. [47] reported the

detection of *Salmonella enteriditis* and *Listeria monocytogenes* in real time using a SPR sensor based on antibodies immobilized on the gold sensor surface at concentrations down to 10^6 cell/ml. *Salmonella typhimurium* detection in liquid samples in less than 2 min by an immunosensor based on the acoustic wave principle was reported by Pathirana et al. with limits of detection around 100 cells/ml [48]. Ercole et al. [49] described a biosensor for the determination of *Escherichia coli* in water samples by an immunochemical potentiometric alternating biosensor, while Hasebe et al. [50] described an amperometric tyrosinase-based biosensor also for the detection of *E. coli* in wastewater. The detection was based on tyrosinase-catalysed oxidation of polyphenolic compounds, which are produced microbiologically from salicylic acid, and the subsequent signal amplification. The sensor was capable of detecting 10^3–10^4 cells/mL after an enrichment step.

The commercialisation of current research in biosensor technology can provide consumers with real-time biosensors capable of maintaining their sensitivity under 100 colony-forming units per milliliter (CFU/mL). Advances in antibody production and the recent emergence of phage-displayed peptide biosensors offer increased possibilities for the rapid detection of pathogens [51, 52], but still require time-consuming pre-enrichment in order to detect low numbers of pathogens in water [53].

Organic compounds

Pesticides

Concern about toxicity, ubiquity and persistence of pesticides in the environment has led the European Community to set limits on the concentration of pesticides in different environmental waters. Directive 98/83/EC on the quality of water for human consumption has set a limit of 0.1 μg/L for individual pesticides and of 0.5 μg/L for total pesticides. Enzymatic sensors, based on the inhibition of a selected enzyme are the most extensively used biosensors for the determination of these compounds. Based on the inhibition of acetyl cholinesterase (AChE) and colin oxidase, various biosensors have been developed for the detection of organophosphorous and carbamate pesticides, such as those described by Choi et al. [54], Vangelis et al. [55], Andres et al. [56] and Law et al. [57], who have developed microelectrode arrays based on sonochemical ablation of a non-conducting polymer-coated electrode for the sensitive determination of dichlorvos, parathion and azinphos.

Although sensitive, the biosensors based on AchE inhibition are not selective [since the AchE is inhibited by neurotoxins, which include organophosphorous pesticides (OP), carbamate pesticides and many other compounds] and cannot therefore be used for quantitation of either an individual or a class of pesticides. One approach to solving the lack of specificity of AchE involves the genetic engineering of cholinesterase enzyme to obtain new specific enzymes for desired analytes or families. Different expression systems for the production of recombinant AChEs for biosensor applications have been described [58, 59], and a specific review was published by Schulze et al. [60].

Organophosphorous hydrolase (OPH), on the other hand, is able to hydrolyse a number of OP pesticides, such as paraoxon, parathion, and chemical warfare agents such as sarin and soman. Hydrolysis of these OP pesticides generates p-nitrophenol, which is an electroactive and chromophoric product. Thus, OPH could be combined with an optical transducer to measure the absorbance of p-nitrophenol or with an amperometric transducer to monitor the oxidation or reduction current of this product [61].

With a different approach the capability of various pesticides such as cyanide [62], diethyldithiocarbamates [63] and hydrazines [64] to inhibit the enzyme tyrosinase has also been reported. Similarly, diazinon and dichlorvos have been detected at limits around 5 and 75 μM, respectively, using a tyrosinase-based oxygen sensor [65]. Dithiocarbamate fungicides have been measured by their ability to inhibit the enzyme aldehyde dehydrogenase (AlDH). Particularly, the detectability of the pesticide Maneb could be improved to a level of 1.5 μg/L by using a bioenzymatic system based on the combination of AlDH and diaphorase [66].

Photosynthesis inhibition is an interesting indicator that rapidly reflects the toxic effect of certain pollutants. Taking advantage of this feature, some biosensors based on Photosystem II (PSII) have been reported to be able to detect herbicides in the environment [67]. An amperometric biosensor developed by Koblizek et al. exhibited, for example, selective sensitivity to phenylurea and triazine herbicides, whereas phenolic herbicides were not registered [68]. Heavy metals were also able to inhibit the activity of the PSII biosensor, but their effect is usually found at much higher concentrations than those typical for herbicides. Another PSII-based biosensor [69] allowed the detection of herbicides such as atrazine, simazine, isoproturon and diuron at sub-μg/L concentration levels.

Biosensors based on immunological detection have also been developed for pesticides. Wilmer et al. determined 2,4-dichlorophenoxyacetic (2,4-D) acid in water by an amperometric immunosensor with a limit of detection of 0.1 μg/L [70]. Based on the evanescent wave (EW)-transducing principle, atrazine was detected at concentrations around 0.1 μg/L [71, 72] and cyclodiene insecticides in the μg/L range [73]. Mallat et al. [74–76] applied the River Analyzer (RIANA) immunosensor for the determination of pesticides such as atrazine, simazine, isoproturon, 2,4-D, alachlor and paraquat in natural waters. A portable SPR flow-through immunosensor has been applied for the analysis of carbaryl in natural water samples [77]. Based also on the SPR principle, another immunosensor was used to measure triazine pesticides achieving a detection limit for simazine of 0.2 μg/L [78]. Analyses carried out on surface and groundwater samples showed a good correlation with parallel chromatographic results. An immunosensor for the determination of Irgarol 1051 was applied to the direct analysis of natural waters [79] and water extracts

[80]. In spite of the lack of specificity and the interferences observed in liquid media, some applications have also been reported on the use of piezoelectric immunosensors for the determination of pesticides such as atrazine [81, 82], 2,4-D [81] and parathion [83]. Recently, a label-free direct piezoelectric immunosensor built on a flow-through cell was used for the determination of 2,4-D in water with a limit of detection around 0.2 μg/L [84].

Hormones

Natural and synthetic hormone residues can be found in the environment as a result of human or animal excretion due to growing population and more intensive farming. Hormones such as estradiol, estrone and ethynilestradiol have been found in water at ng/L levels [85–88], but even at these low concentrations, some of them may have endocrine-disrupting activity in aquatic or even terrestrial fauna [89]. Estrone, progesterone and testosterone, along with other organic pollutants, have been determined with a fully automated optical immunosensor in water samples, reaching limits of detection up to sub-ng/L [90–93].

PCBs

Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants even though their production was banned in several countries many years ago. Different biosensor configurations have been designed to determine PCBs in the environment such as a DNA biosensor with chronopotentiometric detection [94], and various immunosensors with fluorescence [95, 96], SPR [97] and electrochemical [98] detection principles.

Dioxins

Dioxins are polychlorinated compounds released as by-products in a number of chemical processes involving chlorine. They are considered carcinogenic and a potential threat to human health [99], and quite recently they have been included in lists of potential EDCs [97]. The SPR biosensor developed by Shimomura et al. [97] for the determination of PCBs (described above) was also employed in the determination of the dioxin 2,3,7,8-TCDD. Another biosensor for detection of dioxin-like chemicals (polyhalogenated dioxins, furans and biphenyls) based on a recombinant mouse hepatoma cell line has been characterized and optimised by Pasini et al. [100]. More recently, the DRESSA biosensor based on the binding of the dioxins to an aryl hydrocarbon receptor has also been developed in hepatome cells [101].

Phenols

Phenolic compounds, and especially chlorophenols, are significant environmental pollutants because of their high toxicity and possible accumulation in the environment [102]. Parellada et al. developed an amperometric biosensor, with tyrosinase (a polyphenol oxidase with a relatively wide selectivity for phenolic compounds) immobilized in a higrogel on a graphite electrode, which correlated satisfactorily with the official method for the determination of the phenol index in environmental samples [5]. Chlorophenols have been also detected with a flow-injection chemiluminescence fibre-optic biosensor [102].

Bisphenol A

Bisphenol A is a typical product of industrial societies produced in large quantities worldwide. Although only weakly estrogenic, the determination of bisphenol A in the environment is very important due to its extensive use and environmental ubiquity. However, the first biosensors focused on bisphenol A determination have only recently appeared. Some examples are immunosensors based on bacterial magnetic particles [103], on surface plasmon resonance [104, 105] and on total internal reflection fluorescence [106]. Based on a tyrosinase-carbon paste electrode, an optical biosensor for phenolic EDCs, including bisphenol A, nonylphenol and diethylstilbestrol, has also been reported [107].

Surfactants

An amperometric biosensor for detection of anionic surfactants was constructed with *Pseudomonas rathonis T* (bearing a plasmid for surfactant degradation) as the biological element. The limit of detection achieved for sodium dodecyl sulfate (SDS) was 0.25–0.75 mg/L [108]. Taranova et al. also studied the sensitivity and selectivity of biosensors based on bacterial strains (*Pseudomonas* and *Achromobacter*) able to degrade the anionic surfactants [109]. The degradation of surfactants by the bacteria caused a decrease in dissolved oxygen and a change in the oxygen electrode current. The lower limit reached for SDS was near 0.25 μg/L.

Linear alkylbenzene sulfonates (LAS)

Residues of LAS are found in surface waters in the low μg/L range [110], and even though they are not severely toxic, they contribute to the permeation of other pollutants into aquatic animals [111]. A combination of two whole-cell biosensors was applied to river water samples for the determination of anionic surfactants [111]. The first biosensor was based on the detection of the dissolved oxygen consumed in the degradation of LAS by immobi-

lized LAS-degrading bacteria, but the other sensor, which used *T. cutaneum* yeast, did no respond to LAS.

Alkylphenol ethoxylates

Alkylphenol ethoxylates (APEs) belong to the group of non-ionic surfactants whose detection has become more important due to their endocrine-disrupting properties. In wastewater treatment processes and in the environment, APEs degrade to alkylphenols (APs), which tend to be more toxic and show greater estrogenic activity. Rose et al. described the development of a capillary-based immunoassay (CIA) for APEs and APs, utilising glucose dehydrogenase (DH) as a label [112]. An amperometric immunosensor based on a carbon screen-printed electrode has been developed recently for the determination of nonylphenol with a detection limit of 10 μg/L [113].

Alkanes, aromatic compounds

Water-soluble aromatic components of petroleum products (e.g., benzene, toluene, ethylbenzene, and xylenes) are of particular concern for drinking-water quality since they can persist in the environment [114]. A green fluorescent protein-based *Pseudomonas fluorescens* strain biosensor was constructed and characterised for its potential to measure benzene, toluene, ethylbenzene, and related compounds in aqueous solutions. The biosensor is based on a plasmid carrying the toluene-benzene transcriptional activator [114]. Another microbial whole-cell biosensor, using *Escherichia coli* with the promoter luciferase luxAB gene, was developed for the determination of water-dissolved linear alkanes by luminescence [115]. The biosensor was used to detect the bioavailable concentration of alkanes in heating oil-contaminated groundwater samples.

Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are very abundant and ubiquitous carcinogenic compounds. Amperometric biosensors for naphthalene found in contaminated soils were constructed using *Sphingomonas yanoikuyae* B1 [116] and a recombinant *Escherichia coli*-based biosensor for benzene determination in air [117]. A sol-derived array DNA biosensor with fluorescence detection was fabricated to detect PAHs in water and serum samples [118].

Antibiotics

Medical substances have been released into the environment with very little attention so far. The presence of antibiotics in the environment is worrying since they promote antibiotic resistance [119]. The widespread administration of antibiotics raises significant food safety issues since antibiotic resistance can be transferred to humans on ingestion of affected meat and milk products [120]. Therefore, most of the biosensors developed are aimed at determining antibiotics in biological or food samples, although their application for environmental monitoring can be considered. For example, the commercial biosensor BIACORE 3000 was used to study the cross-reactivity between two sulphonamides: sulfamethazine and furosemide [121]. Sulfamethazine has been also determined with an optical immunosensor by Akkoyun et al. [122] in animal urine. Hansen and Sorensen presented the choice of three different recombinant cells modified with a tetracycline-inducible promoter in the development of three corresponding whole-cell biosensors [123]. In the field of food monitoring, different biosensors were able to determine penicillin G [120] or tetracyclines [123], both in milk. More references of biosensors for antibiotic determination can be found in a review by Patel [124].

Toxins

A number of attempts have been made to detect toxins in environmental and clinical samples using receptor sensors [125]. A great number of specific sensors for bacterial toxins and mycotoxins have been developed for food and environmental control [126–130], and a colorimetric enzymatic test with the purpose of developing an electrochemical biosensor has been reported by Campas et al. [131]. A light-addressable potentiometric immunosensor based on the commercial device (Threshold) for the analysis of saxitoxin and ricin has also been described. A portable fibre-optic biosensor and an impedance-based immunosensor have been prepared to determine staphylococcal enterotoxin B [132, 133]. Various evanescent-wave immunosensors have also been reported to be able to detect botulin with very low limits of detection (low pmol) [134]. A rapid and sensitive immunosensor developed for the detection of the *Clostridium botulinum* toxin A enabled the detection of the toxin within 1 min at concentrations as low as 5 ng/mL [135]. With a similar configuration, the detection of the cholera toxin has been described [136].

Inorganic compounds

Metals

Recent progress has been made in the development of biosensors relying on intact bacterial cells to monitor toxic metals. One advantage of the whole-cell sensors is their ability to react only with the available fraction of metal ions whereas common analytical methods are not able to distinguish between fractions of metals that are available and non-available to biological systems [137]. Heavy metals are well known to inhibit the activity of enzymes, and application of this phenomenon to the determination of these hazardous toxic elements offers several advantages, such as simplicity and sensitivity. Durrieu and Tran-Minh

described a biosensor for the determination of heavy metals based on inhibition of the alkaline phosphatase (AP) present on the external membrane of *Chlorella vulgaris* microalgae [138]. Biosensors based on urease inhibition are usually applied for the determination of mercury and have been reported for the determination of other heavy metals ions as well [139–142]. In the biosensor proposed by Nam et al., a combination of specific additive sand-selective rewashing techniques allowed the urease-based biosensor to respond only to mercury ions [142].

Specific recombinant bacterial sensors have been constructed for the selective determination of certain metals. They are based on inducible promoters fused to reporter genes (for example, those that code for bioluminescence proteins, such as luciferase) and are more sensitive than both chemical analysis methods and non-specific toxicity biosensors [143]. Recombinant luminescent bacterial sensors were used for the determination of the bioavailable fractions of cadmium, zinc, mercury and chromium in soil [137, 144] and available heavy metals in sediments and soil [145]. Other luminescence-based bacterial sensor strains, *Pseudomonas fluorescens* OS8 (pTPT11) and *Pseudomonas fluorescens* OS8 (pTPT31), have also been used for mercury and arsenite detection, respectively, in soil extracts [143]. Soil samples were extracted with water, ammonium acetate, hydrogen peroxide and nitric acid, and the results obtained were compared with those obtained with traditional methods.

Inorganic phosphate

Inorganic phosphate in surface waters can be used as a measure of eutrophication status of different water bodies, such as surface and sea waters [1]. Various enzymatic biosensors for phosphate determination have appeared in the literature in recent years [146, 147]. Parellada et al. [5] described a configuration based on the sequential action of three enzymes that opens up a way to construct reagentless enzymatic phosphate sensors.

Nitrate

Urban wastewater treatment regulations aim at reducing pollution, including nitrate pollution, from sewage treatment works and industry. The EU imposes limits for nitrate and nitrite in drinking water of 50 and 0.1 mg/L, respectively. A biosensor containing immobilized denitrifying bacteria was applied to the determination of NO_3^- in tap water. Through the reduction of NO_3^- in a reaction chamber, N_2O was formed and determined by a N_2O microelectrode, which was the sensing element of the biosensor [148]. A whole-cell fluorescence biosensor based on recombinant *Escherichia coli* allowed the determination of nitrate without the interference of phosphate, chloride and nitrite [149]. A conductimetric biosensor for nitrate was developed using nitrate reductase immobilized on a thin-film electrode, reaching detection limits of 5 μM [150].

Biosensors as detectors of separation methods

The application of biosensors as detectors in high-performance separation methods of analysis can allow or improve the detection of substances otherwise undetectable by detectors based on physical-chemical properties [151]. At the same time, biosensors often suffer from lack of selectivity in complex matrices in the determination of certain compounds. The combination of physical-chemical and biosensor methods can enable the identification of molecules that are not detectable separately by either method [152]. A couple of reviews present some examples of the combination of chemical separation, such as high-performance liquid chromatography (HPLC), gas chromatography (GC) or capillary electrophoresis (CE), with biosensor detection using living systems, whole cells, membrane receptors, enzymes and immunosensors [151, 152].

Despite the huge number of biosensors developed so far, there is still a lack of biosensing systems for an important group of emerging contaminants such as phtalates and polybrominated compounds (used as flame retardants), veterinary and human medicines and personal care products (pharmaceuticals, synthetic fragrances, sunscreen agents). However, as the world becomes more concerned about the impact that environmental contamination may have on public health and ecosystems, the demand for rapid-detection biosensors will only increase, and new applications of biosensor systems based on new technologies or targeting new emerging contaminants are appearing constantly.

Application of biosensors to real samples

The application of new biodevices to real-world environmental samples is a must in the final steps of development. However, despite the great number of newly developed biosensors, most of the literature references overlook the real-world step and only report applications of the biosensor in either distilled water or buffer solutions. Most of the reviewed systems still have some way to go before application to real samples can be realised, and the study of matrix effects, stability issues and careful comparison with established methods are crucial steps in this respect [1]. In this section, some aspects related to the implementation of biosensors to different environmental samples are presented and discussed and some examples provided.

Surface, drinking and groundwater have been the targets of the majority of biosensors applied in the environment. Some examples are the application of the optical immunosensor RIANA to the determination of different pesticides and endocrine-disrupting compounds in a waterworks, from the river water source to the finished drinking water [106, 153], and in surface and estuarine

water samples [74–76]. Five different bacterial biosensors were employed to identify toxicity in groundwater samples heavily contaminated with a wide range of chlorinated solvents [154].

The feasibility of using bacterial biosensors as cost-effective tools to complement chemical analysis was assessed as part of a site-evaluation program. Other environmental matrices, such as wastewater samples, have also been considered. Tonning et al. have recently described biosensor arrays aimed at wastewater-sample analysis [155]. Untreated, alarm, alert and normal wastewater samples from a Swedish chemi-thermo-mechanical pulp mill were investigated using an array of enzymatically modified screen-printed amperometric biosensors, and further analysis of the data obtained was performed using principal component analysis (PCA). The commercial toxicity biosensor Cellsense (Euroclon, Yorkshire, UK) has been applied to investigate the toxicity of wastewater and sewage sludge [156–158]. A Lux-based biosensor was used to assess the toxicity of paper mill sludge with some metals (Cd and Cu) and pentachloro-phenol (PCP) as the main pollutants found in those samples [159].

Marine contamination is also cause for concern, although the number of biosensors designed specifically for marine applications is still relatively small. However, many of the systems described for other water bodies are potentially applicable to seawater measurements. Some examples of biosensors applied to marine samples are the microbial biosensors for nitrate and nitrite in seawater [148, 160]. Using different immunosensor devices, Irgarol 1051 [161] and isoproturon [75] have been detected in seawater extracts and estuarine water samples, respectively. Finally, a whole-cell biosensor based on the marine algae *Spirulina subsala*, which reacted both to metals and pesticides, has been described [162].

Soils and sediment samples should undergo an extraction step to draw out contaminants into a liquid solution. Aqueous extracts of solid samples provide information on the bioavailabilty of the pollutants, which is often more relevant for assessing the potential damage caused by a substance. This issue is considered in many biosensors used for the determination of bioavailable metals in soil samples [137, 144, 145].

Air samples can be analysed directly with biosensors although the number of devices developed and applied with this objective is limited. Preliminary studies, but with a possible future application in environmental monitoring, were accomplished by Podola et al. [163] using a multiple-strain algal biosensor to analyse volatile organic compounds, such as formaldehyde and methanol, in air in a workplace environment. Benzene in air has been determined by different biosensors, such as those described by Berno et al. [117] and Lanyon et al. [164].

An example of the application of biosensors to real-world samples is to control remediation processes by determining parameters that influence the growth of bacteria, such as nutrient availability, metal ions, pH, dissolved oxygen and temperature. Different molecular biosensors based on the luciferase expression system have been implemented to monitor these parameters; they have been reviewed by Purohit [165].

Practical aspects for real-world sample analysis

Sample matrix can dramatically affect the application of biosensors to real samples. Matrix effect could be defined as an induced deviation from theoretically predicted parameters, caused by constituents or properties of the sample other than the analyte [166]. Since the exact composition of the matrix of environmental samples is usually unknown and can vary widely from sample to sample, the determination and quantitation of analytes constitutes a challenge in the application of the biosensors in real environmental samples. Some strategies have been undertaken to avoid the influence of the matrix on biosensor measurements.

In the case of analysis of soil samples, the application of a previous treatment step to bring the contaminants of interest to a liquid media can not be avoided. Matrix interferences in water samples may often be prevented by means of simple methods such as diluting and buffering of the sample, adjusting of the sample pH and conductivity, or adding a background protein. In the case of immunosensors, addition of detergents or immolation proteins is a current routine to overcome matrix interferences [153, 167]. Ionic strength must also be controlled, and sometimes mimicking the matrix in the calibration curve standards is a solution for samples with a high content of salts, in particular for sea water samples. The varying organic carbon levels in water samples (e.g. river water or seawater) cause problems in common analysis and in particular in immunological methods. Tschmelak et al. proposed and demonstrated the efficacy of a new method to overcome matrix problems in real-world immunosensor monitoring. They developed an easy matrix referencing method using a synthetic organic carbon standard that could be adapted to other applications with a similar test format [168]. In other approaches, an extraction step prior to biosensor determination of water samples was shown to effectively remove humic substances, and to this end solid-phase extraction (SPE) can be considered as a good option [169, 170].

As explained above, extraction and concentration of some pollutants such as metals and pesticides from solid matrices are commonly carried out with organic solvents. The presence of organic solvents in the extract or water sample can also interfere with the response of the biological recognition element, depending on the nature and the amount of organic solvent. Thus, the choice of the organic solvent is a parameter to take into consideration as part of the method development in order to avoid undesirable effects [171], and there are several works that have studied this effect in certain biosensor applications [161, 172, 173]. Other practical aspects (besides those related to matrix effects) that need to be considered prior to application of a biosensor to real samples are, first, the stability of the components of the biosensor, and second, the appropriateness of the dynamic range of the developed

device for the anticipated analyte concentration in real samples [174].

Little or no required preparation of water samples is one of the advantages of biosensors compared with other analytical techniques, and thus, all pretreatment steps should be as simple and rapid as possible without an excessive increase in analysis time.

Validation of biosensors

Beyond the practical advantages of biosensors, they should be comparable to conventional analytical systems in terms of reliability, sensitivity, selectivity, specificity and robustness. Biosensor measurements therefore need to be verified and validated before being accepted. The validation procedures can vary depending on the technology that is evaluated and include participation in inter-laboratory trials, use of certified reference materials (CRMs) or comparison with conventional chemical analysis. The validation of biosensors may involve some difficulties since, in addition to general quality parameters, it may be necessary to evaluate some other aspects such as stability of the biological element or the element immobilized in the transducer.

On the other hand, although there are several validation procedures accepted for quantitative methods, in the case of qualitative methods, which represent the majority in biosensors, there are not established guidelines [175, 176]. Thevenot et al. recommended several analytical parameters to characterise biosensor performance, such as sensitivity, working and linear concentration range, limit of detection (LOD) and of quantification (LOQ), selectivity and reliability, steady-state, transient response times, sample throughput, reproducibility, stability and lifetime [4]. The overall commercial status and general acceptance of the technology will depend on the performance characteristics, sample throughput, associated costs and acceptance by regulatory authorities, based on independent validation data generated using internationally recognised procedures (e.g. AOAC, ISO and IDF) [124].

Commercial biosensors

A large number of biosensors have been developed in research laboratories, and the corresponding research literature in this area is considerable. However few practical systems have enjoyed commercial success. Nowadays, most commercial biosensors are for medical applications [177], whereas in areas such as food, agriculture, military, veterinary and environment control, there is a potential market still to be established. Nevertheless, much of the instrumentation developed for the medical diagnostics market could be adapted for the environmental market [178]. Even though commercial returns from environmental biosensors are substantially less than from medical diagnostics, public concern and government funding have generated a major research effort [179] aimed at the application of biosensors to the measurement of pollutants and other environmental hazards. Whenever a biosensor technology is proposed for a specific problem it may be helpful to roughly estimate the size of the potential market and also consider some practical issues such as the stability of the device. Stability is required for at least 6 months, and storage and shipment at temperatures below 0 °C leads to an increase in the cost of a technology [174, 180].

Surface plasmon resonance (SPR) biosensors constitute the most successful type of commercial instruments for environmental monitoring. BIACORE AB (Uppsala, Sweden) offers a large range of biosensors, which includes several generations of the original BIAcore (series 1000, 2000 and 3000) as well as other configuration systems offering varying degrees of automation and parameter specifications [53]. Other SPR commercial biosensors are the IBIS system (Windsor Scientific, Berks, UK), CELLIA sensor (Nippon Laser and Electronics Labs, Hokkaido, Japan), Spreeta (Texas Instruments, Dallas, TX, USA), the BIOS-1 system (Windsor Scientific), SENSIA (Madrid, Spain) [77] and BioTul AG (Munich, Germany) [181].

Affinity Sensors (Franklin, MA) manufactures the IAsys line of instruments, which use evanescent-wave technology. Also, REMEDIOS, a whole-cell-based biosensor, can be employed for diagnosis of contaminated land or soil. It detects the levels of any toxic substance that affects the metabolic activity of the biosensor organisms. Unisense (Aarhus, Denmark; http://www.unisense.com/) and NECi´s Nitrate Biosensor (Nitrate Elimination, MI, USA) [182] launched microbial and enzymatic biosensors, respectively, for nitrate determination. The system CALUX (BioDetection Systems, Amsterdam, The Netherlands) is used for detection and quantification of dioxins and dioxin-like compounds [183].

In general, there are several biosensor platforms designed to measure molecular interactions, that can be used for environmental applications, such as the VeriScan 3000 System from Protiveris (Rockville, MD, USA), which is as a multiplexed, label-free cantilever sensor, or Biocom from Kaline (San Francisco, CA, USA), with 1,000 microcantilevers on a chip, that can test 100–200 different compounds [184]. The previously described toxicity biosensor Cellsense incorporates *Escherichia coli* bacterial cells for rapid ecotoxicity analysis and has been applied in some monitoring studies [157, 158]. Industries such as Caliper Technologies, Cepheid, Nanogen, ACLARA BioSciences, MICROGEN Systems and Lawrence Livermore Laboratories are developing microfabricated systems for detection and identification of specific microbial agents [46]. Finally, for the determination of BOD, Dr Bruno Lange GmbH (Düsseldorf, Germany) produces ARAS BOD (http://www.drlange.com).

Current trends and future perspectives

Biosensor technology constitutes a rapidly expanding field of research that has been transformed over the past two

decades through new discoveries related to novel material fabrication techniques, novel means of signal transduction and powerful computer software to control devices [185]. Despite the past and current huge amount of research in biosensor development, there is still a challenge to creating improved and more reliable devices. In this section anticipated trends in biosensor research activities are presented.

Continuous monitoring

Contaminant concentrations in water courses are dynamic, changing both as a result of inputs and changes in water flow. With monthly sampling and analysis, it is extremely unlikely that the maximum concentration for a period of time can be detected. The establishment of network systems (multiple autonomous analytical stations that extensively control the sites of interest in rivers, lakes, wells or even water-treatment plants) has generated huge interest. Many biosensors have been developed for use as continuous monitoring systems that can provide easy, rapid and on-site measurements [154, 186]. They may also be useful for mapping of contamination when it is important to obtain rapid results in the field, such as after accidental spills or pollution events [2]. Gu et al. have recently applied a novel early-warning protocol for monitoring the toxicity of the effluents of a water-treatment plant [22]. One of the main achievements of another biosensor system, AWACSS, based on an optical immunosensor, was the establishment of an early-warning system by means of a network of measurement and control stations [187].

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 [188]. 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 [189], and another SPR biosensor that enabled the division of wavelengths on serial sensing channels by means of a specially designed SPR prism element [190]. 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 [191–193].

A multi-analyte enzyme biosensor based on a disposable, thick film multielectrode was developed for the analyte discrimination of binary mixtures of the pesticides paraoxon and carbofuran [194]. Other studies aimed at the development of multi-analyte immunosensors, such as that described by Gonzalez-Martínez et al. [167], who developed a competitive capture assay for carbaryl, atrazine, and irgarol 1051 as target compounds. Mastichiadis et al. [195] have recently applied an optical capillary immunosensor to the simultaneous determination of the pesticides mesotrione, hexaconazole, paraquat and diquat, by preparing an ordered array capillary of four distinct analyte bands. Determination of pharmaceuticals, antibiotics, hormones, endocrine-disrupting chemicals and pesticides has been accomplished by multi-analyte immunosensors by several authors [90, 196, 197]. The capability for simultaneous multi-analyte analysis will grow, probably through more complicated multipath microfluidic systems, by integration with microarrays or by coded microbeads that carry a number of different capture antibodies [198]. Newly developed methods for spatially resolved immobilization of biomolecular recognition elements in transducer surfaces are playing an important role in the development of multi-analyte devices [90, 92, 199] and, recently, quantum dots (as will be explained in the next section), are also being applied for multi-analyte determinations [200].

Nanotechnology

Nanotechnology—technology comprising a group of emerging techniques from physics, chemistry, biology, engineering and microelectronics that are capable of manipulating matter at the nanoscale—is playing an increasingly important role in the development of biosensors. By using nanomaterials for biosensor construction, the sensitivity and performance is improved, since these materials display unique physical and chemical features [201]. Self-assembly of biomaterials, such as proteins, lipids, or nucleic acids [202, 203], has inspired the development of new biosensors, and molecular self-assembly has been proposed for the synthesis of nanostructures capable of performing unique functions. Such nanostructures are applied for the development of amperometric immunosensors like that developed by Tiefenauer et al. based on a nanostructured gold film electrode [204].

Several research groups have begun to explore alternative strategies for the development of optical SPR biosensors based on the extraordinary optical properties of metal nanoparticles [77, 188, 205, 206]. Cao et al. showed how oligonucleotides and presumably other biomolecules (e.g. proteins) can be used to modify the surfaces of such particles, thereby imparting useful biorecognition properties to them [207]. In another example, Lazarides et al. presented gold colloidal-nanoparticle aggregates that were linked by short pieces of DNA [208] and that exhibited a colour change due to electromagnetic coupling between the gold non-spherical nanoparticles after DNA hybridisation. Other nanostuctures that are being considered for development of biosensors include nanotubes, nanofibers, nanorods, nanoparticles and thin films. Boron-doped

silicon nanowires (SiNWs) were reported by Cui et al. to create highly sensitive, real-time, electrically based sensors for biological and chemical species [209].

Microcantilever and nanocantilever biosensors can perform local, high-resolution and label-free molecular recognition measurements and reach very low limits of detection [210], for example, the cantilever-based immunosensor described by Alvarez et al. [211] that detected DDT at concentrations as low as 10 nM.

One of the new materials to emerge in recent years is the quantum dot (often described as a nanocrystal), which is a nanometer-scale semiconductor particle that may contain a charge in the form of electrons. In solution, they display extraordinary optical properties, tunable by regulating their size. There are many possible future applications of these materials as biological labels or probes by conjugating them to various biological compounds, some of which have already been explored and promise to replace traditional means of detection [185]. One approach has been described by Goldman et al., who developed a biosystem for multianalyte determination using antibodies labeled with four-colour quantum dots [200].

Finally, Lundstrom et al. [212] proposed the use of pigment-containing cells (present in the skin of certain fish and frogs) as biosensors. The cell membrane receptors, the G-protein-coupled receptors, control the pigment-particle aggregation, which is simple to measure. This natural nanosystem shows great promise for new biosensing and bionanalytical systems since G-protein-coupled receptors can be provided through several biochemical modifications for almost any kind of analyte.

Miniaturisation

The advances in microelectronics and microfluidics have permitted the miniaturisation of analytical systems, allowing the handling of low volume samples, a reduction in reagent consumption and waste generation, and increasing sample throughput [213]. Taking advantage of miniaturisation benefits, sensors and biosensors can become inexpensive and easy to handle analytical devices for fast and reliable measurements of chemical species. Biosensor miniaturisation has a particular significance for medical applications when an implantable sensor is sometimes desired for continuous in vivo monitoring [214], and for pharmaceutical industries in the field of high-throughput screening [215].

For environmental monitoring the size of a sensor is usually not so important, the need for miniaturisation is not so evident. However, the use of miniaturised technology reduces the amount of chemicals used and also the energy needed to manipulate fluid volumes, having an impact on the environment and the economy [214]. It has a positive effect also on analytical performance by decreasing analysis time, increasing reliability and sensitivity through automation (which is easier with smaller devices) and integrating several processes in a single device [198]. In fact, the time and the expense devoted to the detection of environmental pollutants are becoming impediments in treating a number of samples for many environmental projects. In this sense, microfabricated sensors or systems can become a powerful tool [214]. On the other hand, small size would be preferred in the design of portable biosensors, especially for on-field screening applications. Another advantage of miniaturisation at the nanometre-scale level is that it will allow high-density information storage.

Miniaturisation involves adaptation of microfabrication and nanofabrication techniques. In addition, the practical application of any sensor requires the use of complete analysis systems for sample handling such as pumps, filters, membranes and sample conditioning [215]. Such formats may be used in bioanalytical microsystems that comprise another new trend in bioanalysis. In such microfluidic systems, miniaturised biosensor arrays, as well as miniaturised sampling, filtering, and so on, have to be accomplished [215].

New sensing elements

Improvement in the affinity, specificity and mass production of the molecular recognition components may ultimately dictate the success or failure of detection technologies [5]. Therefore a crucial aspect in future biosensor development seems to be the production of new sensing elements that are easy to synthesize and have the capability of broadening the spectra of selectivities that can be reached by a biosensor. At present, the preparation and production on a large scale of biomolecules, such as enzymes or antibodies, requires an important commitment of time and knowledge. Synthetic peptides and molecular-imprinted polymers (MIPs) are contemplated as promising alternatives overcoming the above-mentioned limitations, and some works have already been published reporting the application of these sensing elements [216, 217]. Unfortunately the affinity shown by these synthetic receptors is still several orders of magnitude below that of the antibodies.

The possibility of tailor-binding molecules with predefined properties, such as selectivity, affinity and stability, is one of the major aims for biotechnology. Since scientific attention is currently being given to biotechnology, as this review has pointed out, the development of improved molecular recognition elements will be followed by a corresponding enhancement of the biosensor features. The current generation of biosensors is taking advantage of novel biosensing materials based on gene engineering. Gene engineering focuses on two main fields in the area of biosensors: genetically transformed cells and genetically engineered receptor molecules. A recently developed method that generates antibody-like molecules is “phage display”. Phage display affords a unique way of selecting peptides and proteins with binding affinity similar if not higher than monoclonal antibodies [46]. These techniques make possible the production of high amounts of affinity peptides or antibodies without the immunization and sacrifice of laboratory animals. Different authors are now investigating the potential to use phage-displayed peptides

as reagents in sensor applications. Benhar et al. [51], for example, presented single-chain phage-displayed antibodies combined with amperometric detection for application as an immunosensor for the detection of three different analytes (a sugar, a bacteria and an enzyme). Goldman et al. [52] demonstrated the potential of a phage-based biosensor for the determination of staphylococcal enterotoxin B (SEB). Recombinant antibodies derived from antibody libraries with s-triazine selectivities were applied and modified by Hock et al. [29] for the development of suitable antibodies likely to be used in immunoassays or immunosensors.

Modified cell biosensors are being constructed by fusing a reporter gene to a promoter element that is induced by the presence of a target compound [218]. In the case of bioluminescence sensors, the recombinant bacteria employed contain bacterial luminescence genes and gene fusions between the regulatory regions of the mer operon *(merR)* that respond to contaminants. Many biosensors based on this principle have been reviewed in this article [18, 27, 117, 145, 219].

While in days to come antibodies are likely to remain the recognition molecules, a number of alternatives are being investigated. One type of recognition molecule that has received significant attention in recent years is peptide-nucleic acids (PNAs) [220]. PNAs have been used as a platform for attachment of an analyte derivative or capture antibody [221]. Specific nucleic acids, aptamers, have been shown to bind small molecules with high affinity and can thus be considered as a valid alternative to antibodies or other bio-mimetic receptors for the development of biosensors [222].

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