Bioelectrochemical Methods For Environmental Monitoring

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Abstract

In this article we shall outline several biosensor applications which may fill existing technology gaps in the area of environmental monitoring. Biosensors show the potential to complement laboratory based analytical methods for environmental applications. The requirements for these environmental biosensors, as well as difficulties in commercialization, are also addressed.

Keywords: Biosensor, environmental monitoring, field bioanalytical methods.

Introduction

Entering the 3rd millennium, many sectors in our society such as medical diagnosis [1], environmental pollution control [2], food and beverage industries [3], military defense forces [4], sport doping testing [5], space application [6], etc., are highly demanding for easy to use measuring devices allowing, rapid and reliable determinations on site or remote controlled.

Environmental monitoring of the air, water, and terrestrial zones for compounds that may pose a risk to human or ecosystems health is a critical part of the efforts of scientists directed towards pollution prevention, industrial and agricultural waste regulation, and cleanup of hazardous waste sites. The time and expense involved with the detection of environmental pollutants (i.e., sample acquisition, sample preparation, and laboratory analysis) have placed limitations on the number of samples that can be analyzed for any particular project. Limited resources dictate the need for fast and cost-effective field-analytical technologies that can increase the number of analyses and decrease the time required performing them. Increasing the amount of analytical data tends to improve environmental management decisions by increasing the accuracy of hazardous waste site characterization and the certainty of risk assessments and by improving the efficiency of the cleanup procedure [7].

The high cost and slow turnaround times typically associated with the measurement of regulated pollutants clearly indicates a need for environmental screening and monitoring methods which are fast, portable, and cost-effective. In recognition of this need, a variety of field analytical methods have been introduced, a number of which are commercially available or under development. Because of their unique characteristics, however, technologies such as biosensors show promise in complementing laboratory-based methods and in providing field-analytical methods for specific applications and might be exploited to fill specific niche applications in the environmental monitoring area.

Increasing environmental legislation, both in USA and EU, which controls the release and the levels of certain chemicals in the environment, has created a need for reliable monitoring of these substances in air, soil and especially water. Conventional analytical techniques, although highly precise, suffer from the disadvantages of high cost, the need for trained personnel and the fact that they are mostly laboratories bound. Biosensors because of their specificity, fast response times, low cost, portability, ease of use and a continuous real time signal, can present distinct advantages in certain cases and can be expected to be the best available technology not entailing excessive cost. Their biological base makes them ideal for toxicological measurements, which are suited for health and safety applications. Over the last 3-4 years there has been an increase in the number of publications concerning biosensors for environmental monitoring, especially in the field of pesticides, phenols and heavy metals measurements [8,9].

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

This paper will focus primarily on challenges and possible opportunities for the development of biosensors (especially electrochemical ones) for environmental monitoring applications.

Environmental Monitoring

The scope of the environmental monitoring task is enormous. For field analytical methods (such as biosensors) to have a significant impact on this monitoring task, they must address the following challenges associated with environmental applications:

-compound diversity;
-matrix diversity and complexity;
-variety of data quality requirements; and
-diversity in possible monitoring applications.

2.1. Compound diversity

The number and wide variety of chemicals that contaminate the environment are staggering. For example, over 700 chemical species have been identified at hazardous waste sites, and the unidentified compounds may number in the thousands [7]. Furthermore, in addition to the over 600 compounds regulated under the Toxics Release Inventory (TRI), numerous agricultural and industrial compounds are regulated under waste disposal and treatment regulations. Not all of these chemical species, however, pose similar risks to human health and ecosystems. The Agency for Toxic Substances and Disease Registry (ATSDR) has ranked 275 priority hazardous substances based on the frequency of occurrence at sites present on the National Priorities List, available toxicity data, and the potential for human exposure [7]. The ATSDR has further grouped these compounds by chemical class with respect to overall public concern based on public health assessments, public health advisories, and frequency of occurrence.

Although not appropriate for all hazardous compounds, biosensors have been reported to detect compounds in several classes of concern including (**Table 1**): phenols/phenoxy acids (e.g., phenol and catechol [10]), polyaromatic compounds (e.g., benzo [a]pyrene [11]), halogenated pesticides (e.g., triazines [12]; 2,4-D [13]), volatile organic compounds VOCs (e.g., benzene [14]), and inorganic substances (e.g., mercury [15]).

Table 1. Chemical classes of priority hazardous substances*

Compound calss	% **
Volatile organic compounds (VOCs)	26.5
Inorganic elements/radionuclides	17.5
Phenols/phenoxy acids	10.5
Polycyclic aromatic hydrocabons (PAHs)	8.5
Halogenated pesticides/related compounds	8.5
Nitrosoamines/ethers/alcohols	7.5
Reactive intermediates	6.0
Miscellaneous	6.0
Benzidines/aromatic amines	4.0
Phthalates	3.0
Organophosphates/carbamates	2.0

* Compiled and published by the Agency of Toxic Substance and Disease Registry (PHS, Annual Report, 1990).

** Contribution to the US hazardous waste problem from a human-exposure perspective.

2.2. Matrix diversity and complexity

The diversity of contaminated matrices also contributes to the overall environmental monitoring challenge. Typical examples of environmental matrices include: air, drinking water, ground water, soil (of many types and characteristics), and sludge. The complex nature of environmental matrices and the heterogeneous distributions and the broad concentration range of target pollutants and other contaminants within such matrices pose difficult sampling and sample preparation problems (especially for field analytical methods).

Chemical analysis of many environmental samples containing hazardous waste is complicated by co-contamination with a wide variety of elements and compounds. For example, sites contaminated with mixtures of heavy metals, chlorinated hydrocarbons, and reactive organics may pose a particular challenge to field analytical methods if elements of their biological recognition systems (e.g., enzymes, antibodies, or microbes) are inactivated or "poisoned" by co-contaminants.

2.3. Data quality requirements

Yet another challenge facing environmental regulators, contractors, and method developers is the recognition of and compliance with measurement data quality requirements. The data quality objectives (DQOs) for sampling and analysis should derive theoretically from the environmental management decisions to be made on the basis of the expected data; they impact the choice of a particular analytical method (based upon performance characteristics). In practice, however, DQOs for many projects are prescribed under environmental regulations or dictated by the performance characteristics of currently used laboratory-based methods.

Nevertheless, with the introduction of cost-effective field analytical methods, whose quality characteristics are well defined and the movement toward regulatory acceptance based on methods performance, alternative analytical methods may be used more frequently where they can improve our confidence in environmental management decisions.

2.4. Field screening and monitoring

The decision to use a screening or monitoring method in the field must consider a number of issues including: the practical, technical, and data quality characteristics of a particular field analytical method; the specific monitoring tasks for which that method could reduce measurement uncertainty at a similar or reduced cost compared to standard laboratory analysis; and how that method could be incorporated into the overall monitoring plan through the project lifetime. For example, initial characterization of a hazardous waste site may

require a suite of diagnostic methods, whereas remediation (e.g., removal of contaminated soil) may depend on the presence or absence of a threshold concentration level of a particular compound or compound class (e.g., PCBs at 25 mg/kg).

Required characteristics for environmental field screening methods have been previously reported, and we reiterate the following criteria [16]. Field screening tests should accurately differentiate samples with concentrations of a specific analyte or defined analyte class above or below a given level of concern, have a known and stable sensitivity, and be relatively free from (or be well characterized with respect to) positive and negative interferences. We further suggest that field monitoring assays (as compared to field screening assays which are typically designed for operator-assisted discrete sampling), be capable of continuous and in situ operation under defined conditions for a given period of time. In response to the growing need for environmental field analytical methods, a variety of instruments, devices, and kits have been reported in the literature, tested in the field, and to a lesser extent, developed for the commercial market. These methods range from 'mature' to 'emerging' in their developmental status (**Figure 1**). The value of a number of these methods has been already demonstrated in a variety of field applications. With the exception of chemical sensors and biosensors, however, these methods are designed primarily for user-assisted discrete sampling. Biosensors, although still in the emerging technology stage, show potential advantages for both field screening and field monitoring applications and may be particularly well suited for continuous and in situ monitoring scenarios.

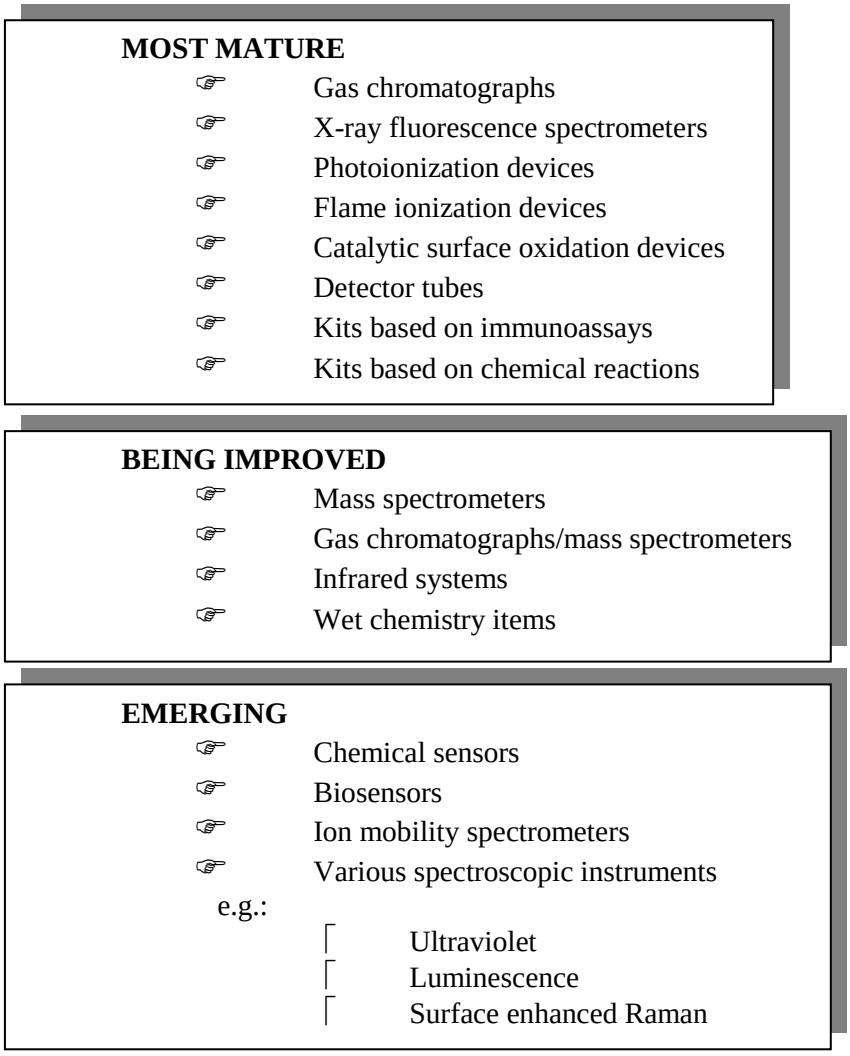
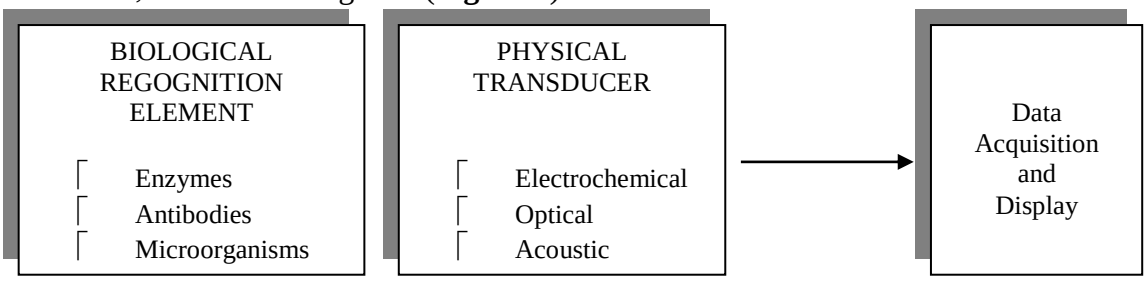


Figure 1. Field screening and monitoring technologies [1].

Analytical tasks for which screening technologies are best suited include the spatial characterization of specific environmental contaminants (e.g., identification of hot spots in contaminated soils or delineation of contaminant plumes). Tasks for which field-monitoring technologies are best suited include the temporal characterization of specific environmental contaminants that may change on a short time scale (e.g., minutes) or in situ monitoring where sample removal is inconvenient, difficult, or dangerous. Specific examples might include: in situ monitoring of a process control stream to determine the efficiency of hazardous waste site remediation; monitoring agricultural run-off during peak application periods; or continuous monitoring of wells to determine whether concentrations of an analyte of interest (during remediation procedures or after site closure) are in compliance.

Biosensor Definition

Due to recent advances in fiber optics, microelectronics, and biotechnology, the definition of a biosensor has evolved from the classical concept of an enzyme-electrode to include a variety of analytical methods and devices based on biocatalysis or bioaffinity [8]. While not officially defined yet, for the purpose of this article, a biosensor will be defined as: analytical devices incorporating a biological material (e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids etc.), a biologically derived material or biomimic intimately associated with or integrated within a physicochemical transducer or transducing micro-systems, which may be optical, electrochemical, thermometric, piezoelectric, acoustic or magnetic (**Figure 2**).



Typical Mechanisms for Potential Environmental Applications

Enzymes:

- Catalytic transformation of the pollutant (e.g., analyte, phenolics; enzyme, tyrosinase);
- Specific inhibition of enzyme activity by the pollutant (e.g., analyte, organophosphates; enzyme, acetylcholinesterase);
- Modification of enzyme activity by a pollutant which acts as a modulator or cofactor (e.g., analyte, Mn (II); enzyme, horse radish peroxidase).

Antibodies:

- Compound or class specific affinity toward the pollutant (e.g., analyte; pesticides, benzo(a)pyrenee).

Microorganisms:

- General inhibition of cellular respiration by pollutant (e.g., analyte, respiratory toxicants; test organism, P phosphoreum; response, luminescence);
- Promotor recognition by specific pollutant followed by gene expression, enzyme synthesis, and catalytic activity (e.g., analyte, Hg (II); expressed enzyme, luciferase; response, luminescence).

Figure 2. Biosensor components and typical mechanisms for environmental applications.

Furthermore, nearly all combinations of these biological and physical components have been reported. Biosensors usually yield a digital electronic signal, which is proportional to the concentration of a specific analyte or group of analytes. While the signal may in principle be continuous, devices can be configured to yield single measurements to meet specific market requirements. Biosensors have been applied to a wide variety of analytical problems including medicine, the environment, food, process industries, security and defense.

Biosensors for use in environmental technology should be automated, on-line, multi-channel, monitor a range of analytes, have detection limits to meet EU Directives (0.1-1.0 ppb), be applicable to target industries (e.g. water, leather, paper, textiles), be robust and be of a convenient size. To meet these needs the complete system must be designed and engineered to meet a specific need including any necessary separation of the analyte from the matrix, appropriate fluidics to move the sample through the devices, an effective measurement process, automatic calibration, maintenance and data processing. There are limited further opportunities for single analyte sensors. The market demands adaptable menus of analytes. This requires advances in the fabrication of arrays, miniaturization, micro-fluidics, regeneration, stability of the sensors, adaptability to meet the demand for new and varied analytes and chemometrics.

The key feature of a biosensor is that the biosensing component is assembled, on the sensor tip, in a manner such as to allow a selective measurement of the analyte of concern with a minimum of sample pretreatment. In its broadest sense, a biosensor may be reusable (reversible response) or disposable (single use miniaturized probe).

An analysis of the conclusions of working groups in biosensors for environmental monitoring field highlights the following target analytes within the EC: oestrogenic substances, blue-green algal toxins, fluorescent whitening agents, sulphonated dyes, pesticides and priority metabolites, pharmaceuticals, pathogens, disinfection by-products, surfactants, phenols, toxicity, carcinogenicity, genotoxicity, immunotoxicity, toxic gases, volatile organic carbons, process-related parameters and organic profile. Various strategies are available to develop suitable receptors for this range of analytes. Natural systems may be selected or screened from those for which interactions like specifically binding, catalyzed or inhibited by the analyte (e.g. antibodies, enzymes or microorganisms) could be pointed out. Alternatively, natural systems can be chosen which exhibit general and preferably relevant effects in the presence of one or more analytes (e.g. photosynthetic bacteria or double-stranded DNA) providing a generic system monitoring effects [9].

The biological receptor can be further engineered to improve its performance with respect to the recognition of one or more analytes (e.g. protein engineering to improve the specificity, sensitivity or stability of an enzyme or genetic engineering to improve the performance of an enzyme, micro-organism, or antibody fragment) or to include intrinsic transduction (such as the attachment of a reporter group to an enzyme or antibody, or the inclusion of the lux gene in a specific micro-organism). Most recently, the possibility of using synthetic biomimetic systems has attracted considerable attention. Techniques using synzymes, molecularly imprinted polymers and synthetic receptors generated using combinatorial chemistry, have shown that the advantages of biological receptors can be mimicked in more stable and less expensive analogues. The use of chemometrics in combination with either natural, semi-synthetic or synthetic receptors yields a pattern-based approach to the recognition of analyte profiles that would appear to be well suited to the needs of environmental technology.

In summary it may be concluded that in order to enhance the performance of biosensors for environmental technology advances are required in: the identification or creation of stable receptors for key analytes or effects; the design of sensing systems to yield

required analytical performance in real matrices; the fabrication and operation of multi-channel arrays; and the engineering of integrated systems for specific applications.

Yet electrochemical biosensors have been most investigated and a several companies provide amperometric biosensors for a variety of components to be measured (**Table 2**). The latter devices may comprise a disposable sensing part, based on miniaturized electrodes supporting the immobilized biocomponent, which is inserted into a pocket size instrument (**Figure 3**) or it may comprise a biosensor operating reversibly and which is implemented in a larger (automated) experimental set up for discrete or continuous monitoring (**Table 2**).

Table 2. *Example of companies commercializing electrochemical biosensors*

Company	**Compounds detected**
Yellow Springs Instruments-YSA (USA)	Glucose,ethanol,lactose,fructose,lactate….
Baxter Travenol (USA)	Glucose
Universal Sensors (USA)	Glucose,aspartame,urea,uric acid
Molecular Devices (USA)	DNA,pollutants,toxics…
AndCare Inc,ESA (USA)	E.Coli, Salmonella, Poliovirus, DNA hybridization
Bioanalytical Systems, BAS (USA)	Hydrogen peroxide
VEB-MLW Prufgerate-Medigen (D)	Glucose, lactate, BOD
Institute of Molecular biology-Berlin (D)	Glucose,lactate,uric acid…
Setric SGI (F)	Lactate, Glucose
Seres (F)	Glucose,choline,lysine
Radiometer (F)	Glucose
Metertech Inc. (Taiwan)	Glucose
Nissan Electric (JPN)	BOD
Daiichi-Kyoto (JPN)	Glucose
Pegausus Biotechnology (Canada)	Fish freschness

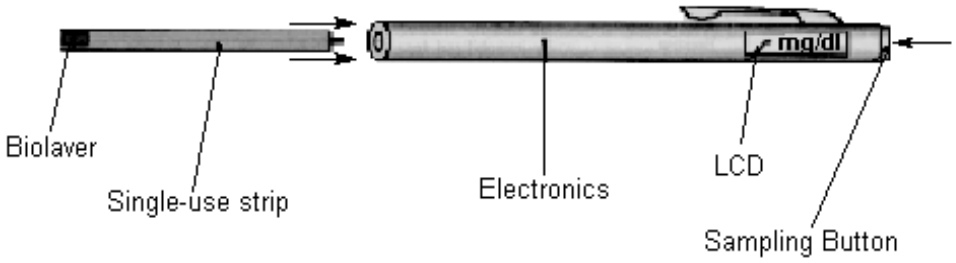


Figure 3. A pen-size electrochemical biosensor with single use enzyme immobilized electrode strip

Amperometric Biosensors

Amperometric biosensors provide an electrical signal, at a controlled potential, directly related to the concentration of the analyte of interest. These sensors have been investigated the most because of the inherent high sensitivity, good selectivity and mass production facilities [5].

Since the first description in the early sixties of enzyme (glucose oxidase) based electrodes and the commercialization in 1974 of an instrumentation comprising an electrochemical biosensor for glucose determination, much water has passed under the bridge. Interest in biosensors development and commercialization went on relatively smoothly in

these early days, till the commercial launching in the nineties of a new biosensor concept, single use enzyme electrode strip, for glucose assay in a droplet of blood, which considerably stimulated research in this area. The biosensors market in the healthcare is dominated by amperometric biosensors for glucose monitoring e.g. in the 1995-96 period, the ExacTech pen sized glucose analyser of Medisense (marketed by Baxter-USA) was ranked top one in the field with sales of about $170x10^6$ $. Considering that more than 5 % of the world population suffers from diabetes, it is understandable that constructors have primarily focused their efforts to glucose sensing devices. It is amazing (or frightening!) to imagine what would have been the biosensor research and development world (would it ever had showed up?) in the absence of the remarkably active, selective and stable enzyme glucose oxidase (GOx) [17].

Actually the concept of portable, automated, hand sized and reliable instruments for testing is adding substantial value for the customer in comparison to "heavy" modern laboratory instrumentation. It opens the way of testing at sites or in a satellite laboratory, decreasing the laboratory turnaround time i.e., sample transport laboratory test- transfer data-action.

Since the concept of the first enzyme based electrode much progress has been realized in the detection principle. Actually, electrochemical enzyme based biosensors may be classified into 3 generations (**Figure 4**).

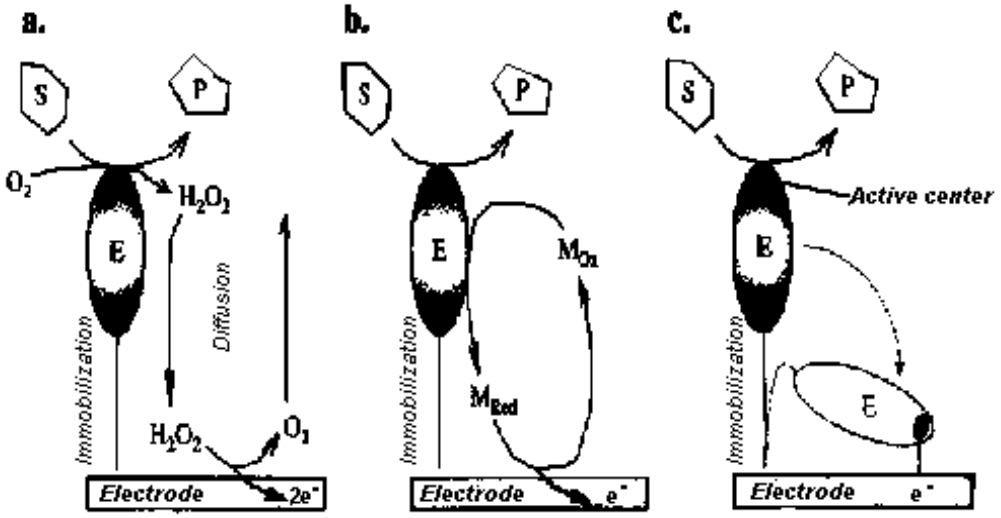


Figure 4. Schematic view of the three generations of electrochemical enzyme (oxidase) based biosensors.

The first generation of EC biosensors operates by enzymatic action on the analyte to be investigated (**Figure 4a**). At the working electrode, some product formation (e. g. hydrogen peroxide on platinum, *o*-quinone on glassy carbon, H^+ on glass pH electrode or ISFET, ammonia on gas electrode etc.) or reactant consumption (Clark type oxygen electrode) is monitored either amperometrically or potentiometrically. This category, especially the amperometric biosensors, are still employed (e. g. by YSI) and are even seeing renewed interest thanks to micromachining and use of improved perm-selective membranes, thanks also to microfluidic and sample volume miniaturization. The physical transducer is generally platinum (Pt), since it is well suited for the quantification of hydrogen peroxide formation or molecular oxygen consumption. Miniaturized GOx immobilized Pt electrodes can be implanted subcutaneously for in vivo glucose monitoring during periods as long as one week. High selectivity in detection is achieved by the additional casting of one or more perm-selective thin membrane(s) onto the electrode surface (cellulose acetate, Nafion etc.).

Monitoring of oxygen consumption can be exploited e.g., in bacteria (Trichosporon cutaneum) immobilized electrodes by following the change in respiratory action of the bacteria caused by the consuming of organic matter (e.g. BOD-2000 of Nissin Electric Co. Ltd., Tokyo JPN) or e.g., in fish freshness determination by monitoring trimethylamine at a Penicillium decumbens immobilized oxygen electrode. Also belonging to the first generation are biosensors, which detect the enzymatic product, *o*-quinone, generated from the enzyme tyrosinase. This enzyme is quite stable and it possesses polyphenol oxidase and catechol oxidase activities and is suitable for the development of biosensors for phenol and catechol derivatives of physiological interest, or for water pollution control. Purified enzyme or vegetable tissues may be immobilized as well onto the electrode [18,19].

The second generation consists mainly on amperometric biosensors which comprise an artificial redox mediator for enzyme cofactor (FAD) or coenzyme (NAD^+) regeneration and electrochemical signal transduction (**Figure 4 b**). The redox mediator is generally a small molecule (e.g. iron complex such as in the ferrocene derivatives) which shuttle the electron from the cofactor to the electrode surface and which exhibit rapid electron transfer at the electrode. Advantages are oxygen partial pressure independency (for oxidases), minimization of transducer fouling (NAD^+ dependent enzymes) and the use of cheap carbon based electrode miniaturized strips with immobilized enzyme and mediator. Ideally, the mediator should be immobilized for reversible response of the biosensor. This is not observed at the reagentless carbon strip with adsorbed ferrocene/glucose oxidase (ExacTech glucose pen) the reason why the constructor solved this problem by designing a single use probe. Current investigations are focused on attaching the mediator directly on the enzyme proteinic shell or to molecular redox wires (e.g.osmium-bipyridine-polyvinylpyrrole polymer) [20].

In the third generation of EC biosensors (**Figure 4 c**), constructors are attempting to reach direct electron transfer between the enzyme active center and the electrode surface. Most enzymes have their redox center embedded in the proteinic shell allowing no close contact with the electrode surface i.e., no electron transfer. Yet some proteic structures such as cytochrome c and peroxydases (hemoprotein) have their active center at the outer sphere and, provided that a proper orientation of the biocomponent is achieved, direct electron transfer will be achieved. The modification of the electrode surface by a monolayer of selected molecules offers a suitable anchoring surface for subsequent optimal biocomponent (enzyme, antibody etc.) attachment and orientation close to the electrode surface. This domain is under intensive studies especially using gold substrates modified by self-assembled monolayers of thiol species [21,22].

As mentioned, miniaturized electrodes may serve as well for antigen and antibody immobilization. Direct immunobiosensors follow in real time the binding event (potential or conductivity change) while indirect immunosensors measure voltammetrically the result of a binding event i.e., increased or decreased amount of the bound label/enzyme, electroactive indicator). Contrary to classical Enzyme Linked Immuno Assays, here the immobilization of the biocomponent on the conducting support can be better controlled, high sensitivity is achieved yet non-specific interactions must be minimized [23,24].

The recent development of DNA hybridization biosensors holds great promise for obtaining sequence-specific informations (DNA recognition, DNA damage detection etc.). Electrochemical DNA or peptic nucleic acid biosensors rely on the strong adsorption of a single-stranded DNA sequence onto solid carbon electrodes for subsequent hybridizing with its complementary strand. This DNA biosensor may give rise to voltammetric (guanine oxidation signal), potentiometric or impedimetric or chemiluminescent signals at the ng/ml sensitivity range [25].

Environmental Biosensors

Biosensors typically consist of a biological sensing element (enzyme, receptor, antibody or microbe) in intimate contact with a chemical or physical transducer (electrochemical, optical, mass or thermal). Because of their selectivity and high binding affinities toward certain environmental pollutants, a variety of biological macromolecules may prove to be useful candidates as sensing elements for environmental biosensors [26-32].

Examples of enzymes, which have been used in biosensors, include acetylcholinesterase, which binds with high affinity to organophosphate insecticides, and Japanese pine-comb fish luciferase, which has been used to measure Hg^{2+}.

Receptors that may prove useful as sensing elements include the γ-aminobutyric acid receptor (GABA) which has been shown to bind cyclodienes, pyrethroids, bicyclophosphates and orthocarboxylates; the muscarinic receptor, which binds organophosphate insecticides; and the aryl hydrocarbon receptor, which binds with high affinity to dioxins.

Other biological macromolecules, which have been shown to be versatile and powerful as biological sensing elements, are antibodies. Analytes, which have been measured using antibodies, include dozens of insecticides and herbicides as well as environmental pollutants such as polychlorinated biphenyls (PCBs), dioxins, pentachlorophenol, benzo(a)pyrene and benzene, toluene and xylene (BTX).

Microbes have also been used as biological sensing elements. Cyanobacteria have been used to measure herbicides, Trichosporon cells have been used to measure biochemical oxygen demand and a genetically altered *Pseudomonas* has been used to measure naphthalene.

The sensitivity and specificity of a biosensor is primarily conferred by its biological sensing element. Although enzymes, receptors and some antibodies show high binding affinities to their respective analytes, they typically bind to a group of closely related molecules. This feature lends itself to application such as screening for the presence of closely related environmental pollutants (for example, organophosphate insecticides, triazine herbicides and BTX). On the other hand, as some antibodies show little cross-reactivity even among closely related analytes, biosensors using these molecules, as sensing elements may be highly specific.

For environmental monitoring, there are several general areas in which biosensors may have distinct advantages over current analytical methods. Miniaturization of biosensors has, in the clinical field, led to the development of economical (i.e. disposable or reusable) sensor modules, which can be detached, from the display module. For example, a blood glucose analysis can typically be made for under 1$. This is an attractive feature for environmental monitoring, considering that the average cost of a laboratory analysis for an environmental sample can range from $ 130 to $200. The development of portable biosensors could also eliminate the time, expense and chain-of-custody considerations required to transport samples to a laboratory. Other possible advantages of biosensor-based assays include the measurement of analytes in complex matrices with a minimum of sample preparation, operation of the biosensor by untrained personnel, completion of the analysis in less than 1 hour and continuous or remote monitoring capabilities.

Specific Areas of Application

There is a critical and growing need for more cost-effective techniques for identification and quantitation of pollutants in complex environmental matrices. For biosensors to become competitive, their characteristics must be exploited to fill existing technology gaps left by classical laboratory analysis and emerging field screening methods such as portable gas chromatography, mass spectrometry, chemical sensors, ion mobility

spectrometry and immunoassay test kits. This is not a trivial problem considering that out of hundreds of existing designs, relatively few biosensors have been developed into commercial products. As chromatographic, immunoassay and spectroscopic-based field screening assays improve, biosensors must meet or exceed the quality of data produced by these methods for the specific analyte of interest. Issues, which must be addressed in the development of biosensors, include detection limits, dynamic range, positive and negative controls, interferences and matrix effects. Some general requirements for biosensors, which might be, used in field assay applications are listed in (**Table 3).**

Table 3. *General requirements for biosensors in environmental field applications*

Requirement	Specification range
Cost	$ 1-15 per analysis
Portable	Can be carried by one person; No external power requirements
Fieldable	Easily transported in a van or truck; Limited external power required
Assay time	1-60 minutes
Personnel training	Can be operated by minimally trained personel after 1-2 hours training period
Matrix	Minimal preparation for ground water, soil extract, blood, urine or saliva
Sensitivity	Parts per million/ parts per billion
Dinamic range	At least two orders of magnitude
Specificity	*Enzyme/receptors*: -specific to one more groups of related compound *Antibodies*: -specific to one compound or one group of closely related compounds

Specific requirements may vary depending on the application

As problematic toxic waste repositories are identified and remediation procedures are implemented, "real-time" monitoring assays become crucial for directing appropriate clean-up strategies. Existing and emerging chromatographic and spectroscopic field screening technologies appear well suited to the detection of certain pollutants such as volatile organic compounds and heavy metals.

Furthermore, immunoassays have been demonstrated for a wide range of insecticides (e.g. chlorinated hydrocarbons and organophosphates), herbicides (e.g. thiocarbamates, phenoxyaliphatic acids, bipyridiliums and triazines), fungicides (e.g. benzimidazoles, acylalanines and triazoles) and rodenticides (e.g. coumarins). Biosensors can exploit the sensitivity and specificity afforded by antibodies used in these assays and take advantage of technological design improvements in areas of speed, simplicity and user friendliness.

Another area in which biosensors may find a niche involves continuous and/or remote monitoring of industrial or agricultural groundwater or runoff. Chemical sensor technologies have made recent progress in this area. These devices use mass, optical, electrochemical or thermal sensors coupled to chemically selective coatings. Because of the similarities in signal transducers, chemical sensors and biosensors share many of the same characteristics (e.g. size, speed and cost of analysis). Continuous and/or remote monitoring applications, however, have specific requirements in that unattended operation over extended periods is needed. Chemical

sensors have the advantages of ruggedness and durability for this application; however, biosensors have the advantages of sensitivity and selectivity.

Challenges for biosensors in this area include the stabilization of the biologically active membranes and coatings and the development of methods for continuous delivery of the biological macromolecules into the system.

Human exposure assessment is another area in which biosensors could make a significant contribution. Regulations governing the release of chemicals into the environment are typically influenced by the perceived risk to public health and local ecosystems. Determination of human exposure requires measurements of the compound of interest at locations, within the time frames and in the media (e.g., air, soil or water) most likely to result in exposure. This determination is complicated by the fact that an individual typically engages in a variety of activities and moves through a number of microenvironments in which exposure may occur.

A useful tool in the determination of exposure to airborne contaminants is a sampling device worn by an individual (e.g. a dosimeter). These dosimeters are typically configured as a sample concentration medium from which the compounds of interest are extracted and analyzed by a classical method. Current efforts in this area include work on configurations in which the analyte is trapped by an immunospecific medium and analyzed by immunoassay techniques. A cumulative exposure over a given time period can then be calculated. A gas permeable membrane interfaced to an immunosensor might also allow instantaneous measurements, as well as time integrated average exposures. This information would prove invaluable for integrating exposure patterns into risk assessment models.

Biosensors may also contribute to the area of risk assessment that attempts to correlate exposure to effective dose, adverse biological effect and ultimately to clinical disease. Because of the diversity of possible routes of entry, however (e.g. skin, lung and gut), exposure is not easily correlated even to effective dose.
Extrapolations from animal systems must be used and these approximations are often confounded by differences in pharmacokinetics between species.

The measurement of a pollutant or its metabolites in easily sampled biological fluids such as blood, urine or saliva has provided a useful tool for determining dose in humans.

Measurement of statistically significant populations using classical analytical methods, however, is expensive and time-consuming. Biosensors may again provide a solution for fast and economical field assays for estimation of human exposure to hazardous pollutants.

Conclusions

The area of environmental chemical analysis in the USA and worldwide is expanding; market projections suggest that "the environmental market is one of the few growth opportunities". Because field-analytical methods have been shown to reduce the time required for and the cost of environmental monitoring applications such as cleanup projects [33]**,** these methods will most likely make a significant contribution to this market. While the environmental market (for biosensors) is largely dwarfed by the market for clinical applications of biosensors, there are market opportunities for a competitive product, and relatively few 'players'.

Although clear opportunities exist for the development of field-analytical methods for environmental applications, from a regulatory perspective, new methods most likely to find widespread acceptance and use will target high risk compounds covered under current or proposed regulations and which are difficult to measure using currently accepted methods. From a practical perspective, these methods must be either extremely versatile both in the

range of compounds and matrices for which they can be adapted or occupy a niche for which other potential field analytical methods cannot be easily developed. Although combining physical components of a sensor platform with highly specific biological recognition elements may allow biosensors to respond to both regulatory and practical requirements, commercial development of biosensors for environmental applications is not a trivial matter.

As evidenced by the number of recently reported biosensors for environmental applications and the variety and innovation demonstrated for detection of significant pollutants, it appears probable that biosensors will be among the field analytical methods that provide the future tools for monitoring the environment. Nevertheless, there is a number of significant obstacles that must be overcome before these devices achieve their potential in the environmental arena.

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