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Environmental Sensor Selection : classification and its applications

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Abstract This review focuses on the developed and the being developed environmental sensors in particular biological sensors. As well as discussing the classification and some main principles, presenting current trend of the environmental sensors is given. Two main categories are immunosensors and catalytic sensors. In addition to those, DNA or RNA sensors or protein based sensors are discussed. Some crucial examples of the applications of such sensors are given to show how the sensor technology is used for environmental and biological monitoring, biomarkers of exposure

Key Words : environmental biosensors, sensor technology, classification, application

Introduction

The amount of chemicals released into the environment has grown enormously over recent decades. These chemicals may undergo biochemical transformations, leading to new compounds of unknown toxicity. In addition, some may be introduced into the trophic chain and bioaccumulate in organisms. These facts have created serious concerns regarding their adverse effects on the ecosystem and public health. Recently, government agencies, private companies and research institutions use many resources to provide fast and reliable environmental monitoring methods.

The development of monitoring techniques focussed mainly at three levels: (i) fast, highly sensitive and specific screening tools that may easily be adapted to use on-site (bioindicators or immunoassays); (ii) sophisticated and reliable analytical techniques that can identify and/or quantify with a high accuracy a broad variety of pollutants at the trace level (GC-MS, HPLC-MS, etc); (iii) combinatorial devices that combine the sensitivity, flexibility and reliability of the above mentioned techniques.

Let us have a definition of a biosensor: a miniaturized device integrating a biological sensing element (antibody,enzyme,cell,receptor,etc) on contact with an appropriate transducer (optic, electrochemical, piezoelectric, etc) for conversion of the recognition success to a primary signal that can be amplified and subsequently processed eventually to take automatic remediation actions (see Fig. 1). The sensor should respond directly, selectively and continuously to the presence of analyte(s). Consequently, the biological reaction should be highly reversible to provide *in-situ,* real time measurements. Table 1 shows a listed information about biosensors. In a practical manner, all sensors do not meet these criteria though. In this review the fundamentals and some examples of recent progress in biosensors for environmental monitoring are presented.

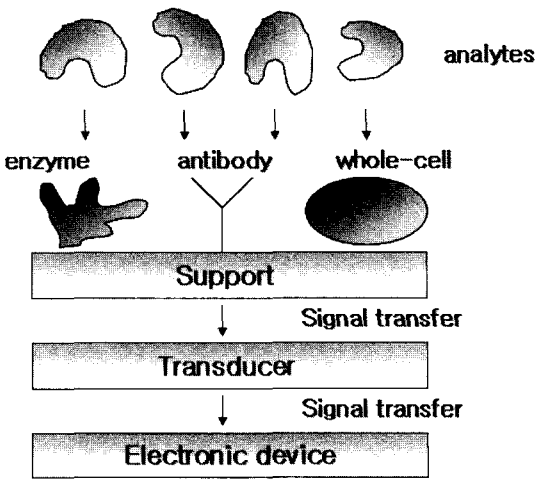

Fig. 1. General working mechanism in various biosensors

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Table 1. Sensors (transducers) and their uses in environmental analysis

Transducers	Measuring Mode	Signals	Biomolecules
Electrochemical			
Conductimetric	Conductance	Ions, Iodine,change in conductivity	Enzymes; antibodies (anti-pesticide,antibody)
	Amperometric	O_2,H_2O_2,NADH, mediators	Enzymes;antibodies;whole cells; organelles; receptors
Field effect transistors	Potentiometric	Change in gate voltage	Enzymes;antibodies; antigens
Ion-selective electrodes	Potentiomtric	H^+, H_2O_2, K^+	Antibodies,enzymes
Gas-sensing electrodes	Potentiometric	CO_2,NH_3	Whole cells; enzymes; receptors
Piezo-electric crystals, surface acoustic wave devices	Micro-mass change	Change in resonant frequency	Antibodies;antigens; receptors
Optoelectronic, fiber optics and waveguide devices	Optical	Absorbance, fluorescence, chemiluminescence, evanescent light wave, change in refractive index	Enzymes; antibodies; antigens,organelles; receptors
Thermistors, diodes	Thermometric/ calorimetric	Change in temperature	Enzymes

1. Immunosensors

Before 1980's biosensors based on immunochemical detection was not used for environmental control. Immunosensors or antibody based sensors were used mainly for medical diagnostic testing, mostly for macromolecules[1]. In detecting macromolecules, piezoelectric, surface acoustic wave, field effect transistor, surface plasmon resonance, and optoelectronic transducers are used for direct measurement of the antigen-antibody reaction[2]. Simplified working mechanism of an immunosensor is shown in Fig. 2.

Biosensing based on the use of antibodies has been paid attention to as reported in recent literature[3-5]. The scope of selectivity of the antibodies is almost unlimited and their abundance covers a variety of trace contaminants (pesticides, industrial residues and degradation products)[6-8]. Also antibodies can be easily tailored in their affinity and selectivity. Immunosensors also profit from monoclonal antibody technology which offers a longer supply period of antibodies with defined chemical and biological properties[9]. Recent research for recombinant antibody production in hosts other than mice at lower cost has raised new hopes for new immuno assay technologies[10,11]. Research in the antibody field is still growing and future perspectives also count on the use of small antibody fragments which are better designed regarding their chemical structure. Readers can refer to the related articles to the antibodies[10-12].

Immunosensors are based on the principles of solid-phase immunoassays. Physicochemical events derived from the antibody-analyte recognition phe-

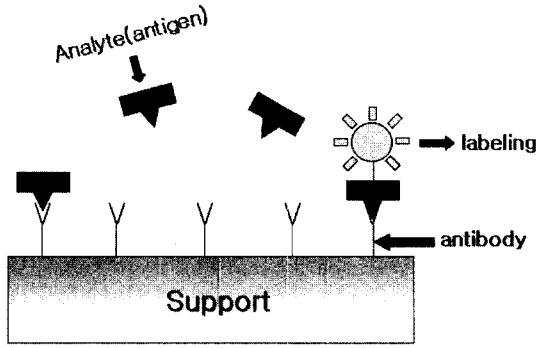


Fig. 2. Principle of immunosensors showing how the analyte can be detected

nomenon are subtle and therefore hard to detect. Environmental contaminnats are often small-size molecules which make the detection of the binding even more difficult. This is why indirect measurements using competitive immunoassy configurations such as enzymes, fluorescent chemicals or electrochemically active substances are used in immunosensors.

For detecting trace level contaminants, it is necessary to use amperometric and potentiometric transducers. Some approaches towards direct detection of the analyte use piezoelectric or optical devices[13,14]. In this case, the detection limits are still not sufficient for reliable quantification of trace pollutants.

Immunosensors are generally irreversible, single-use because the antibody-antigen interaction is not readily reversible. Some immunosensors can be regenerated by equilibrium displacement of the immunoreaction[15,16] using low affinity antibodies, organic solvents or digesting enzymes[17].

Several commercially available instruments for label-free, real-time monitoring of intermolecular interactions and suitable as immunosensors for environmental analysis are listed as below.

(i) The BIOS-I grating coupler - uses the change in the effective refractive index, which is caused by adsorption or binding of molecules to the surface of a glass chip.

(ii) IAsys - uses multi-frequency phase modulation in concert with total internal reflection fluorescence for direct detection of affinity reactions.

(iii) BIAcore

(iv) D-Tech sensor - consists of latex particles with immobilized antibodies. A membrane filter is used to separate bound and unbound material. Determined by a reflectometer.

(v) Smart sense - includes conducting polymer coated platinum electrodes as transducers for atrazine and toxic organic analytes.

All these devices and instruments need labeled compounds to detect the antigen-antibody interaction, which will always be more complex in the performance, separation and regeneration steps are necessary. These formats are successful because they are the only ones sensitive enough for pesticide analysis. To avoid indirect measurements, one recent approach directly measured the antigen-antibo interaction by CEP (conducting electroactive polymer) to determine pesticides as well as PCBs and PAHs. Antibodies were immobilized into the CEP such as polypyrrole during polymerization, thus providing a conduction biomolecular interface easily usable for electrochemical detection. A reversible signal was obtained through application of a pulsed potential[18].

Immunochemical methods are limited by the availability of immunoreagents. Compared with clinical chemistry, where both mono and polyclonal antibodies are readily available to the developer of immunosensor systems, there are very few immunochemicals for pesticides. Immunoreagents are mainly available through university laboratories, which cannot provide that kind of standardization of reagents usually guaranteed by a company.

Acceptance of new techniques, particularly in environmental analysis, generally takes some time. The only methods currently being tested worldwide are immonoassay test kits, which are used for comparison with or in addition to conventional techniques such as LC and GC/mass spectrometry.

2. Receptor biosensors

Because of the specific functions of organisms, protein receptors or genes have been adapted to develop environmental monitoring biosensors. For example, the nicotine acetylcholine receptor (nAChR) has been coupled to a signal transducer based on TIRF in order to measure potential agonists and competitive agonists[19]. The nAChR is the molecular target of a large number of neurotoxins and drugs which can be detected at very low concentration. A flow injection analysis (FIA) system coupled with a biosensor employing TIRF and DNA as binding molecule has also been reported. This biosensors can detect poly-aromatic hydrocarbons (DHAs) such as dimethylbenz, anthracene and naphthalene[20]. A highly fluorescent DNA intercalator, such as ethidium bromide, is used as the reference compound for detection.

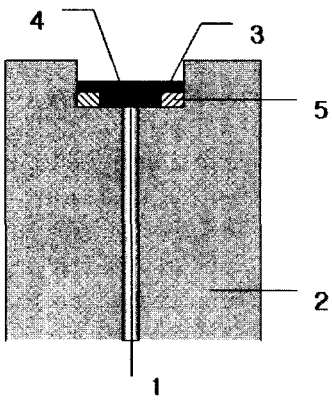
More sensitive or more specific receptors must

also be designed, as for the RC of *R. spaeroides*. Use of DNA as the biological compound will lead toward DNA based sensors and may provide suitable sensors for detecting genetically modified microorganism, viruses, and agricultural products in the environment.

3. Enzyme biosensors

In general, detection of environmental pollutants is not based on their enzymatic transformation but on their capability to act as inhibitors of an enzyme reaction. Although in nature enzymes may exist which are able to transform or degrade the pollutants, they are not always available. Though electrochemical transducers are the most frequently used, the use of fiber optics has been reported. Regarding the sensing element employed, esterases or oxidative enzymes are the most frequently used (see Fig. 3 for a widely used glucose oxidase sensor as an example). Marty et al [21]have recently received the potential of enzyme biosensors for environmental applications. Some of the earliest tests using enzyme-based biosensors for environmental analysis were carried out with the immobilized enzyme butyryl cholinesterase in contact with a platinum electrode as a transducer for electrochemical detection[22].

Enzyme inhibition was caused by the presence of pesticides, such as paraoxon and sevin. This early development was established to monitor air or water for the presence of pesticides. The instrument (called as CAM) could operate for three days. The CAM demonstrated the feasibility of continuous automatic environmental analysis. Interest in this research was facilitated by the commercial availability of cholinesterase enzymes, which were used in diverse applications, such as in the pharmaceutical industry (mode of action), and as tools to monitor chemical warfare agents. However, these assays lacked specificity and were therefore not usable for enforcing legal maximum residue levels in the environment, which are based mainly on single compounds.



1 : Copper wire, 2 : Teflon, 3 : Platinum disk
4 : Epoxy resin (silver), 5 : Epoxy resin (Insulated)

Fig. 3. Schematic of a glucose oxidase (enzyme) biosensor

Additional enzymes are used to detect other environmental contaminants, such as nitrate, nitrite, sulfate, phosphate, heavy metals, and phenols. Tyrosinase is frequently used to determine phenols, chlorophenols, cyanide, carbamates and atrazine. Tyrosinase catalyses the o-hydroxylation of monophenols and the oxidation of o-diphenols to o-quinones while molecular oxygen is reduced to water. This allows the use of this enzyme in amperometric devices to detect contamination by phenolic compounds. With optical detection by fiber optic biosensor, fluorescein isothiocyanate conjugate was immobilized to the surface of a quartz fiber. The H^+ ions, which are produced during hydrolysis of acetylcholine, were detected by the pH-dependent fluorescence yield of fluorescein. Another transducer for determining protons released during acetylcholine hydrolysis was the so-called LAPS system (light addressable potentiometric sensor[23]). LAPS is a silicon-based device with an electrolyte-insulator-semiconductor interface for measuring potential changes[24]. Determination of 3,4-dichlorophenol, atrazine, and carbamate pesticides by tyrosinase inhibition was described by Besombes et al[25], who used an amperometric biosensor made by a pyrrole amphiphilic monomer-tyrosinase coating.

Several enzyme reactions may be coupled in one biosensor to overcome the limitation of monoenzymatic sensors. An example of a multi-enzyme sensor used glycogen phosphorylase to determine inorganic phosphate combined with an alkaline phophatase/mutarotase/glucose oxidase enzyme sequence[26].

4. Micobial whole-cell biosensors

The earliest whole-cell biosensors were used to monitor biodegradable organic compounds in wastewater and sewage treatment plant outflows. Typical microorganisms used for BOD determination were *T. cutaneum, R. erythropolis, C. butyricum,* etc[27]. The cells were immobilized by different techniques, such as entrapment in alginate or agar gels and immobilization by cellulose acetate or cellulose nitrate membranes to the surface of a conventional oxygen electrode. These probes have been relatively successful in monitoring high BOD levels in wastewater. Fig. 4 presents the principle of a cell biosensor.

Other microorganisms were used to determine environmentally relevant chemicals, for example, *E. coli* for heavy metals. Rawson discussed the advantages such as higher stability and drawbacks (low sensitivity, slow response) of whole-cell biosensors[28].

Phototrophic organisms (e.g. eucaryotic algae) use another principle to detect herbicides. Eucaryotic algae or chloroplasts form higher plants possess all photosynthetic complexes with oxygen production and therefore can fulfill the complete redox cycle of the photosynthetic reaction. Also used are cyanobacteria, which have a different redox cycle without oxygen, and therefore require the mediator $K_3[Fe(CN_6)]$ as an artificial electron acceptor for amperometric detection of the photosynthetic reaction[29].

BOD sensors are affected by the presence of heavy metals or by changes in temperature, pH, and salinity, which vary with the microorganism used and must be optimized for each sensor. A recent BOD sensor, for example used *T. candida* and *T. cutaneum*[30]. The cells were immobilized to a cellulose nitrate membrane, which was coupled with a dissolved oxygen probe with the aid of a dialysis tube. The sensor was tested under different environmental conditions to show the influence of heavy metals, temperature, pH and salinity on sensor performance. The sensor performed well at 298-303 K, pH 6.0-8.0 and less than 1.5% of salinity.

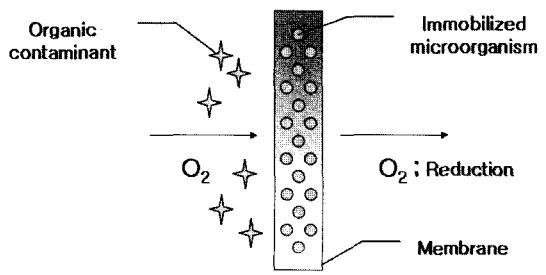


Fig. 4. Principle of microbial sensor using oxygen electrode

For monitoring pollution by phenols, the microorganisms used were *E. coli, S. cerevisiae,* and *Synechococcus* in a mediated ferricyanide amperometric biosensor[31].

Generally, whole-cell biosensors have the potential to be used as an early warning sensor to monitor pollutants in waterways because of their broad specificity.

Conclusions

Biosensors and especially immunosensors for analysis of environmental contaminants such as pesticides are of major interest in this paper. Although not as successful as predicted a decade ago, these techniques will become more prevailed after some of the major technological barriers, such as availability of biological compounds and reproducibility are solved. they will complement existing conventional analytical methods either as stand-alone or additional methods and as integral parts of other techniques. As a result, more samples can be analyzed at lower costs and the use of organic solvents will be reduced. Furthermore, they will serve as tools to measure contaminants where no fully equipped laboratory facilities or well-trained personnel are at hand in developing countries.

Although biosensors are not yet implemented for regulatory purpose in environmental monitoring, they should fulfil the some requirements as conventional robust techniques: acceptable reproducibility; sufficient robustness when applied to a variety of environmental matrices. A key question about future biosensors is obviously the limit of quantitation that a biosensor must reach [32].

More interdisciplinary teamwork between various specialists in research and development and their collaboration with industry and potential users will be essential for the success of biosensors.

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