# Progress and Perspectives in Biosensors for Environmental Monitoring

ANTON CIUCU

*Department of Analytical Chemistry, Faculty of Chemistry, University of Bucharest, 90-92 Panduri Av., sector 5, 76235, Bucharest, Romania*

## Abstract

*Monitoring the environment for the presence of compounds which may adversely affect human health and local ecosystems is a fundamental part of the regulation, enforcement and remediation processes which will be required to maintain a habitable environment. Because of the diversity of current environmental monitoring methods, the choice of techniques used for sample collection, extraction and analysis depends on the compound or compounds of interest and the matrix they contaminate. Although classical analytical techniques are being continually refined and improved, these laboratory-based methods are, for the most part, relatively expensive and time-consuming. Because biosensor technology lends itself to fast, economical and portable analysis, these devices may provide solutions for some of the problems currently encountered in the measurement of environmental contaminants.*

**Keywords:** biosensor, environmental monitoring, field analytical methods

## Introduction

Our community face faces the same types of environmental monitoring problems as any other large community.

Discharges of both radioactivity and conventional substances from the Company's sites are subject to stringent regulatory controls and consents. Regular environmental monitoring is carried out at all sites. Thousands of samples of air, water, soil and silt, seaweed, fish, shellfish, meat, milk and vegetables are analyzed to ensure the company complies with strict legislation [1].

Over 600 chemical compounds have been identified at hazardous waste sites alone and there may be thousands of unidentified pollutants. The majority of compounds found on the Agency for Toxic Substances and Disease Registry's (ATSDR's) priority list are volatile organic compounds; however, other prominent chemical classes include inorganics, phenols, phenoxy acids, polyaromatic hydrocarbons, nitrosamines and aromatic amines. In addition to radiometric measurements, regular measurements of parameters such as heavy metal concentrations, Nitrates, suspended solids, C.O.D., fluoride, pH, ammonia, chloride, phosphates, carbonates, $NO_x$, VOC's, carbon dioxide and $SO_x$ in various effluent streams are made. This usually involves sampling followed by analysis in a central laboratory using large and complex apparatus with a sample turn around time in the order of several hours.

In many cases, identification and quantitation of specific compounds in mixtures of these chemicals must be made in media such as air, water, soil and sludge as well as biological matrices such as blood, saliva and urine. For many analytes of interest no on-line technique is available. Present sensors are not robust or reliable and suffer from interference. Often sensors are required that can function with very little maintenance in very hostile environments. In addition existing laboratory techniques available are inappropriate or involve difficult pretreatment procedures. Presently research is centered in developing novel sensor techniques, chemometric approaches and toxicity measurements in order to improve its environmental monitoring [2].

Biosensors are capable of making a unique and valuable contribution to monitoring the state of the environment, providing a stream of data which can be used to monitor unattended sites and used for direct feedback to optimize processes such as waste water treatment and remediation of contaminated land. There are many advantages which can be foreseen in developing biosensors for monitoring the environment including measurements in complex matrices and capability to provide new information which is not possible using existing technologies, e.g., toxicity assessment and endocrine responses. Although most biosensors must operate in aqueous environments, the development of gas-permeable membranes and appropriate soil extraction procedures can extend the range of media accessible to analysis by these devices.

Biosensors can also provide fast and cost-effective screening and early warning for one or more chosen broad spectra of chemicals in effluents, which may contain thousands of individual substances. This information permits fast remedial action and can be followed up by subsequent detailed analyses using other biosensors or conventional analytical technologies [3].

The present state of the art in biosensors for environmental monitoring includes devices based on catalysis and affinity interactions that have proven to be cost and time-effective alternatives to traditional techniques and, as a result, there are several commercially available instruments to monitor microbial contamination, BOD or total toxicity. The specific analytical requirements of environmental analysis has driven biosensor research into challenging areas not only to fulfil the current legislation but also to search for new applications and possibilities complementary to traditional means. The evidence of synergetic effects in a mixture of pollutants, the necessity to measure unexpected chemicals and adverse biological effects seem to be obvious examples where biosensors could find a new "niche" market for application.

This paper will expand on these themes with specific examples of the types of environmental monitoring challenges, and the future needs of source water in terms of environmental monitoring.

## Biosensors in the Field

Traditionally biosensors are seen as self contained analytical devices that can compete with and complement classical analytical techniques providing low cost, portable, as well as easy-to use instrumentation and new capabilities either through miniaturization or through detection of parameters not achievable with other methods.

Biosensors for monitoring the environment were *defined* by the participants of the 5th European Workshop on Biosensors for Environmental Monitoring and Stability of Biosensors held in Freising in May 1997. The agreed definition is: "Biosensors are considered to be analytical devices incorporating a biological material (e.g. tissue microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids etc.), a biologically derived material or a biomimic intimately associated with or integrated within a physicochemical transducer or transducing microsystem, which may be optical, electrochemical, thermometric, piezoelectric or magnetic". They are distinct from bioassays where the transducer is not an integral part of the analytical system [4].

Biosensors are particularly valuable for environmental monitoring when they either provide continuous or near-continuous information about rapid and unpredictable fluctuations in the concentration of one or more parameters simultaneously, or single measurements of the concentration of analytes which are difficult to measure using conventional methods. Biosensors may also offer competitive advantage over conventional methods of analysis if they are less expensive, more rapid and/or easier to use. In all cases biosensors should be amenable to miniaturization and integration into multisensor arrays to facilitate analysis via, for example, neuronal networks and chemometrics.

Biosensors are needed to measure biological effects (e.g. genotoxicity, immunotoxicity, biotoxins and endocrine effects) and the concentration of specific analytes which are difficult to detect and are important contaminants of water, waste, soil or air (e.g. surfactants, chlorinated hydrocarbons, sulphopheni1 carboxylates, sulphonated dyes, fluorescent whitening agents, naphthalensulphonates, carboxylic acids, dioxins, pesticide metabolites etc). In the case of emissions, waste and remediation, the analytes will be dictated by the process in question, but the biosensors proposed should offer improvements over conventional analytical techniques and contribute to greater process efficiency and/or safety. The development of practical systems integrated in the process and capable of operation under realistic conditions will have a high priority and there is particular interest in the development of stable arrays of sensors, possibly combined with other analytical or separation techniques [5].

Biosensors exploit biological selectivity to obtain analytical information. Therefore the major feature of biosensor is the ability to extract quantitative analytical information on a selected chemical compound in a complex matrix; classical example in clinical applications is the determination of glucose in the blood.

When this concept is transferred for environmental problems the challenge is different. Usually the public demand is not for determining a selected chemical compound but for obtaining broader information like: this water is suitable for drinking, or it is suitable for swimming or for pharmaceutical preparation, etc.

Therefore the term biosensor for solving environmental problems has not the *selectivity* as analytical feature but the possibility to respond at broad questions.

Biosensors show potential for practical analytical application, particularly in environmental protection. Currently, there is only one type of biosensor, among many designs, that has been commercialized for environmental applications: the microbial BOD biosensor. Also this parameter is quite broad and generic.

The obstacles to commercialization of biosensors in environmental protection are, in general [6]:

I. insufficient stability in comparison to chemical and physical methods for practical application,
II. insufficient selectivity and sensitivity,
III. discrepancy between high development expense and low market volume, and

IV. restrictions due to legislation.

Other example of recent research in the field of biosensors in similar direction is the anticholinesterase probe, which is measuring the decrease of activity (mainly in solution) of acetyl-cholinesterase. Such decrease is related to the presence of pesticides (phosphoric and carbamic) and moreover of heavy metals and unknown chemical compounds.

Moreover, another example is the DNA biosensor where on the surface of a carbon electrode a single strand or double strand calf thymus DNA is immobilized. By dipping the biosensor in the sample solution and by interrogation the electrode surface (by chronopotentiometry at constant current) we can evaluate if there is any substance intercalating, complexing, reacting with the DNA structure and thereby very probably harmful. This is the new way for directions of biosensor research for solving environmental problems.

Thus only the BOD biosensor has been developed commercially. Biosensor-based measuring instruments for the determination of BOD are already on the market. The great advantage of the biosensor BOD system is that its short response time permits true on-line process control, which is not possible with the conventional BOD test.

Optimization of biocomponents for increased sensitivity, selectivity, and stability should enable miniaturization of biosensor systems and the development of portable systems for environmental monitoring [7-10].

## Water Monitoring Requirements

In the future, European water source quantities will be critical in some areas, with increasing need to use water of poor quality, requiring adequate protection and monitoring policies to be implemented, particularly in the Eastern European countries. The availability of the resources in terms of quality and quantity, the water supply systems and the needs and expectations of the customers are factors affecting the water supply, where biosensors are applicable at all three levels.

Water is one of the most important resources and its environmental analysis requires fast and reliable results. Large numbers of pollutants (in excess of 100,000 substances) have been detected in surface water. It is impossible to measure all these substances using conventional analytical methods [11].

The system to be monitored is complex. Traditionally water and wastewater systems comprise the following parts:

- ☞ the water source,
- ☞ the distributing and collecting networks,
- ☞ the treatment plant and
- ☞ the receiving water.

Several factors are potential contributors to monitoring problems in these systems, e.g. the number and type of pollutants, the complexity of environmental matrices such as wastewater, and a wide dynamic concentration range of pollutants. Biosensors could play a key role in ensuring the quality of drinking water, surface and ground water, and in assessment of wastewater for suitability for treatment prior to reuse.

The information required, the properties of the water system, the layout of the monitoring network and cost benefits analysis, are important factors for implementation of water and wastewater quality monitoring. Although real-time results are wanted, e.g. in treatment plants, results of water quality measurement from complex systems are most often obtained *a posteriori*. New technique developments should take into consideration that methods used for water and wastewater treatment process control can be different from methods used for quality control, and the performance requirement of laboratory, field and online methods need not to be the same.

The commercial success of biosensors to date has been mainly restricted to medical applications, and the only significant product in the environmental area, as we already mentioned, has been the BOD (Biochemical Oxygen Demand) sensor offering a 20-minute test to replace the conventional 5-day assay. Atrazine is monitored by ELISA in Mississippi, and the more conservative attitude of Europe towards the use of e.g. immunosensors for the control of water quality must be changed. Considerable energy has been expended in researching new possibilities for environmental monitoring using biosensors, but too little attention has been paid to practical on site applications.

Among enzymatic sensors, the ones involving the catalytic transformation of the pollutant, such as phenols, are the more advanced, and deserving actual commercialization efforts. The efforts in this direction are driven mainly by environmental applications with the goal to develop hand-held, battery operated instruments for field analysis. The state of the art in this type of biosensors is still at its discovery stage. Electrochemical biosensors can readily be adapted to transduce all types of biological signals. Electrochemical transduction is still low cost, and ingenious designs and transduction chemistries have proven the key to the development of reliable devices.

Antibody based techniques, i.e. immunosensors are potentially simple to handle and may provide unattended monitoring. Most immunosensor assays rely on competitive binding with some tracer component. Some work is reported on label free detection systems, but the advantages as compared to labeled systems are limited. Electrochemical immunosensors have not yet reached the level of development of other transduction schemes. Although the principal feasibility of immunoanalytical devices has been demonstrated, several problems remain, e.g. availability of a broad range of high affinity antibodies, multichannel/multianalyte capability, and true and proven simplicity and stability of immunoanalytical devices. Future developments are expected within multiarrays, chromatographic surfaces, "non" antibody binding molecules, chemical libraries and microsystems.

The potential of biosensors/integrated systems to monitor pollutants in water and wastewater is demonstrated by the application of continuous working microbial electrodes and bioassays at different tropic levels (e.g. fish, algae, mussels, and water ileas), which cover a wide range of biocides, surfactants, endocrine disrupting substances, heavy metals etc. Biosensors are useful for measurement of short-term responses, i.e. as alarms, and for long time measurements e.g. of ecological effects. Measurement of genotoxic and immunotoxic potential of surface water, effluents and drinking water are among future prospects for field application of whole cell biosensors.

Four key areas have emerged for the integrated management of water resources: urban and industrial wastewater, surface water, soil and ground water, and ecological monitoring. These highlight issues important for potential use and development of biosensors for water and wastewater monitoring.

### 3.1. Urban and Industrial Waste Water

New analytical systems and biosensors are required for monitoring of urban and industrial wastewater. Both cities and industries of the future will need suitable sensing systems to ensure that both clean technologies and closed loop systems are reliable. Where discharge is permitted this must be maintained within limits. Domestic and industrial sewage will need to be monitored, as will agricultural effluents. However, the area of treatment plants will require the greatest innovation. Improved measurement technology is required to adjust the process in response to variations in intake and to protect against accidental discharge. This must be fast, quantitative and process related. General toxicity sensors or organic profile detectors would be most appropriate. For process control, semi-continuous measurement of process-related parameters such as $0_2$, low molecular weight carbon uptake, organic profile, specific physico/chemical parameters and microorganisms are required. The product from sewage treatment may be discharged to the environment or reused. In addition to compliance with standard methods, rapid biologically relevant alarm systems would be beneficial but these must have optimized sensitivity and verified specificity [12].

### 3.2. Surface Water

New measurement systems are required for four categories of analytes: pharmaceuticals and endocrine disrupters, pesticide metabolites, disinfection by-products, and microbiological parameters. Pharmaceuticals and endocrine disrupters are not specified in the current EU regulations and there is a general lack of information about concentrations present. In addition, a laboratory method is needed to quantify pesticide metabolites. No reliable analytical method exists and although pesticide metabolites are in the regulations, they are not measured. New measurement systems are required for pesticide metabolites for which no reliable method already exists. Chlorinating by-products of disinfection should be minimized in water treatment and the results of different treatment processes studied. On-line or in-line measurement techniques would be beneficial. New measurement systems are also required for microbial contamination, including both regulated organisms, e.g. coliforms, and non-regulated organisms, e.g. Cryptosporidium. Methods are generally too long and laborious, and there is a need for alternative rapid, sensitive and cheap methods. Advanced and hybrid analytical methods and biosensors are the appropriate methodology to address the needs for monitoring surface water. All analytical techniques for integrated control of the water system, i.e. source, treatment plant and network, should have a recognized level of performance, including screening methods [13].

### 3.3. Soil and Ground Water

Sustainable management and quality of soil and ground water demand characterization of background values diffuse pollution and point pollution. However, research and activities addressing background values are not currently seen as a priority. Due to the important role of ground water for drinking water supply, pollution from diffuse sources in the ground water protection zone should be characterized. New technologies and tools for analysis have to be implemented for improvement of the cost-effectiveness of laboratory analysis. The large number of polluted sites endangers the environment by the spread of pollutants by groundwater flow. Early warning systems should be implemented around these sites using in-situ detection systems to provide timely information on chemical contamination of the surrounding area.

### 3.4. Ecological Monitoring

The ecosystem must be intact in structure and function otherwise the system has no potential for self-purification. In addition specific uses worthy of protection include: supply of drinking water (drinking water protection zones, groundwater filtration processes, self purification in river banks, self purification in rivers, reservoirs and lakes), leisure and recreation, coarse and game fishing, as well as aquatic communities.

Loading of rivers by chemicals and nutrients must be considered in the light of the capacity of the receiving waterways and the potential for self-purification.

Early warning of long-term effects is required in the field as quickly as possible and so measurement is required as often as cost permits.

Measurement systems and biosensors are required for ecologically relevant summarizing effects i.e. genotoxicity, immunotoxicity, and for antibiotics, endocrine disrupting substances and biotoxins. Quantitative parameters are needed to characterize the health status of the food web: immune status and quantification of the populations. This may be achieved using instruments and sensors containing genetically engineered bacteria (e.g. with reporter genes) and organelles of higher organisms. Biocoenose sensors may include double- or single-stranded nucleic acids, natural or synthetic receptors, phagocytes and immunotoxicity sensors [14].

## Legislation

It is obvious that regulation is the key driver for environmental improvement. It is a fact that new EU regulation exists but implementation of the EU regulation is lacking. In this respect, the need to facilitate the implementation of the different water directives, like the Framework Water Directive, IPPC, and the Municipal WasteWater Treatment Directive is needed. The full implementation of the various Directives affects different levels of responsibility from local authorities (like Water Treatment Agencies), operators of the Water Treatment Plants (WTP), constructing companies involved in the design and construction of WTP and the EU together with research institutes and universities.

Concerning Water Treatment, the Directive 91/271/EEC is a key reference and it aims to reduce pollution in Community surface waters caused by municipal waste. Under its provision, all waste water in the territories of the EU will have to be properly collected and subjected to secondary (biological treatment with secondary settlement) or equivalent treatment before being discharged into estuaries or coastal waters. Municipalities over 15.000 inhabitants will have to have secondary waste treatment by the year 2000. Under the terms of this directive, industrial wastewater will be subjected to the same provisions as those established by municipal wastewater. Industries which discharge waste into municipal collecting system and Water Treatment Plants (WTP) have been required to seek prior authorization since December 1991 [15].

In practice, many SMEs are discharging to the domestic collecting system and so many municipalities receive wastewaters, which are coming from industrial water slightly treated at source, and mixed with domestic activities.

It is then obvious that for the correct implementation of this Directive, there is a need to investigate the operation of WTP, by characterizing the influent and effluent waste waters form either domestic and/or industrial discharges in order to improve the operation of WTP. There is a lack of information about the correct operation of WTP, especially those receiving industrial effluent discharges. Indeed, the 'reality' is that many municipal WTP receive waters from industrial origin, from SMEs, which are often not treated or are only slightly treated at source and mainly treated at the WTP.

## Advanced Measuring Systems

As regards the methods used for the control of organic pollution present in WTP it can summarized that:

(a) there are many unknown (today)analytes with potential endocrine disrupting effects and /or different kinds of toxicity that are not measured but it is obvious that they need to be measured;

(b) the routine parameters currently measured, like VOC, methylene blue active substances and the phenol index, give very limited information on the organic pollution present in the WTP and as a consequence there is a need to develop and apply a broad spectrum of analytical methods including different types of biosensors in the field, on-line methodologies and mass spectrometric characterization to achieve a higher degree of knowledge of this organic pollution present in WTP, especially of that coming from industrial processes, and

(c) new, internationally recognized and comparable advanced measuring systems should be implemented, in WTP. In this way a correct diagnostic of WTP will be achieved thus providing a better operation of the WTP by reducing the pollution of the industrial effluents-process related- and by improvements in the type of treatment performed in the WTP.

Updated monitoring tasks can be achieved by advanced measuring systems that will act as precautionary and preventive against organic/toxic pollution and for process control in industries. These systems will permit the protection of all water resources, from wastewaters, receiving surface waters to estuarine and coastal waters.

## Conclusion

The use of biosensors for on-line monitoring of surface water and for the determination of intermittent samples offers a powerful and promising tool for environmental pollution control and water management. They are needed to provide sensitive information about substances which are important contaminants of the environment and which are difficult to detect. Biosensors can measure important biological effects, e.g. genotoxicity, immunotoxicity, biotoxins and endocrine effects. The development of practical systems capable of operation under realistic conditions is a high priority. There is particular interest in the development of stable arrays of sensors, possibly combined with other analytical or separation techniques.

Biosensors have an important role in contributing to ensuring the quality of European water sources in the next decade, monitoring of source water (surface and ground water), monitoring in water treatment and in assessment of suitability of wastewater for water production and groundwater recharge. The small size and easy operation of such equipment promises an attractive new European product range to sell in the global marketplace.

The environmental biosensor area is facing two important challenges connected with the nature of the market. On one hand; the specific analyte market is highly fragmented and on the other, the official environmental regulations are tuned to the cumbersome, expensive, possibly unreliable, but consistent sophisticated hyphenated techniques. For biosensors to offer a viable alternative and to cover the necessities of the environmental monitoring and management needs of the future, they have to be based in generic licensable technologies that can address large market segments, and to meet the reliability requirements necessary for consideration in environmental legislation. At the same time that these requirements are met, biosensors should remain of low cost and facile use so that new market possibilities open (as is for example routine use by concerned citizens). In these directions several efforts are being made on the research level. They involve both the biorecognition element design and the transducer part of the biosensor. In the former area, tailored antibodies, genetically engineered organisms with marker genes, exploration of new enzymes and new uses of old enzymatic systems, exploitation of oligonucleotides as sensing elements are some of the technologies followed at the research level. In the latter area, not only are advances in selective, immobilization techniques for the development of multisensors, or advances in optical and electrochemical transducer materials relevant, but also advances in the electronics and packaging of the devices. Increasingly, 'satellite' areas to traditional biosensor development, such as the increasing blurring of the biosensor-chemical sensor border with the use of combinatorial chemistry produced recognition elements and the field of signal processing, become important for the development of biosensors suitable for environmental monitoring [15].

As a conclusion, biosensor development for environmental monitoring becomes even more interdisciplinary and promising technologies are equally important at the basic research and at more advanced development levels. Whether or not these approaches have a real impact on environmental monitoring will very much depend on integrated research projects that are goal-oriented without underestimating novel basic approaches for the development of environmental biosensors that are generic, reliable, easy to use and can be produced at low cost to solve the future necessities of environmental monitoring and management.

## References

1. A. CIUCU, "*Aplicaţiile Analitice ale Biosenzorilor în Controlul Poluării Mediului*", Ed. Ars Docendi, Bucureşti, pp. 52-55, 2001.
2. A. CIUCU, "*Biosensors for Environmental Monitoring*" , Ed. Niculescu, Bucureşti, pp. 103-155, 2000.
3. J. M. KAUFFMANN, M. PRAVDA, *Int. Bioforum Bioresearch Plus Biotechnol.*, **2**, 22-25 (1998).
4. K. R. ROGERS, *Bios. Bioelectron.*, **10**, 533-541 (1995).
5. F. MUNTEANU, L. GORTON, T. RUZGAS, J. EMNEUS, E. CSORREGI, A. CIUCU, I. G. GAZARYAN, I. V. OUPOROV, E. A. MAREEVA, L. M. LAGRIMINI, *Appl. Biochem. Biotechnol*., **6 ,** (2001).
6. A. LINDGREN, L. STOICA, T. RUZGAS, A. CIUCU**,** L. GORTON, *The Analyst*, **124**, 527-532 (1999).
7. F. MUNTEANU, A. LINDGREN, J. EMNEUS, L. GORTON, T. RUZGAS, A. CIUCU, E. CSORREGI, *Anal Chem*., **70,** 2596-2600 (1998).

8. M. J. DENNISON, A. P. F. TURNER, *Pergamon Biotech. Adv*., **13**, 1-12 (1995).
9. K. R. ROGERS, J. N. LIN, *Bios. Bioelectron.*, **7**, 317-321 (1992).
10. C. NISTOR, J. EMNEUS, L. GORTON, A. CIUCU, *Anal. Chim. Acta*, **387,** 309-326 (1999).
11. J. WANG, *Nat. Exp.Res. Lab*., **94**, 3-17 (2001).
12. K. R. ROGERS, L. R. WILLIAMS, *Trends Anal. Chem*., **14**(7), 289-294 (1995).
13. K. RIEDEL, "*Application of Biosensors to Environmental Samples*" in, "*Commercial Biosensors: Applications to Clinical, Bioprocess, and Environmental, Samples*", G. Ramsay, ed., John Wiley & Sons, Inc., pp. 80-90, 1998.
14. C. NEGULESCU, C. CIUCU, A. CIUCU, *Terapeutică, Farmacologie şi Toxicologie Clinică*, **5(3)**, 31-38 (2001).
15. E. DOMINGUEZ, "*Identification of Technologies which are More Promising for Environmental Monitoring*", in, "*Biosensors for Environmental Monitoring*: *Technology Evaluation*", O'Sullivan, G.G. Guilbault, S. Alcock, A.P.F. Turner, eds., University College Cork, Ireland, pp. 11-15, 1998.