Preface

This book on “Biosensors for the Environmental Monitoring of Aquatic Systems” is based on the scientific developments and results achieved within a group of European Union (EU) funded projects that will be briefly discussed below. Indeed, everything started with the development of biosensors for environmental measurements in 1991 as part of the EC Environment Programme (1991–1994) under Area II: Technologies and engineering for the environment, section 1: Assessment of environmental quality and monitoring. In the context of this Programme, a biosensor was defined as a compact analytical device containing a biological or biologically derived sensing element (e.g., enzymes antibodies, microorganisms, or DNA) either integrated with or in intimate contact with a physicochemical transducer (e.g., electrochemical, optical, thermometric, or piezoelectric). The first European Workshop on Biosensors for Environmental Monitoring took place at the Technische Universität Berlin (BIT - Berlin Institute of Technology) in February 1993, organized by Prof. P.D. Hansen and Dr. J. Büsing. This was the first focused workshop of a series of workshops in London (1994) organized by P. Bennetto and J. Büsing, Florence (1995), Barcelona (1996), Freising (1997), Kinsale (1998), Paris (1999), Cascais (2000), and Alcala de Henares (2001). All these workshops covered a large variety of technical and scientific topics related to biosensors for environmental monitoring with additional presentations of the progress reports of the specific EU projects. The workshop in Florence had already presented ten biosensor projects supported under the EC Environment Programme, dealing with pesticide detection, microbiological contamination, and xenobiotics in water and air. This workshop involved some 100 scientists from 15 European countries and from Japan. The objective of the workshop in Florence (1995) was to present the state of European research activities supported under the EC Environment and Climate Programme (1995–1998) in the field of biosensor development and to identify priorities for future Research & Development.

During that period the European Commission’s Environment and Climate Research Programme supported a three-year co-ordination project to guide technological developments in Biosensors for Environmental Technology (BIOSET). The aim of BIOSET was to enhance the development of biosensors for practical applications in monitoring pollutants in the environment. BIOSET maintained close contact with European industry and explored possibilities for collaboration with the commercial sector. This ensured eventual transfer of the technology demonstrated at the workshops and Technical Meetings to marketable devices. BIOSET provided assistance for researchers from European laboratories to meet to exchange ideas, use equipment, and establish a basis for new joint projects. The secretariat of the Concerted Action BIOSET supported the Technical Meetings.

There were three Technical Meetings held, two in Berlin in 1997 and 1998, and the third in Barcelona, in April 2000. The goal of these technical meetings was to join different research and industrial teams to evaluate the performance of their biosensor technology in field conditions with common and standardized surface and waste waters.

As a result of these field experiments, the additional information that biosensors can offer to environmental monitoring was also evaluated. Thus, these three Technical Meetings were useful accompanying measures and practical additions to the currently organized yearly workshops. The concerted action BIOSET was followed by the SENSPOL network. The 1st SENSPOL Workshop was held on the 9–11 May 2001 on Sensing Technologies for Contaminated Sites and Groundwater at the University of Alcala. There was one special Workshop on "Genotoxicity Biosensing (TECHNOTOX)" supported by the European Commission DG XII D-1 and BIOSET in the year 2000. The TECHNOTOX meeting at the Flemish Institute for Technological Research (VITO) in Mol was organized by Phillippe Corbisier (VITO), Peter-D. Hansen (TU Berlin) and Damia Barcelo (CSIC Barcelona). Thirty scientists participated in this meeting and 14 genotoxicity tests were performed on-site simultaneously on a maximum of 11 samples (chemical compounds and environmental samples). The AMES Test (ISO 16240) and the well-accepted umuC Test (ISO 13829) were performed as reference genotoxicity tests. The panel of genotoxicity tests included six prokaryotic tests, one eukaryotic test, four mammalian tests, one DNA test, and one bacterial test. Four assays were commercially available. It was demonstrated that no single test is capable of detecting all relevant genotoxins. Therefore, a Test Battery for genotoxicity is recommended (see the chapter by T. Grummt et al., this volume). The concept was to transform the standardized and harmonized microplate assay into an automated on-line assay and/or finally into a biosensor. The EuCyanobacteria Electrode was one of the first biosensors developed under the EC Environment Programme (1991–1994) initiated by Jürgen Büsing. The EuCyanobacteria Electrode was developed and optimized as an on-stream biosensor for pesticide detection (EV5V-CT92-0104). The EuCyanobacteria Electrode, in principle a so-called Rawson sensor, was transformed into a single rod electrode. The EuCyanobacteria Electrode with detection limits for atrazine <10 microgram per liter and diuron <2.5 microgram per liter was implemented in the monitoring programme on the river Rhine after the SANDOZ disaster in 1986 and optimized in several research projects in the following years. Special catch system devices were coupled with secondary electrochemical methods of signal transduction based on enzyme or whole-cell reactions. With the aid of mass spectrometry it was possible to evaluate the bio-recognition and detection components.

The Technical Meetings showed that biosensors are useful tools for controlling the degree of pollution of surface waters and of WWTP (wastewater treatment plants).

All biosensors evaluated showed much higher “alarm” values for the influent waste waters as compared to the final treated WWTP effluent. This is already remarkable, since the main advantage of the different biosensors evaluated, like BOD, ToxAlert, or phenols is that the measurements are being carried out very rapidly—a maximum of 20 minutes—so the information is available immediately. This is certainly an advantage over conventional methods that require several days, either for the BOD-5 or for conventional chromatographic/mass spectrometric methods.

The contributions in this book intend to summarize some of the practical achievements in the field of biosensors for environmental monitoring in Europe focusing, mainly, on examples of chemical groups of analytes (or effects) in different water matrices and more specific aspects of biosensor technology. The target objective of this book is to provide an overview of biosensors as a practical alternative and complementary or additional measurement methodology to traditional chromatographic techniques. Emphasis will also be given to the validation of the applied technology and its application to real-world environmental samples. The various chapters cover examples in different areas that are listed below:

General parameters of water quality. Biochemical Oxygen Demand (BOD) which is usually determined by measuring the oxygen consumption of a mixed culture over 5 days (BOD5). In the Third Technical Meeting held in Barcelona, the commercial firm Biosensores presented an on-line biosensor technique for the determination of BOD. This on-line biosensor device was installed in the la Llagosta wastewater treatment plant and after the Technical meeting, was installed in two more WWTP for a period of one month. This is an example of the implementation of biosensors into real-world situations, since the authorities are already using two of their devices for routine control.

Trace organics in surface water. The RIver ANAlyzer (RIANA), an optical immunosensor coupled to a FIA system based on a competitive solid-phase fluorescence immunoassay with immobilized analyte derivative and free fluorescence-labeled anti-atrazine or anti-simazine antibodies has been developed and applied for the measurement of chlorotriazine pesticides in river water samples. A pilot monitoring survey in the Ebre area (Tarragona, Spain) was carried out over a three-month period. Simazine and atrazine were detected at levels ranging from 0.5 to 1 microgram per liter and in soil samples from 19 to 40 g kg^{-1}. Phenylurea herbicides and paraquat were also determined by this immunosensor approach. The validation of the RIANA biosensor for the determination of phenylurea herbicides was carried out by LC-MS whereas in the case of paraquat capillary electrophoresis(CE)-UV detection was used. Enzymatic biosensors based on catalytic inhibition have been widely used and applied for the analysis of organophosphorus and carbamate pesticides in water samples. A cholinesterase biosensor using acethylthiocholine chloride as substrate was able to distinguish between different batches of water containing 3–10 ppb levels of pesticides.

Toxic and endocrine-disrupting effects. There are different methods of measuring toxicity. Two of the most commonly used methods are Microtox® and the recently developed ToxAlert@ system. Although they are not strictly biosensors, they are a kind of bioprobe used in a standardized way to measure toxicity of the effluents. They are widely accepted and internationally recognized. During the Third Field Experiment held in Barcelona, Spain more data was generated and a variety of cytotoxic compounds were identified in the wastewater treatment plant during this field experiment. The efficiency of the WWTP was evaluated by collecting and analyzing samples at various stages of the water treatment process. The chemical compounds identified could explain the toxicity observed at the different steps of the WWTP. Endocrine-disrupting effects are today widely studied. One of the most current measurements is the so-called vitellogenin (VTG) level in male fish. If VTG levels are high as compared to control values, exposure to endocrine-disrupting chemicals can be concluded. This approach has been used for the development of a biosensor called VITELLO within the EU projects PRENDISENSOR and SANDRINE. Within the EU project SANDRINE different biosensors, bioassays, and receptor assays are being developed for detecting endocrine-disrupting compounds in WWTP. In this respect, MCF-7 and vitellogenin by the ELRA and Vitello sensor of the SANDRINE project are a few of the different assays in use. A practical example was the determination of the presence and the effects of endocrine-disrupting compounds in two tributaries of the Llobregat river (NE Spain). Water samples and carp, *Cyprinus carpio*, were collected from selected sites along a transect, for chemical and biological determination, respectively. Western blot analysis of male carp plasma using a polyclonal antibody raised in the cyprinid Koi carp, detected this protein in all samples, the VTG increase being more evident at the vicinity of the treatment plants. A certain correlation was also found between NP in water and VTG induction in fish ($r = 0.75$). Within the EU project ALGAETOX a bio-analytical system was developed with the immunotoxic relevant endpoint. Phagocytosis was measured by oxidative burst (ROS) and luminescence. There is now a recent application of this system in the COLUMBUS/BIOLAB programme. The bio-analytical system contributes to risk assessment concerning immunotoxicity under space-flight conditions. A schematic work flow demonstrates the close connection between environmental chemistry and the development and validation of a biosensor.

All these promising results demonstrate some of the European efforts to implement biosensor technology and other assays, including toxicological relevant assays and ELISA, in environmental analysis. The developments achieved during the last years by the different EU Environment Programs have permitted us to show some key examples of the application of biosensors for environmental monitoring, demonstrating not only cost-effective alternatives for screening but also effect-related readouts where new and unpredictable approaches are expected. Biosensor technology has reached maturity for BOD, pesticides, and phenols incorporating whole cells, affinity or catalytic events with electrochemical or optical transduction. Complex and difficult analytes (e.g., surfactants) are progressively being approached by biosensors. The large variety of analytes, the complexity of environmental matrices, the low limits of detection, and the legislative compliance should not shadow the progress achieved in this area. On the contrary, biosensor research is offering new strategies for the evaluation of biological effects where cocktails of compounds may show synergistic, additive, or antagonistic effects that will remain unsolved by chromatographic or spectroscopic techniques. The challenge for environmental biosensors remains open and further research in this area will only prove that new parameters are needed for safer and more complete environmental risk assessment. It is expected that collaborative work, focused research, and interdisciplinary approaches may lead to biosensor indexes that will certainly contribute to a better knowledge and real-time characterization of environmental samples. The book is organized to provide information on relevant principles, novel biosensor techniques and their applications. It is hoped that it will stimulate much discussion and bring new ideas to one of the most exciting and environmentally beneficial areas of environmental chemistry and eco-toxicology. It is hoped that the book and its methods contribute to the broader goal of conserving and protecting our aquatic ecosystems and their biota for future generations.

The book will be of interest to a broad audience of analytical chemists, biologists, environmental chemists, water management operators, and technologists working in the field of wastewater treatment, or newcomers who want to learn more about the topic using new measurement devices such as biosensors. Finally, we would like to thank all the contributing authors of this book for their time and effort in preparing this comprehensive compilation of research papers. Special thanks to Dr. Juergen Büsing for "pushing" this topic since 1991 at the EU level thus allowing excellent scientific and technological developments. The book that you have in your hands is part of such achievements.

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