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New Trends in Biosensors for Water Monitoring

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1. Introduction

Intensive industrialisation and farming associated to domestic uses of a growing number of chemicals have led to the release of many toxic compounds in the environment, causing an important pollution of aquatic ecosystems. In Europe, the Water Framework Directive WFD 2000/60/EC lays down the monitoring of a large number of substances, the so-called “priority substances”, with the objective of restoring a good chemical and ecological status of all water bodies by 2015 (Allan et al., 2006). To implement effective monitoring and treatment programs, complementary analytical methods are required:

- low cost and high throughput screening methods for semi-quantitative determination of families of compounds and/or prediction of their harmful biological effects (overall toxicity, genotoxicity, estrogenicity),
- conventional methods based on chromatographic separation techniques (LC/MS, LC/MS/MS, GC/MS or ICP-MS), which are more time consuming, costly and require trained operators. These methods do not provide informations on water toxicity but allow the rescanning of positive samples for more accurate analytes identification (Rodriguez-Mozaz et al., 2007).

Biological techniques, such as bioassays and biosensors, constitute the first category of methods. Many works in the past have been focused on the development of bioassays and have led to the commercialization of bacterial bioassays and immunoassays (Allan et al., 2006; Farre et al., 2005). In recent years, biosensors have received particular attention owing to their high sensitivity, low cost and possible easy adaptation for on-line measurements (Barcelo & Hansen, 2009).

A biosensor is an electronic device used to transform a biological interaction into an electrical signal. This device is based on the direct spatial coupling of the immobilised biologically active element, the so-called “bioreceptor”, with a transducer that acts as detector and electronic amplifier. Different types of bioreceptors (enzymes, receptors, antibodies, DNA or microorganisms) combined with electrochemical, optical or mechanical transduction have been used for the elaboration of biosensors in view of water monitoring applications (Badihi-Mossberg et al., 2007; Rogers, 2006). To answer to the ever increasing requirements of water monitoring legislation, not only in terms of amount and reliability of informations provided, but also in terms of rapidity of response, selectivity, sensitivity and cost, tremendous efforts have been devoted in the last few years to improve the different elements contributing to the overall response of the biosensors, i.e. bioreceptor, transducer

and cell/transducer interface. All these aspects will be addressed in the present chapter. New advances recorded in the field during the last five years will be more particularly emphasized.

2. Transduction modes

2.1 Electrochemical transduction

Electrochemical sensors are classified according to their transduction mode, which may be potentiometric, amperometric, conductimetric or impedimetric. In a general way, electrochemical transducers measure the electron transfers occurring between electroactive species (molecules or ions) present in a solution and an electrode, in well defined analytical conditions (Grieshaber et al., 2008). Over the past 10 years, electrochemical transduction technology has evolved significantly. Novel electrode materials such as boron doped diamond (BDD) have emerged as possible alternative materials to conventional gold, platinum or carbon (Luong et al., 2009). Owing to the recent advances in microfabrication techniques, it is also now possible to prepare microelectrodes of various sizes and geometries as well as to construct parallel arrays of microsensors on a same chip (Wei et al., 2009). Such systems are powerful tools able to answer to most of the environmental monitoring requirements such as rapidity of response, sensitivity, and parallel analysis of a large number of parameters and/or samples. Moreover, the small size is useful for the design of portable biosensors intended for on-field applications.

2.1.1 Potentiometric transduction

The two classical types of potentiometric transducers are ion selective electrodes (ISEs) and semiconductor-based field-effect devices (FEDs). The inherent miniaturization of ISEs and FEDs and their compatibility with advanced microfabrication technology make them very attractive for the integration into sensing arrays and microfluidic platforms and thus, the creation of miniaturized analytical systems suitable for environmental monitoring (Bakker et al., 2008; Bratov et al., 2010).

ISEs involve ion exchange equilibria at the interface between the solution and a membrane made of an ionic conducting material (inorganic solid electrolyte or organic liquid membrane). The nature of the membrane depends on exchanged ions, special glasses being typically used for H⁺, ionic solid for halide ions, polymers including specific ionophores for other ions. Practically, potentiometric biosensors measure the difference of potential E_p between the selective electrode on which the bioreceptor is immobilised and a reference electrode when no significant current flows between them. E_p can be expressed by Nernst equation:

$$E_p = E^0 + RT/nF \ln a_{A^{n+}} \quad (1)$$

where E^0 is the selective electrode constant, $a_{A^{n+}}$ is the activity of Aⁿ⁺ ion

Significant efforts have been made during the past decade to improve the robustness of conventional ISEs, widen the range of ions detected and miniaturize the electrodes (Bakker et al., 2008; Tymecki et al., 2006).

FEDs belong to the second class of potentiometric transducers and include ion-sensitive field-effect transistors (ISFETs) and light-addressable potentiometric sensors (LAPSs). At present, only ISFET sensors measuring H⁺ ions are commercially available. By deposition of enzymes or bacteria, it is possible to monitor enzymatic and metabolic reactions generating

H⁺. LAPS devices are also extensively used to monitor cellular acidification in response to pollutants. Several recent reviews document the main features of these devices and their application to biosensing (C.-S. Lee et al., 2009; Schoening & Poghosian, 2006; Poghosian et al., 2009).

2.1.2 Conductometric transduction

Conductometric biosensors rely on the direct measurement of conductance variations in electrolytic media containing mobile electric charges. For that, an alternating voltage is applied between the working electrode, on which the bioreceptor is immobilised, and a reference electrode. The frequency value is chosen in order to minimize polarization effects. The conductance can be expressed by the following equation:

$$G = \gamma \frac{S}{l} \quad (2)$$

where γ (S. cm⁻¹) is the specific conductance or conductivity, characteristics of the medium; S (cm²) is the working electrode surface; l (cm) is the distance between the electrodes.

Recent advances in the field have led to the production of miniaturized interdigitated electrodes that have been used to the elaboration of enzyme-based and cell-based biosensors for water monitoring (Hnaïen et al., 2011; Jaffrezic-Renault & Dzyadevych, 2008). Enzymatic reactions between the pollutant and the bioreceptor induce a local change of conductivity due to the production of charged species.

2.1.3 Impedimetric transduction

Impedimetric transduction measures charge transfer processes occurring at electrode/electrolyte interfaces. Practically, measurement is performed using three electrodes, a working electrode modified by the bioreceptor, a reference electrode and an auxiliary electrode. A small amplitude sinusoidal voltage is imposed between reference and working electrodes and the resulting current generated between working and counter electrodes is measured. The applied voltage over measured current intensity ratio defines the impedance of the electrochemical system. Impedimetric data can be modelled by an equivalent electrical circuit from which electrical parameters that define charge transfer processes can be deduced (Katz & Willner, 2003). Impedimetric transduction is particularly well-suited to investigate reactions based on molecular affinity such as antigen-antibody or receptor-target interactions. Cell adhesion to the electrode surface is also expected to increase impedance value due to the insulative properties of the cell membrane. In the presence of cytotoxicants, morphological changes or functional alterations, and even death of the cells are also observed, inducing impedance variations. Therefore, these properties have been extensively exploited for water pollutants biosensing and toxicity assessment using electrodes modified with antibodies, receptors or cells.

2.1.4 Optical transduction

Optical transducers measure the effect of biological entities used as bioreceptors on light absorption, fluorescence, luminescence, refractive index or other optical parameters. In general, two different protocols can be implemented in optical biosensing. The first one requires a preliminary functionalisation of the bioreceptor or the target analyte with an optically active tag (labeling). Although this process produces highly sensitive biosensors, it

is time-consuming and may interfere with the function of a biomolecule. In contrast, in the second protocol, target molecules are not labeled or altered, and are detected in their natural forms. This type of detection is relatively easy and cheap to perform (Fan et al., 2008). The most recent innovations in optical transduction applied to environmental biosensing are related to the development of new solid-state devices, microarrays and microfluidic systems for continuous monitoring (Ligler et al., 2009)

2.1.4.1 Optical fibre sensors

An optical fibre is a waveguide that classically consists of a silica core (optical index: n_1) surrounded with a cladding of index n_2 , slightly lower than n_1 . The fiber is placed in a medium of index n_0 . The light-guiding conditions are defined by:

$$n_0^2 \sin^2\theta_0 = n_1^2 - n_2^2 \quad (3)$$

where θ_0 represents the numerical aperture of the fibre or the limit injection angle of the incident beam.

Fibre optical biosensors are all of extrinsic type. In some of them, called punctual biosensors, the physical or chemical effect is measured at the tip of the fibre on which the bioreceptor is deposited. The biosensor operates in reflection mode. In the so-called continuous biosensors, measurements are performed on a well defined length of the fibre. Cladding is removed in this zone and the bioreceptor layer is directly deposited on the core. This system can operate in reflection or in transmission modes. Bioreceptors are generally immobilised by adsorption or covalent attachment to a membrane, or recovered by a semi-permeable membrane. The most conventional fibre biosensors are based on absorption, fluorescence or luminescence detection (Goure & Blum, 2009). Others exploit the physical properties of the evanescent wave corresponding to the light power lost at the core-cladding interface. These biosensors, for which a stripping of the fibre is required, are more fragile than the massive optical systems based on the same principles and described in the following sections.

2.1.4.2 Mach-Zehnder interferometers

This type of sensor relies on the perturbation of the light propagating in one arm of an optical waveguide. In a typical Mach-Zehnder interferometer configuration, the light guide is divided into two branches via a Y-junction. A branch, functionalised with the biosensing element, is used as the sensitive arm, while the other is the reference branch (Fig. 1). The two branches recombine at the output, resulting in interference, and a photodetector measures the intensity. A change in the refractive index at the surface of the functionalised arm results in an optical phase change and a subsequent variation in the light intensity measured at the photodetector. This latter is proportional to $\cos(\Delta n_2 k_0 L)$, where Δn_2 represents the refractive index change, k_0 the amplitude of the wave vector and L the length of the sensitive region. These structures are made in glass or silicon and may be easily integrated into lab on chip laboratories (Sepulveda et al., 2006).

2.1.4.3 Surface plasmon resonance (SPR) sensors

These sensors are based on the physical principle of surface plasmon resonance (Hoa et al., 2007). The bioreceptor is deposited on a metal surface covering a glass support attached to the base of a prism (Kretschmann configuration). Interaction between the target and biorecognition molecules can be investigated in real time, with high precision and sensitivity, without specific labelling, through the measurement of the variations of

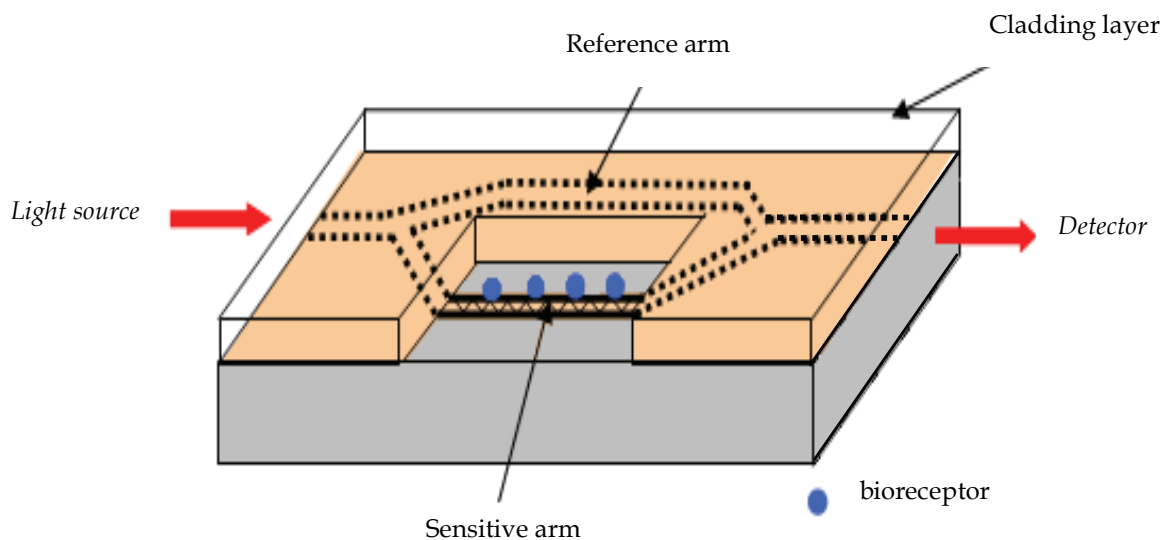


Fig. 1. Design of a Mach-Zehnder interferometer

refractive index near the interface. These sensors have been extensively used for the study of affinity interactions (eg antigen-antibody). SPRi systems allowing real-time and simultaneous imaging of several spots functionalised with different affinity systems are currently in full expansion (Scarano et al., 2010).

2.1.4.4 Optical waveguide light mode spectroscopy (OWLS)

This is a new detection technique based on evanescent field for *in situ* and label free investigation of surface processes at molecular level. It is based on accurate measurement of the resonance angle of a linearly polarized laser light, diffracted by a grating and coupled in a thin layer of the waveguide. Resonance coupling occurs at a specific angle characteristic of the refractive index of the medium covering the waveguide surface. The light is guided by total internal reflection on the edges where the detection is performed via photodiodes. By varying the light incidence angle, a spectrum is obtained, which allows the calculation of effective indices for both the electric and magnetic fields (Luppa et al., 2001).

2.1.4.5 Total internal reflection fluorescence (TIRF)

This technique has been used with planar waveguides and optical fibres as optical transducers in many biosensors. The light propagates along the waveguide, generating an evanescent wave on the surface of the optically denser part of the waveguide (quartz) as well as in the adjacent less dense medium (aqueous medium). The evanescent wave amplitude decreases exponentially with distance in the lower refractive index medium. The fluorescence of a fluorophore excited by the evanescent field can then be detected. Only fluorophores bound to the surface are excited. Real time kinetics of interaction of bioanalytes with molecules immobilised on the surface of the waveguide can be measured using TIRF. This is a rapid, nondestructive and sensitive technique used for the development of automated detection systems for environment monitoring (Tschemelak et al., 2005).

2.1.5 Mechanical transduction

Various mechanical methods have been used as detection in biosensors. These transducers have become increasingly popular over the years.

2.1.5.1 Transducers based on piezoelectric effect

A quartz crystal, to which a sinusoidal electric field is imposed, undergoes mechanical deformation due to the electrical potential appearing at its surface (piezoelectric effect). The crystal oscillates at its resonance frequency that depends on its structure (orientation, thickness...). Any change in mass (Δm) occurring at the crystal surface causes a proportional decrease in its resonance frequency (ΔF). This linear relationship is expressed quantitatively by the Sauerbrey equation:

$$\Delta F = -\frac{2F_0^2}{\sqrt{\mu_Q \cdot \rho_Q}} \cdot \frac{\Delta m}{A} \quad (4)$$

where F_0 is fundamental frequency; A the geometric surface; μ_Q the shearing mode; ρ_Q the density of piezoelectric crystal

The Sauerbrey equation applies only for thin and rigid layers, excluding viscoelastic films, e.g. polymer or polyelectrolyte films. The most common transducer based on piezoelectric effect is the quartz crystal microbalance (QCM).

2.1.5.2 BioMEMS

BioMEMS (Bio-Micro-Electro-Mechanical-Systems) are mechanical systems of nanometer size that allow the translation of biomolecular interactions into mechanical data originating from the deflection of a cantilever. A biomembrane, attached to a silicon platform, constitutes the sensitive part of the device. This membrane, functionalised with specific molecules of interest (probes), vibrates in its fundamental mode by means of a piezoelectric patch. When the biomembrane is in contact with an aqueous solution containing species to be detected (target), the molecules are captured by the probe and membrane mass increases. By detecting the vibrations of this biomembrane, it is possible to measure the resonance frequency variations and thus estimate the quantity of biomolecules present in the solution. BioMEMS offer many advantages including rapidity, high sensitivity, low signal-to-noise ratio, ability for real time monitoring and possible integration of a large number of sensors on a small area (Hassen & Thundat, 2005).

2.1.6 Bioreceptor immobilisation

Immobilisation of the active biosensing element (enzymes, antibodies, cells ...) onto the transducer surface is a key point in the development of a biosensor. Apart from preserving the functionality of the biomaterial, the immobilisation method must ensure the accessibility of the cells towards target analytes as well as a close proximity between the bioreceptor and the transducer. The selection of an appropriate immobilisation method depends on the nature of the biological element and of the transducer, the physico-chemical properties of the analyte and the operating conditions of the biosensor. Several methods have been proposed in the literature, including chemical and physical methods (Fig. 2) (D'Souza, 2001). Physical methods include adsorption, retention into a membrane or entrapment within a polymeric network. Adsorption is based on the establishment of low energy interactions between the functional groups of the bioreceptor and of the substrate surface. This type of immobilisation offers the advantage of preserving bioreceptor properties but results in the formation of weak bondings that favours its desorption. To avoid leakage processes, biological elements can be covered by a thin polymer membrane that allows diffusion of the target molecule or entrapped in a chemical or biological polymeric matrix. Sol-gel silica or

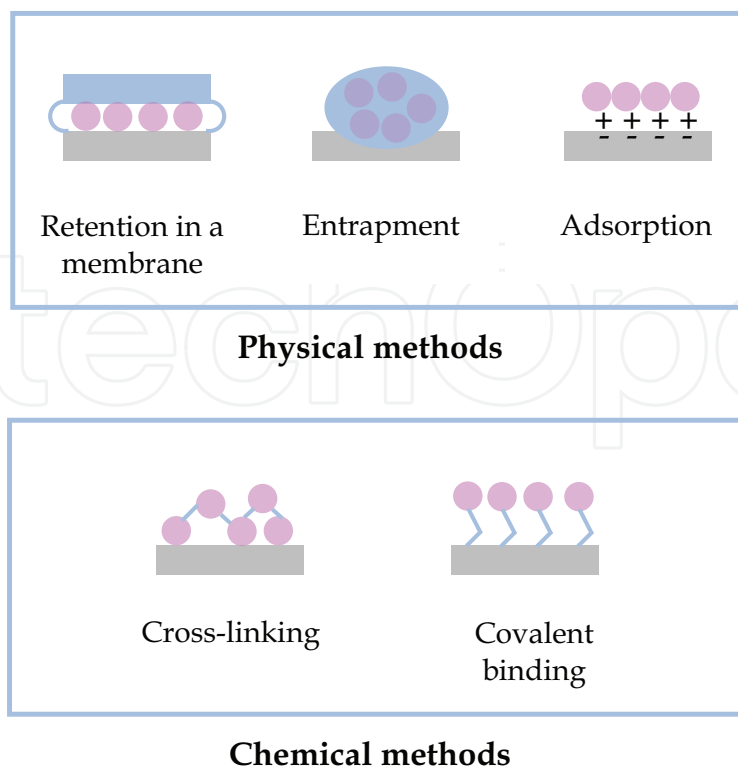


Fig. 2. The different methods for bioreceptor immobilisation

hydrogels are typically used for that purpose. These polymers can efficiently protect the bioreceptor from external aggressions but may form a diffusion barrier that restricts the accessibility to the substrate and/or decrease light and electronic transfers to the transducer. The swelling properties of hydrogels may also limit their practical application in some cases. In chemical methods, biosensing elements may be attached directly to the transducer through covalent bindings or to an inert and biocompatible matrix through cross-linking using a bi-functional reagent. Proteinic supports, e.g. bovine serum albumin or gelatine are typically used to constitute the network with glutaraldehyde (GA) as cross-linking agent. This second technique is primarily used to attach enzymes or antibodies to the transducer, less often to immobilize microorganisms. Indeed, cross-linking involves the formation of covalent bindings between the functional groups located on the outer membrane of cells and GA. This mode of immobilisation is consequently not suited when cell viability is absolutely required or enzymes involved in the detection are expressed at the cell surface. Finally, covalent grafting is based on the reaction between functional groups of the biological element and previously activated groups of the transducer. Functional groups of the bioreceptor are typically ϵ -amines of lysine, carboxyl groups of aspartate or glutamate, sulfhydryl groups of cysteine and hydroxyphenolic groups of tyrosine, which belong to the side chains of aminoacids in proteins (enzymes, antibodies or external cellular proteins). To ensure the formation of covalent bondings with the transducer, this latter has to be functionalised first. Metal surfaces such as gold and silver can be functionalised with amine, hydroxyl or carboxyl groups through reaction with aminoalkanethiols, hydroxyalkanethiols and carboxyalkanethiols, respectively. Oxide surfaces are functionalised with organosilanes. More recently, metal electrodes on which films of functionalised conducting polymer (polypyrrole, polythiophene, polyaniline) are deposited electrochemically, have been used to immobilise active biomolecules through covalent bonding formation (Teles & Fouseca,

2008). In recent years, particular attention has been also paid to the use of nanomaterials, typically gold nanoparticles, magnetic beads, carbon nanotubes (CNTs) or quantum dots (QDs) for the elaboration of biosensors (Xu et al., 2006; X. Zhang et al., 2009). Their particular chemical and physical properties make them very attractive to improve bioreceptor stability as well as biosensor sensitivity.

2.2 Application to the determination of specific (groups of) pollutants

2.2.1 Enzyme-based biosensors

A large number of enzymes has been used in the construction of water pollution biosensors. They may be classified into different families corresponding to the type of reaction they catalyze, typically oxidation, reduction and hydrolysis. The enzyme is immobilised on a transducer that detects the consumption of a co-factor, e.g. oxygen in the case of oxidase enzymes, or the appearance of a product following enzymatic reaction. Hydrolase enzymes are generally associated with optical fibres, potentiometric or conductometric transducers to detect local changes in pH or in conductivity. For their part, reductase or oxidase enzymes are generally immobilised on an amperometric or conductometric transducer to record electronic transfers towards the electrode. These electronic transfers may be promoted by the use of redox mediators that allow the application of lower potentials and limit interferences from other electroactive species.

Tyrosinase is a copper monooxygenase that catalyzes the hydroxylation of monophenols and the oxidation of diphenols into reactive quinones. This reaction has been extensively exploited for the determination of phenolic compounds using tyrosinase-based amperometric and optical biosensors. In electrochemical systems, substances produced by the reduction of quinones at the electrode can be detected at a low potential value, in the absence of mediator. Various electrode materials, such as gold (S. Wang et al; 2010), platinum (Yildiz et al., 2007), carbon paste (De Albuquerque et Ferreira, 2007; Mita et al., 2007), glassy carbon (Carralero et al., 2006; J. Chen & Jin, 2010; Hervas Perez et al., 2006; Kochana et al., 2008; Kong et al., 2009; Y.-J. Lee et al., 2007; S. Wang et al., 2008; L. Wang et al., 2010) or BDD (Notsu et al., 2002; Zhao et al., 2009, Zhou & Zhi, 2006) have been used for that purpose. Very recently, Yuan et al. (Yuan et al., 2011) developed an amperometric biosensor using a carbon fiber paper (CFP) electrode. This biosensor exhibited short response times (10-20s) and very high sensitivities to phenolic compounds such as catechol, phenol, bisphenolA and 3-aminophenol, corresponding detection limits being 2, 5, 5 and 12 nM, respectively. As seen in Table 1, these values are much better than other figures reported in the literature and are 4 to 10 times lower than the values obtained in the same experimental conditions, by the same authors, using a commercial screen-printed carbon electrode (SCPE). In this work, tyrosinase was immobilised in photoreticulated polyvinylalcohol (PVA-SbQ) matrix. Many other modes of immobilisation have been proposed to stabilize tyrosinase on the transducer including, among others, entrapment into titania sol-gel (Kochana et al, 2008), polyacrylamide microgel (Hervas Perez et al., 2006), Fe₃O₄ or multi-walled carbon nanotubes (MWNT)-chitosane composites (Kong et al., 2009; Wang et al., 2008), MWNT-epoxy resin (Perez-Lopez et al., 2007), physical adsorption on ZnO nanorods (Zhao et al., 2009), or covalent binding (Zhou et al., 2006; Wang et al., 2010). Optical tyrosinase-based biosensors have been also reported (Abdullah et al., 2006; Jang et al., 2010). Table 1 presents some recent biosensors based on tyrosinase enzyme, with the type of transducer and immobilisation used, as well as the detection limits obtained for typical phenolic contaminants.

Pollutant	Enzyme	Transduction	Detection limit (μM)	Reference	
<i>Phenolic compounds</i>					
17 β -estradiol	tyrosinase	Amperometry BDD electrode	10	Notsu et al., 2002	
Bisphenol A	tyrosinase	Amperometry BDD electrode	1	Notsu et al. Mita et al. Yuan et al.	
		CP electrode/SWCNT	0.02		
		Carbon fiber paper/PVA	0.005		
2,4-dichloro-phenol	tyrosinase	Amperometry GC electrode/MWNT+chitosane	0.06	Kong et al., 2011	
Catechol	tyrosinase	Amperometry GC electrode/MWNT/TiO ₂ /Nafion	0.087	Y.-J. Lee et al., 2007	
		MWNT/epoxy resin	10	Perez-Lopez et al., 2007	
		GC electrode/MWNT/TiO ₂ /Nafion	0.09	Kochana et al., 2008	
		GC electrode/Fe ₃ O ₄ /chitosane	0.025	Sang et al., 2008	
		GC/Teflon/ Au nanoparticles	0.003	Carralero et al., 2006	
		GC/polyacrylamide microgel	0.3	Hervas Perez et al., 2006	
		Pt electrode/EDP	0.01	Yildiz et al., 2007	
		GC electrode/ TiO ₂	0.09	Kochana et al., 2008	
		CP electrode/ CoPc	0.25	De Albuquerque et al., 2007	
		GrC-acethylcellulose/ CoPc	0.45	De Albuquerque et al., 2007	
		Carbon fiber paper/PVA	0.002	Yuan et al., 2011	
		SPCE/PVA	20	Yuan et al., 2011	
			Optical Glass/SiO ₂ /Nafion	2.1	Abdullah et al., 2006
Phenol	tyrosinase	Amperometry GC electrode/MWNT/TiO ₂ /Nafion	0.095	Y.-J. Lee et al., 2007	
		Functionalised Au electrode	0.1	L. Wang et al., 2010	
		GC electrode/palygorskite	0.05	J. Chen & Jin, 2010	
		Functionnalised BDD electrode	0.2	Zhou et al., 2006	
		GC/polyacrylamide microgel	1.4	Hervas Perez et al., 2006	
		Pt/EDP	0.1	Yildiz et al., 2007	
		GC electrode/ TiO ₂	0.13	Kochana et al., 2008	
		BDD electrode/ ZnO nanorods	0.5	Zhao et al., 2009	
		Carbon fiber paper/PVA	0.005	Yuan et al., 2011	
		SPCE/PVA	20	Yuan et al., 2011	
			Optical Glass microarray/PEG-DA/QD	1	Jang et al., 2010
			Glass/SiO ₂ /Nafion	1.9	Abdullah et al., 2006
		4-chlorophenol	tyrosinase	Amperometric GC electrode/MWNT/TiO ₂ /Nafion	0.11
GC electrode+Teflon+Au nanoparticles	0.02			Carralero et al., 2006	
Functionnalized BDD electrode	0.1			Zhou et al., 2006	
GC/polyacrylamide microgel	0.03			Hervas Perez et al., 2006	
GC electrode/ TiO ₂	0.17			Kochana et al., 2008	
BDD electrode/ ZnO nanorods	0.4			J. Zhao et al., 2009	

Pollutant	Enzyme	Transduction	Detection limit (μM)	Reference
<i>Trophic pollutants</i>				
Nitrates	Nitrate reductase	Amperometry Pt electrode/Ppy	10	Sohail et al., 2009
		Conductometry Au electrode /GA /Nafion	5	Xuejiang et al., 2006
Nitrites	Nitrite reductase	Amperometry GC electrode/[ZrCr-AQS]-LDH/GA	0.004	H. Chen et al., 2007
		Conductometric GC electrode/modified MV	0.06	Quan et al., 2010
		Au electrode /Nafion	0.05	Z. Zhang et al., 2009
Phosphates	Maltose phosphorylase	Conductometric Au electrode /GA	1	Z. Zhang et al., 2008
		Amperometry Pyruvate oxidase Pt electrode/Nafion/PCS hydrogel SPC electrode/acetate cellulose	3.6 <300	Gilbert et al., 2010 Khadro et al., 2009
Organic Matter (proteic fraction)	Protéinase K + pronase	Conductometric	0.583 $\mu\text{g/L}$ for TOC	
<i>Organophosphorous pesticides</i>				
Dichlorvos	tyrosinase (inhibition)	Amperometric GC electrode/NQ/Nafion	0.17	Vidal et al., 2008
		GC electrode/NQ/o-PPD	0.19	
		GC electrode/Nafion	0.07	
methyl parathion	OPH	Amperometric GC electrode/ MWCNT/ Au/QD	0.004	Du et al., 2010
		GC electrode/ MWCNT	0.8	Deo et al., 2005
demeton-S	OPH	Amperometric CSP electrode/MWCNT	1	Joshi et al., 2006
paraoxon	OPH	Amperometric GC electrode/ MWCNT	0.15	Deo et al., 2005
		GC electrode/mesoporous C/C black	0.12	J.H. Lee et al., 2010
		Piezoelectric Microcantilever/LbL	0.1	Karnati et al., 2007
		SPR Au/SiO ₂	20	Luckarift et al., 2007
		OWLS Glass/TiO ₂ array	2.5	Ramanathan et al., 2007
		PMMA/sol gel	0.004	Zourob et al., 2007

AQS: anthraquinone sulfonate, BDD: boron doped diamond, CoPc: cobalt phtalocyanine, CP: carbon paste, CSP: carbon screen-printed, EDP: electrodeposition polymer, GA: glutaraldehyde, GC: glassy carbon, GrC: graphite carbon, Ppy: polypyrrole, LbL: layer by layer, LDH: Layered double hydroxide, MV: methylviologen, MWCNT: multi-walled carbon nanotubes, NQ: naphthoquinone, PCS: poly(carbamoyl) sulfonate, PVA: polyvinylalcohol, QD: quantum dot, SPC: screen-printed carbon, SWCNT: single-walled carbon nanotubes.

Table 1. Examples of enzymatic biosensors for the detection of chemical pollutants.

Another enzyme, organophosphate hydrolase (OPH), has been also commonly used for the development of electrochemical, optical and mechanical biosensors for organophosphorous pesticides detection, while nitrate reductase, nitrite reductase, maltose phosphorylase, pyruvate oxidase have been employed for the determination of trophic pollutants such as nitrates, nitrites or phosphates (Table 1).

2.2.2 Immunosensors

Immunosensors are based on highly selective antibody (Ab) - antigen (Ag) reactions. The immobilized sensing element can be either an Ab or an Ag which can be chemically modified (hapten). In the first case, analyte binding is measured directly. In the second case, the method is based on the competition between immobilized Ag, the analyte (Ag) and a fixed amount of Ab. All types of immunosensors can either be run as nonlabeled or labeled immunosensors. Label free immunosensors rely on the direct detection of antigen-antibody complex formation by measuring variations in electrical properties using electrochemical impedance spectroscopy (EIS), or changes in optical properties using SPR. The second type of immunosensors use signal-generating labels which allow more sensitive and versatile detection modes. Peroxidase, glucose oxidase, alkaline phosphatase, catalase enzymes and electroactive compounds such as ferrocene are the most common labels used for electrochemical detection, while fluorescent labels (rhodamine, fluorescein, Cy5, etc...) are employed for optical detection. Some recent examples are presented in Table 2. Over the two past decades, a large number of immunosensors targeting individual pollutants or groups of pollutants and based on these different configurations have been reported. Recent developments have been focused on label free immunosensors using EIS and SPR detection (Mitchell, 2010; Prodromidis, 2010) as well as on improvements in antibody design (Conry et al., 2009).

2.2.3 Cell-based biosensors

Many works have been focused on the development of cell-based biosensors. Bacteria, algae and yeasts are the main organisms used for that purpose. Various types of strains have been exploited, from commercial and well-characterized cells harbouring a broad range of substrates to genetically-engineered organisms specially constructed to detect specific molecules or groups of molecules, passing through environmental cells isolated from polluted sites offering higher robustness and more specific enzymatic properties. Cell membranes can be permeabilized in order to improve accessibility to internal enzymes. Compared to their individual components (enzymes, antibodies or DNA), cells are easier to produce in large quantities and are more tolerant to pH, ionic strength and temperature variations. Owing to the large number of enzymes and cofactors that the cells contain, a large variety of biosensors has been proposed for the detection of specific (groups of) analytes or for aquatic toxicity assessment, this latter application being addressed in section 2.3. Several reviews have been published on the topics (Lei et al., 2006), some of them being more specifically dedicated to yeast-based sensors (Baronian et al., 2004), genetically-modified bacteria sensors (Daunert et al., 2000; Girotti et al., 2008; Hansen & Sorensen, 2001; Van der Meer & Belkin, 2010; Woutersen et al., 2010), or electrochemical cell biosensors (Lagarde & Jaffrezic-Renault, 2011).

2.2.3.1 Electrochemical biosensors

Amperometry is the most common electrochemical transduction mode used in whole cell biosensors. It allows detecting oxygen consumption or production during respiration/

photosynthesis processes, consumption or production of specific compounds in course of analyte metabolisation, or induction of a specific enzyme activity by genetically modified microorganisms (Lagarde & Jaffrezic-Renault, 2011). Different microbial strains exhibiting a wide range of substrates have been used for the determination of biological demand of oxygen (BOD), an index of the amount of degradable organic compounds present in the sample (Nakamura, 2010; Ponomareva et al., 2011). Oxygen consumption during biological respiration is generally detected by means of conventional Clark type electrodes, but miniaturized systems based on small-size carbon screen-printed electrodes (SPEs) have been also proposed in recent years. In the same way, biosensors able to detect surfactants, phenolic derivatives, alcohols or organophosphorous pesticides have been constructed by immobilizing bacteria degrading specifically these groups of pollutants on classical oxygen,

Pollutant	Detection mode	Transduction	Detection limit (ng L ⁻¹)	Reference
<i>Pesticides</i>				
Isoproturon	Competitive/ Cy5.5 fluorescence labelling	TIRF	20	Tschmelak et al., 2005
Chlorpyrifos	Competitive/no labelling	SPR	55	Mauriz et al., 2006
DDT and derivated products	Competitive/no labelling	SPR	15	Mauriz et al., 2007
Atrazine	Direct	EIS	10	Hleli et al., 2006
	Competitive/ Cy5.5 fluorescence labelling	TIRF	10	Tschmelak et al. 2005
	Competitive	SPR	500	Farre et al., 2007
Picloram	Competitive HRP labelling	Amperometric	< 1	L. Chen et al., 2010
<i>EDCs</i>				
Testosterone	Competitive/labelling	TIRF	1.7	Tschmelak et al. 2006
	Competitive/no labelling	Amperometric	170	Eguilaz et al., 2010
Estradiol	Competitive	SPR	300	Ou et al., 2009
Bisphenol A	Direct	EIS	400	Rahman et al., 2007
	Competitive/labelling	TIRF	8	Marchesini et al., 2005; Tschmelak et al., 2005
Nonylphenols	Direct/HRP labelling	Amperometric	10000	Evtugyn et al., 2006
2,4-dichloro-phenoxyacetic acid	Competitive/Cy5.5 fluorescence labelling	TIRF	90	Long et al., 2008
	Competitive/no labelling	SPR	100	Kim et al., 2007
	Competitive (signal amplification)	SPR	8	Kim et al., 2007
<i>Toxins</i>				
Microcystin-LR	Competitive/Cy5.5 fluorescence labelling	TIRF	30	Long et al., 2008

Table 2. Examples of immunologic biosensors for the detection of chemical pollutants.
EDC: endocrine disrupting compound

graphite carbon or carbon paste electrodes, more recently on SPEs (Lagarde & Jaffrezic-Renault, 2011). Table 3 presents the most recent examples of electrochemical biosensors developed for BOD measurement and for the determination of specific (groups of) analytes. To modify cell resistance and sensitivity towards toxic compounds, microorganisms may be genetically modified. Buonasera *et al.* recently combined on a single biosensing platform amperometric and optical modes of transduction as well as several genetically modified algal strains harbouring various degrees of sensitivity and resistance towards pesticides. The system allowed detecting different subclasses of pesticides in the 0.1 to 10 nM range (Buonasera *et al.*, 2010). To enhance selectivity, genetical modification of the cells is also possible by fusing a natural regulatory circuit existing in the microorganism with a promoterless gene encoding for an easily measurable protein expressed only when the analyte(s) is present. The most common gene used for electrochemical detection is *lacZ* encoding β -galactosidase. Activation of β -galactosidase is generally followed through the increase of its enzymatic activity using *p*-aminophenyl β -D-galactopyranoside (PAPG) as substrate. PAPG is transformed into *p*-aminophenol oxidized at the amperometric electrode. Tag *et al.* (Tag *et al.*, 2007) proposed another method of detection using lactose as deputy substrate.

Potentiometric and conductometric biosensors have been also developed for the determination of specific pollutants. For example, a biosensor based on *P. aeruginosa* J1104 immobilized on a chloride ions-selective solid-state electrode has been reported for trichloroethylene detection in waters. More recently, Hnaïen *et al.* (Hnaïen *et al.*, 2011) proposed a fast, sensitive and miniaturized whole cell conductometric biosensor for the determination of the same pollutant. The biosensor assembly was prepared by immobilizing *P. putida* F1 bacteria at the surface of gold microelectrodes through a three dimensional alkanethiol self-assembly monolayer/carbon nanotubes architecture functionalised with *Pseudomonas* antibodies. pH electrodes have also been widely used to detect H⁺ ions produced through enzymatic reactions (Kumar *et al.*, 2008).

2.2.3.2 Optical biosensors

Optical biosensors rely on the modulation of cell optical properties (UV-Visible absorption and bioluminescence, reflectance, fluorescence) following interaction with compounds present in the sample. Most of the optical biosensors proposed are based on bioluminescence or fluorescence detection. The so-called "light-off" systems measure a decrease in the cellular light emission following exposure to the pollutant(s). They are mainly used for water toxicity assessment (§2.3.5). The detection of specific analytes or groups of analytes is rather performed using "light-on" type biosensors, where the interaction causes an increase of light signal proportional to the analyte concentration. "Light-on" microorganisms are produced naturally or via genetic engineering. The most frequently used genes are *lux* gene encoding for luminescent luciferase and *gfp* gene encoding for fluorescent GFP (green fluorescent protein). A variety of well-characterized promoters is available for genetic manipulations and has been used to construct new organisms able to sense specifically different classes of pollutants, including metals (copper, mercury, lead, cadmium, arsenic ...), hydrocarbons and organic solvents or pesticides. Many examples have been reported in the literature (Daunert *et al.*, 2000; Lei *et al.*, 2006). Naturally emitting bacteria have been also used for BOD determination (Lin *et al.*, 2006; Sakaguchi *et al.*, 2007).

Target	Microorganism	Transduction	Detection limit	Reference
<i>BOD</i>				
	<i>Saccharomyces cerevisiae</i>	Amperometry	6.6 mg L ⁻¹	Nakamura et al., 2010
	Microbial consortium (BODSEED)	Amperometry	< 5 mg L ⁻¹	L. Liu et al., 2010
	<i>Escherichia coli</i> DH5a	Potentiometry	1mg L ⁻¹	Chiappini et al., 2010
	<i>Photobacterium phosphoreum</i> IFO 13896	Luminescence	1mg L ⁻¹	Sakaguchi et al., 2007
	<i>B. licheniformis</i> , <i>D. maris</i> , <i>M. marinus</i>	Optical fibre	0.2 mg L ⁻¹	Lin et al., 2006
<i>Phenolic compounds</i>				
Phenol	<i>Pseudomonas putida</i> DSM 50026	Amperometry	500 µM	Timur et al.
<i>p</i> -nitrophenol	<i>Pseudomonas</i> sp.	Amperometry	< 10 µM	Timur et al.
<i>Organophosphorous pesticides</i>				
Paraoxon, parathion	Modified <i>P. putida</i> JS444	Amperometry	0.001 µM	Lei et al., 2005
Fenithron, EPN			0.005 µM	Lei et al., 2007
<i>Heavy metals</i>				
Cu	<i>S. cerevisiae</i> 19.3C/ <i>CUP1::lacZ</i>	Amperometry	0.1 µM	Tag et al., 2007
	<i>S. cerevisiae</i> SEY6210/ <i>CUP1::lacZ</i>		33 µM	
<i>Endocrine disrupting compounds</i>				
	<i>S. cerevisiae</i> Y190 <i>medER ::lacZ</i>	Amperometry	-	Ino et al., 2009
<i>Antibiotics</i>				
Cephalosporins	<i>P. aeruginosa</i> MTCC 647	Potentiometry	100 µM	Kumar et al., 2008
<i>Organic solvents</i>				
Benzene	<i>P.putida</i> L2	Amperometry	10 µM	Lanyon et al., 2006
Trichlorethylene	<i>P. aeruginosa</i> JI104	Potentiometry	0.22 µM	Han et al., 2002
	<i>P. putida</i> F1	Conductometry	0.07 µM	Hnaien et al., 2011

Table 3. Some recent examples of cell based biosensors for the detection of specific (groups of) pollutants

2.3 Application of biosensors to the assessment of aquatic toxicity

Most of biosensors developed for toxicity assessment exploit toxic effects of the pollutants, including enzyme inhibition, such as AchE inhibition by neurotoxic compounds, interaction with a specific receptor (androgenicity, estrogenicity), interaction with and damage of DNA or RNA (genotoxicity). The detection of some biomarkers of toxicity may be also used.

2.3.1 Enzyme biosensors

A major contribution of enzyme biosensors to ecotoxicological studies concerns aquatic neurotoxicity assessment. This latter may be due to organophosphate and carbamate

pesticides, heavy metals or detergents that inhibit esterase enzymes. Neurotoxicity biosensors proposed in the literature are mostly based on two enzymes belonging to this family, acetylcholinesterase (AChE) and butylcholinesterase. Many works and two reviews have been published on this type of biosensor (Jaffrezic-Renault, 2001; S. Liu et al., 2008). Current developments aim to improve enzymatic activity, either by genetic modification (Bucur et al., 2006) or by a better immobilisation on the transducer. The use of new materials based on gold, silver or iron nanoparticles (Du et al. 2008; Gan et al., 2010; Gong et al. 2009; Shulga et al., 2007; W. Zhao et al., 2009) or on carbon nanotubes (Viswanathan et al., 2009) also allows significant increase of the sensitivity of electrochemical and optical biosensors. A portable system using a potentiometric transduction and AChE as bioreceptor has been recently validated on different samples of water (Hildebrandt et al., 2008). Cortina et al. (Cortina et al., 2008) proposed an enzyme-based array that used three AChE enzymes: the wild type and two different genetically modified enzymes. Multianalyte devices combining informations from several different types of enzymes have been also proposed. For example, Soldatkin et al. recently developed an amperometric multibiosensor using the inhibition of acetylcholinesterase, butyryl- cholinesterase, urease, glucose oxidase, and three-enzyme system (invertase, mutarotase, glucose oxidase) for water toxicity assessment (Soldatkin et al., 2009).

2.3.2 Estrogen receptor-based biosensors

Endocrine disruptors (EDCs) are chemical substances that cause hormonal imbalances and impair endocrine or nervous systems. Some of these compounds affect the synthesis of endogenous hormones or that of their receptors. Others are structurally similar to estrogens and bind to their receptors, leading to their inactivation or to abnormal behaviours. Many molecules, such as synthetic hormones or chemical substances such as phthalates, surfactants, PCBs, alkylphenols, parabens, PAHs, dioxins and some pesticides, are EDCs. To date, 320 priority substances suspected to disrupt endocrine system have been identified by the European Community. Some of them (nonylphenol, di-2-ethylhexylphthalate and polybrominated diphenyl ethers) have been included in the list of priority substances of the Water Framework Directive. A review has addressed the use of biosensors for environmental EDCs monitoring (Rodriguez-Mozaz et al. 2004).

Toxicity biosensors rely on EDCs binding on estrogen receptors immobilised on the surface of a transducer. The estrogen receptor of human origin (ER- α) is the most often used. Different transduction modes such as fluorescence, cyclic voltammetry, SPR, electrophoretic mobility, have been proposed. Portable systems, mainly based on SPR detection have also been developed (Habauzit et al., 2007). Recently, a biosensor containing carbon nanotubes functionalised with the α -type human estrogen receptor and using a FET as transducer has been reported. The response time was extremely rapid (2 min) (Sanchez-Acevedo et al., 2009). Another biosensor, using impedance as transduction mode, was fabricated by immobilizing ER- α in a supported bilayer lipid membrane modified with Au nanoparticles. (Xia et al., 2010) The results indicated that the biosensor was able to detect the natural estrogen 17 β -estradiol with an acceptable linear correlation ranging from 5 to 150 ng/L and a detection limit of 1 ng/L. The biosensor could also detect bisphenol A and 4-nonylphenol. Im et al. (Im et al., 2010) propose to bind ER- α receptor covalently on a gold electrode for impedimetric detection of 17 β -estradiol. The detection limit was 1 μ M.

Toxicity mechanism	Pollutants	Microorganisms	Transduction	Reference
Inhibition of AChE activity	Cd, Zn	<i>C. vulgaris</i>	Conductometry	Chouteau et al., 2005
Inhibition of AP activity	OPs	<i>C. vulgaris</i>	Conductometry	Chouteau et al., 2005
	Cd	<i>C. vulgaris</i>	Conductometry	Guedri et al., 2008
	Cd, Zn	<i>C. vulgaris</i>	Amperometry	Chong et al., 2008
Inhibition of respiratory activity	Antibiotics	<i>E.coli</i> JM 105	Amperometry	Mann & Mikkelsen, 2008
	Hg, Cu, Zn, Ni, phenolic compounds	<i>E.coli</i>	Amperometry	H. Wang et al., 2008
	KCN, As ₂ O ₃ , Hg ²⁺	<i>E.coli</i> DH α	Amperometry	C. Liu et al., 2009
Inhibition of photosynthetic activity	Atrazine, DCMU Formaldehyde	<i>C. vulgaris</i>	Amperometry	Shitanda et al., 2009
		<i>C. vulgaris</i> / <i>P. subcapitata</i> / <i>C.reinhardtii</i>	Amperometry	Tatsuma et al., 2009
Inhibition of luminol peroxidase activity	Pb ²⁺ , Hg ²⁺ , Cu ²⁺	<i>Vibrio fischeri</i>	Luminescence	Komaitis et al., 2010
Genotoxicity	Nalidixic acid, mitomycin C, H ₂ O ₂	<i>E.coli</i> RFM443 <i>nrdA</i> :: <i>luxCDABE</i>	Luminescence (induction)	Hwang et al., 2008
	Mitomycin C, nalidixic acid, MNNG, 4-NQQ	<i>E. coli</i> RFM443 with <i>recA</i> , <i>NrdA</i> , <i>dinI</i> , <i>sbmC</i> , <i>recN</i> , <i>sulA</i> or <i>alkA</i> promoters and <i>luxCDABE</i> reporter	Luminescence (induction)	Ahn et al., 2009
	Nalidixic acid	<i>E. coli</i> RFM443 <i>sulA</i> :: <i>phoA</i>	Amperometry (induction of AP)	Ben Yoav et al., 2009
	IQ	<i>S. typhimurium</i> TA1535 <i>umuC</i> :: <i>lacZ</i>		
	Mitomycin C, ethidium bromide, H ₂ O ₂ , toluene, pyrene, benzo[a]pyrene, MMS	<i>Acinetobacter baylyi</i> ADP1 <i>recA</i> :: <i>luxCDABE</i>	Luminescence (induction)	Song et al., 2009
	Mitomycin, H ₂ O ₂	<i>E. coli</i> RFM443 <i>grpE</i> :: <i>luxCDABE</i> and <i>recA</i> :: <i>luxCDABE</i>	Luminescence (induction)	Eltzov et al., 2009
Mitomycin C, pentachlorophenol, H ₂ O ₂	<i>E. coli</i> K12 <i>recA</i> :: <i>luxCDABE</i> and <i>ColD</i> :: <i>luxCDABE</i>	Luminescence (induction)	Kotova et al., 2010	

Toxicity mechanism	Pollutants	Microorganisms	Transduction	Reference
Protein damage	Phenol	<i>E. coli DnakA::lacZ</i> <i>E. coli grpE::lacZ</i>	Amperometry (induction of β -galactosidase)	Popovtzer et al., 2006
Membrane damage	Phenol	<i>E. coli fabA::lacZ</i>	Amperometry (induction of β -galactosidase)	Popovtzer et al., 2006
Heat shock	Mitomycin C, pentachlorophenol, H_2O_2	<i>E. coli grpE::luxCDABE</i> <i>E. coli plbpA::luxCDABE</i>	Luminescence (induction)	Kotova et al., 2010
Oxidative stress	Cd^{2+} , Cu^{2+} , Pb^{2+} , Zn^{2+} H_2O_2 , menadione, selenite, arsenite, triphenyltin naphthalene	<i>E. coli</i> DH5 α <i>pRSET::roGFP2</i>	Fluorescence (induction)	Arias-Barreiro et al., 2010
	Mitomycin C, pentachlorophenol, H_2O_2	<i>E. coli</i> K12 <i>katG::luxCDABE</i> and <i>SoxS::luxCDABE</i>	Luminescence (induction)	Kotova et al., 2010
	Paraquats and derivatives, H_2O_2	Various strains and promoters with <i>luxCDABE</i> reporter	Luminescence (induction)	J.Y. Lee et al., 2007

AchE: acetylcholinesterase, AP: alkaline phosphatase; DCMU: 3-(3,4-dichlorophenyl)-1,1-diethylurea, IQ : 2-amino-3-methylimidazo[4,5-f]quinoline, MNNG: 1-methyl-1-nitroso-N-methylguanidine, MMS: Methyl methanesulfonate, 4-NQQ: 4-nitroquinoline N-oxide

Table 4. Some recent examples of toxicity cell-based biosensors.

2.3.3 Immunosensors

As seen in section 2.2.2, a large number of applications of immunosensors relate to the determination of pollutants or groups of target pollutants. It is also possible to exploit them for the detection of substances, called biomarkers, that are produced by an organism following exposure to specific pollutants. Vitellogenin, for example, is a phospholipo-serum glycoprotein secreted in large quantities by fish exposed to endocrine disruptors. Its presence is suitable for identifying oestrogenotoxic effects of natural or anthropogenic substances. Vitellogenin may be detected using electrochemical, optical or piezoelectric biosensors based on carp anti-vitellogenin antibody (Bulukin et al., 2007).

2.3.4 DNA biosensors

DNA structure is extremely sensitive to the influence of environmental pollutants such as heavy metals, polycyclic aromatic compounds and PCBs. These substances possess high affinity for DNA, at the origin of mutagenic and carcinogenic effects. Biosensors, measuring the interactions between these substances and single or double strand DNA molecules immobilised on a transducer, have been developed and used for water genotoxicity assessment. Electrochemical transduction is the most commonly used (Nowicka et al., 2010). The compounds bound to DNA are detected, either directly when electroactive species are involved, or through the modification of DNA electrochemical signal. Toxicity biosensors based on the detection of DNA bases oxidation (mainly guanine, but also guanosine and

adenosine) or on the degradation of the strands using an electrochemical probe, have been developed and applied to the analysis of water samples containing different types of genotoxic aquatic contaminants (metals, pesticides, PCBs, aromatic amines ...). Some of these biosensors were favorably compared to commercial genotoxic assays.

Other types biosensors using either optical (SPR, fluorescence) or mechanical transduction have been also proposed (Palchetti & Mascini, 2008).

2.3.5 Biosensors based on whole cells

Bacteria, yeasts, algae and fish cells have been also used for the development of toxicity biosensors (Baronian, 2004; Daunert et al., 2000; Hansen & Sorensen, 2001; Lagarde & Jaffrezic-Renault, 2011; Lei et al., 2006; Girotti et al., 2008; Van der Meer et al., 2010; Woutersen et al., 2010). The biosensor response may be due to a change in cell metabolism (inhibition of enzyme activity, respiration or photosynthesis), cell alteration, death, or change in the expression of certain genes (modified organisms).

Many examples of electrochemical and optical biosensors proposed for toxicity assessment may be found in the different reviews cited above. The most recent ones are given in Table 4. Optical biosensors are mainly based on luminescent modified bacteria, using typically the *recA*, *uvrA*, *NrdA* promoters for DNA damage detection, the *grpE* and *dnaKp* promoters for protein damage detection, and the *fab A* promoter for cell membrane damage (Woutersen et al., 2010).

3. Conclusion

Despite the large number of works carried out on the field of biosensors for water analysis, and although they have many benefits, very few systems have so far been marketed, unlike bioassays. Most commercial biosensors are versatile and suitable for applications in various fields such as environment, biological analysis or medical (Rodriguez-Mozaz et al., 2005).

Significant efforts still have to be done to obtain selective, robust, rapid and sensitive tools usable in the field. The main limitation of the proposed systems come from the biological elements. Current developments include enhancement of their sensitivity, selectivity and their stability by genetic engineering (Girotti et al., 2008; Campas et al., 2009; Conroy et al., 2009). Recent progress in this area as well as in data numerisation, transmission and processing allows now the construction of arrays of microorganisms or of enzymes arranged on a single detection platform for the determination of several parameters at the same time. In parallel, the development of new biomimetic receptors such as that molecularly imprinted polymers (MIP) or aptamers (synthetic oligonucleotides) is expanding to overcome the fragility of natural bioreceptors (Wang et al., 2007; Guan et al., 2008). Methods allowing a more efficient immobilisation of the bioreceptor will also have to be developed to improve the robustness and sensitivity of biosensors. The exploration of new materials, including gold nanoparticles, carbon nanotubes or quantum dots is an extremely promising route to achieve this goal.

Essential progress has also been made in recent years in the miniaturization of transducers (nanoelectrodes, nanowaveguides, BioMEMS) and will contribute to reduce significantly the amount of biological entity required, but also to improve integration of the systems in labs on chips (Ligler, 2009; Wei et al., 2009).

4. References

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This book is a collection of contributions from leading specialists on the topic of biosensors for health, environment and biosecurity. It is divided into three sections with headings of current trends and developments; materials design and developments; and detection and monitoring. In the section on current trends and developments, topics such as biosensor applications for environmental and water monitoring, agro-industry applications, and trends in the detection of nerve agents and pesticides are discussed. The section on materials design and developments deals with topics on new materials for biosensor construction, polymer-based microsystems, silicon and silicon-related surfaces for biosensor applications, including hybrid film biosensor systems. Finally, in the detection and monitoring section, the specific topics covered deal with enzyme-based biosensors for phenol detection, ultra-sensitive fluorescence sensors, the determination of biochemical oxygen demand, and sensors for pharmaceutical and environmental analysis.

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