MICROBIOLOGICAL SENSORS FOR THE MONITORING OF WATER QUALITY

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1. Introduction

The environmental monitoring of pollutants with automatic systems, applied on-line and allowing rapid responses constitutes one of the most successful ways to improve the quality of the environment. Real time toxicity analysis offers the advantage of rapidly detecting sources of pollution and preventing any accidental release of pollutants. There is a need for in situ continuous biomonitoring of underground waters, surface waters and rivers, of pollution sources, and of the efficiency of depuration processes.. Such a strategy is not possible with conventional toxicity tests, carried out in the laboratory and in static conditions. In situ continuous biomonitoring has thus encouraged the development of biological alarm systems, giving a rapid response, in real time, which can be automatically recorded.

In order to fulfil these requirements, the concept of these alarm systems must differ from classical aquatic toxicity tests :

- their response must be delivered after a short period of exposure of the biocatalyst to the flow : this implies following other relevant criteria than growth, mortality or reproduction,
- the biological response must be easily recorded and converted into an electric signal which may set off an alarm. A suitable association of the biocatalyst and the transducer is needed to ensure the performance of the system,
- an immobilization is necessary when using whole cells as biocatalysts, as they are used in flow-through conditions.

Environmental biomonitoring and toxicity assessment require devices with a broad spectrum sensitivity rather than highly specific ones. As the array of contaminants is wide and the threat unknown, the choice of cellular rather than molecular systems is more suitable.

If there is a need to increase testing for an improved protection of the environment, there is also a need for fast, simple and cheap ecotoxicological methods. Microbial biosensors probably show a greater potential for pollution monitoring and toxicity assessment than other alarm warning systems using aquatic invertebrates and vertebrates, or immobilized enzymes and antibodies.

2. Microbial biosensors

Whole cell biosensors probably offer the greatest technological changes among early warning systems, with an immobilized biocatalyst (in most cases) in close contact with a transducer unit. Their development has benefitted from biotechnological progress in enzyme electrodes used to control fermentation processes in food technology and medical analyses. To date, bacteria, yeasts and photosynthetic cells (cyanobacteria and microalgae) constitute the main biocatalysts used in microbiological biosensors.
Whilst some microbial biomonitors propose the use of free cells in bioreactors, most systems utilize biocatalysts immobilized on membranes or entrapped in gel matrices.

Microbiological biosensors combine the advantages of the two other categories of early warning systems (E.W.S.) developed today for aquatic environmental biomonitoring : in one hand, enzyme and immunological electrodes, and E.W.S. using vertebrates and invertebrates in the other hand. Microbiological biosensors share the advantages of molecular electrodes :

- the miniaturization of systems using immobilized cells,
- the immobilization, which ensures practical handling in flow-through conditions.

Cells must be immobilized by gentle processes in order to maintain cellular integrity : these processes are restricted to adsorption or entrapment in non-toxic polymers. The weakness of adsorption can lead to leakage problems of the biocatalysts in the flow. On the other hand, gel matrix is permeable to organic substances but impermeable to cations, the toxicity of which will not be detected by entrapped cells.
In contrast to molecular electrodes which exploit only one combination enzyme/substrate or antigen/antibody, microbial biocatalysts are living cells, i.e. complete organisms with multiple biochemical pathways governed by multiplicity of enzymes, which thus offer the greatest potential of investigation. Therefore, microbial biosensors share the property of presenting a wide spectrum of response to toxicants, with vertebrates and invertebrates : this has already been underlined as an incomparable advantage in environmental biomonitoring. Yet, it must not be forgotten that microbial biocatalysts are alive and must be kept alive to remain active. Consequently, nutrients must be provided during the servicing time of the biosensor : this is a source of problems in the conception and the construction of the device and in its sensitivity.

All these systems must deliver a signal after a short period of exposure to toxicants : this influences the choice of the parameter(s) to be monitored because biological changes in the short term must be representative and predictive of toxicity in the long term. The biomonitoring of energetic cellular processes is privileged because of their relevance as criteria for toxicity. Indeed, ATP levels control a number of essential physiological events, such as motility, synthesis of cell constituents, growth, division, viability,....and any impairment of ATP synthesis will have dramatic consequences on these processes. Respiratory activity and photosynthesis are the main metabolic pathways monitored in microbial biosensors (tables 1-4). Respiration can be easily followed by measuring oxygen consumption or CO_2 production with an electrochemical transducer ; photosynthetic activity will also be controlled by measuring O_2 production after exposing the cells to light.

Optrodes are transducers of interest due to their high sensitivity for measuring the fluorescence of photosynthetic pigments or bioluminescence produced during respiration in naturally luminescent bacterial cells (tables 2 and 3).

Measuring electron transfer between redox components of the respiratory or photosynthetic pathway has also been recommended. Yet, the transfer of electrons to the electrode often requires the use of mediators, such as quinone derivatives or ferricyanide. Mediators may be toxic to the biocatalyst in the long term, which will impair its longevity.

New systems using genetically transformed microorganisms are now emerging : these systems exploit the simplicity of luminescent measurements in order to identify the activation of genes as a result of exposure to pollutants. Lux gene is inserted in the promoter region of the genes controlled, whose transcription will be expressed by light emission. Thus these systems detect very early events ; their specificity will depend on the role of the genes controlled, whether they express an exposure to a specific toxicant or not. The activation of genes which code for stress proteins for instance, can be considered as a general response. In other cases, the response can be very specific to a particular toxic substance (table 2).

3. Field of application of microbiological biosensors

The degree of sensitivity and specificity of biosensors condition their field of application. A response to a wide spectrum of toxicants is necessary when controlling surface waters, underground waters and wastewaters, whose contamination sources are multiple and pollutants unknown. In contrast, biosensors of high specificity will be devoted to situations with identified polluting substances (Osbild et al., 1995).

Non specific bacterial biosensors generally detect chemicals at concentrations from 10^2 - 10^3 µg/l or µM (tables 1 and 2). This level of sensitivity is a little lower than other E.W.S. using fish or bivalves, and apparently far below the sensitivity of algal biosensors with fluorimetric transducers (table 3).

The field of application of bacterial biosensors as alarm systems is the following :
the detection of high pollution loads likely to induce toxicity in the short term ; this includes the monitoring of rivers used for the production of drinking water,
the survey of complex effluents and wastewaters before their discharge into surface waters,
the monitoring of treatment plants, performed (i) to protect biofilms from an input of high toxic charges, and (ii) to control the efficiency of depuration processes.

The performance of algal biosensors appears better than non-specific bacterial sensors. Nevertheless, most experiments have been carried out with herbicides which are specifically toxic to photosynthetic activity : it cannot be excluded that their sensitivity to other kinds of contaminants may be lower. Nonetheless, photosynthetic cells are the ideal biocatalysts to detect pollutants toxic to the vegetal world. Consequently, they must necessarily be included in a strategy for water biomonitoring.

The sensitivity of microbiological biosensors depends on three main parameters, that is to say :

- the sensitivity of the biocatalysts themselves,
- the nature of the transducer connected to the biocatalyst. For instance, increased sensitivity over two orders of magnitude was seen in algal biosensors when substituting fluorescent receptors to O_2 electrodes (Schmitz et al. 1993 ; Pandard and Vasseur, 1992).
- the environment of the biosensor, which influences the bioavailability of pollutants and thus their toxicity. Physico-chemical composition of the analysed medium, which may contain complexants in addition to suspended solids likely to adsorb pollutants, pH, temperature, oxygenation,... are factors which may interfere in the response of the biocatalyst and need to be taken into account or adjusted (Osbild, 1997).

It is worthy remembering that microorganisms are live cells, which need to be fed during their use. Conversely, high concentrations of nutrients in the flow may decrease the sensitivity in some cases, by maintaining an over active metabolism, or by modifying the bioavailability of contaminants. Therefore, a compromise has to be found in concentrations ensuring simultaneously longevity and stable sensitivity.

Table 1 : Characteristics of the main biosensors using bacteria as biocatalysts and measuring respiration changes

PARAMETRE OR RESPONSE / SPECIES	BACTERIA	SUBSTANCE(S)	LOEC* µM	LOEC* µg/l	RESPONSE ....TIME	REFERENCES
O_2						
Methylomonas flagellata	Free	Methane	5		1 min	Karube *et al.*, 1982
	Immobilized Nylon membrane	BOD		2 to 22.10^3	10 min	Strand and Carlson, 1984
Bacillus subtilis	Immobilized PVA gel	BOD		2 to 22.10^3	30 s	Riedel *et al.*, 1988
Activated sludge "RODTOX"	Free 10 l.	3,5-dichlorophenol Cu^{2+} CN^-	1.10^4 (IC50) 11.10^3 780		5 min 15 min 5 min	Kong *et al.*, 1993
Pseudomonas putida "Toxalarm"	Free	PCP		10^3	Immediate	Schmitz *et al.*, 1993
Pseudomonas putida "Stiptox - norm"	Free			$1.5.10^3$		
Activated sludge "Toxiguard"	Biofilm			50		
Activated sludge "Biox-1000T"	Fixed on rings			10^3		
Nitrosomonas eurapae	Immobilized Cellulose acetate membrane	NH^{4+} Arsenic Pb^{2+} CN^- ortho-chlorophenol Trichloroethylene Tetrachloroethylene	0 to 10^3 (ICs) > 50 (ICs) > 100 (ICs) 40 (ICs) 60 (ICs) 60 (ICs) 40 (ICs)		10 min	Tanaka *et al.*, 1993
Escherichia coli	Immobilized	PCP Tributyric tin chloride Formaldehyde Phenol 2-chlorophenol MCPA Mercury chloride Atrazine Cadmium Copper chloride	9 11 5.10^3 $>10^3$ 50 150 27 $>10^3$ $>10^3$ 500			Hatton *et al.*, 1994
Bacillus subtilis and *Bacillus licheniformis*	Immobilized Polycarbonate membrane	BOD		0 to 7.10^4	5 min	Li *et al.*, 1994
LAS degrading strain	Immobilized Calcium alginate gel	LAS (Alkylbenzene linear sulphonate)		0 to 6.10^3	15 min	Nomura *et al.*, 1994
Activated sludge	Free	3,5-DCP		$5.5.10^3$ (IC50)	30 min	Rawson and Rogerson, 1994
Pseudomonas putida	Immobilized cellulose acetate membrane	Benzene	71 to 11.10^3		2-10 min	Tan *et al.*, 1994
Activated sludge "Biox-1000T"	Fixed on rings	BOD		5.10^3	3 min	Teutscher and Grosser, 1994

Activated sludge "RODTOX"	Free 10 l.	BOD PCP Hg^{2+} Cu^{2+} CN^- 3,5-DCP o-cresol Toluene	10^4 to 5.10^8 10^3 10^4 10^4 10^4 10^4 10^5 10^6	30 min 1 h	Vanrolleghem *et al.*, 1994
Thiobacillus thioparus	Immobilized Nitrate cellulose membrane	Thiosulphate Methanethiol	1 to 10^3 200 to 3.10^3	1 min	Kubo *et al.*, 1995
Magnetic activated sludge	Immobilized	BOD	0 to 60.10^3	30 min	Sakai *et al.*, 1995
Sphingomonas species *Pseudomonas fluorescens*	Immobilized Polymethane gel	Naphtalene	30 to 2.10^3	2 min	König *et al.*, 1996
Activated sludge "Rodtox"	Free	Creoline	5.10^3 (IC_{20})	1 h	Kong *et al.*, 1996
Pseudomonas fluorescens	Immobilized Cellulose nitrate membrane	HCN	10^2 to 10^3	3 min	Lee and Karube, 1996
Pseudomonas putida	Immobilized PVA gel	Paraoxon	1 to 10^3	20 min	Rainina *et al.*, 1996
Bacillus stearothermophilus	Immobilized Gel	Phenol o-cresol m-cresol p-cresol 3-chlorophenol Catechol 4-methylcatechol 2-naphtol	2.5 to 50 1 to 3 1 to 3 1to 3 2,5 to 25 5 to 200 5 to 200 2.5 to 70	2 min	Rella *et al.*, 1996
Brevibacterium species	Free	Acrylamide Acrylic acid	10^4 10^4	15 min	Ignatov *et al.*, 1997
Activated sludge (Optrode)	Free	Hg^{2+} Cu^{2+} Pb^{2+} Ni^{2+} Zn^{2+} Cr^{3+}	6.10^3 (IC_{50}) 8.10^3 (IC_{50}) 19.10^3 (IC_{50}) 21.10^3 (IC_{50}) 28.10^3 (IC_{50}) 40.10^3 (IC_{50})		Wong *et al.*, 1997
CO_2 *Escherichia coli*	Immobilized Nucleopore membrane	Phenol HCN Cd^{2+} AsO_2^- Pb^{2+} Cu^{2+}	1.10^6 (IC_{40}) $3.5.10^3$ (IC_{40}) 300 (IC_{40}) $1.5.10^3$ (IC_{40}) 130 (IC_{40}) 1.10^3 (IC_{40})	5 to 30 min	Dorward and Barisas, 1984
Hyphomicrobium	Immobilized	Methylsulphate	10^3	30 min	Schär and Ghisalba, 1985
Pseudomonas aeruginosa	Immobilized Nylon membrane	Nitrate	2.10^4 to 2.10^5	15 min	Hikuma *et al.*, 1993
electronic transport *Escherichia coli* "Ferrocyano-Bacteria Electrode"		PCP	10^3		Schmitz *et al.*, 1993
Escherichia coli E *Pseudomonas putida* P		PCP PCP	500 E 10^3 P		Hansen, 1994

* Lowest concentration inducing a response (LOEC). The concentrations of inorganic elements are expressed as μg/l or μM of the corresponding cation or anion.

Table 2 : Characteristics of the main biosensors using luminescent bacteria as biocatalysts. Comparaison with IC 50, for the same species.

RESPONSE / SPECIES	BACTERIA	SUBSTANCES	LOEC* µM	LOEC* µg/l	RESPONSE TIME	IC_{50} Microtox µg/l 15 min - *5 min	REFERENCES
***Vibrio fischeri* Lux CDABE / In :**							
. *Staphylococcus aureus* S	Free	Bismuth	1 E		120 min S		Corbisier *et al.*, 1993
. *Escherichia coli* E		Cd^{2+}	2 E 3 S		60 min E		
		Pb^{2+}	1 E 10 S				
Escherichia coli	Free	Hg^{2+}	5.10^{-4} to 10^{-3}		40 min to 100 min		Selifonova *et al.*, 1993
Escherichia coli	Free	$HgCl_2$	2 to 800		60 min		Tescione and Belfort, 1993
	Immobilized	Naphtalene	550		21 min		Heitzer *et al.*, 1994
Pseudomonas fluorescens	Glycerol alginate gel	Salicylate	500		24 min		
Escherichia coli stress proteins	Free Microplates	Cu^{2+}	9.10^{4}		10 min	*2.10^{6} (a)	Van Dyk *et al.*, 1994
		2,4-dichloro phenoxyacetic acid	2.10^{3}				
		Methanol	2.10^{5}				
		2-nitrophenol	1.10^{4}			*13. 10^{3} (a)	
		4-nitrophenol	4.10^{3}			* 34.10^{3} (a)	
		Phenol	2.10^{4}			*16.10^{3} (a)	
		Xylene	3.10^{5}				
Vibrio fischeri	Free Freeze-dried	Na Laurylsulphate	2.10^{3} (IC50)		15 min	$1.5.10^{3}$	Heuriet *et al.*, 1990
"Automicrotox"		Toluene	2.10^{4} (IC50)		15 min	15.10^{3}	
		Chlorobenzene	$7.5.10^{3}$ (IC50)		15 min	8.10^{3}	
		Manebe	23 (IC50)		15 min	39	
		Phenol	2.10^{4} (IC50)		15 min	$18.5.10^{3}$	
		CN^{-}	2.10^{3} (IC50)		15 min	1.10^{3}	
		Zn^{2+}	580 (IC50)		15 min	1	
		Cu^{2+}	730 (IC50)		15 min	1	
		Cd^{2+}	7.10^{3} (IC50)		30 min	7	
		Cr^{6+}	2.10^{4} (IC50)		15 min	11.5	
		Pb^{2+}	900 (IC50)		total duration of a test : 50 min	1	
	Immobilized Cellulose nitrate membrane	Benzalkonium chloride (biocide)	23 (IC50)		few minutes		Lee *et al.*, 1992
		Cr^{6+}	85 (IC50)				
"Photox" = RBT	Free Freeze-dried	PCP	50		15 min		Schmitz *et al.*, 1993
"Biotoximeter"	Free batch		$1.5.10^{3}$				
"Automicrotox"	Free Freeze-dried	Malathion	50 (IC39)			*12.10^{3} (a)	Chevillon, 1994
		Chromium	10^{5} (IC51)				
		Formaldehyde	65 (IC74)				
		Diesel	750 (IC51)				
Firefly luciferase in *Escherichia coli*	Free	Chloramphenicol	10^{4} (IC12)		30 min		Lee *et al.*, 1991
		Sodium azide	50 (IC20)				
		Fluoroacetic acid	10^{4} (IC12)				
		Neomycine sulphate	10^{4} (IC12)				
***Photinus pyralis* Luc FF in** *Escherichia coli*	Free Microplates	$HgCl_2$	10^{-9} to 0.1		60 min		Virta *et al.*, 1995
		$CdCl_2$	0.5 to 100				

* Lowest concentration inducing a response (LOEC). The concentrations of inorganic elements are expressed as µg/l or µM of the corresponding cation or anion.
(a) Kaiser and Ribo (1988)

RESPONSE	MICROALGAE	SUBSTANCE(S)	LOEC µM	LOEC µg/l	RESPONSE TIME	REFERENCES
O_2 production	*Chlorella vulgaris*	Phosphates	8.10^3 to 7.10^4		1 min	Matsunaga *et al.*, 1984 a
		Isoproturon		100 (IC50)	30 min	Pandard and Vasseur, 1992 Pandard *et al.*, 1993
		Propanil		500 (IC50)	30 min	
		Hg^{2+}		$1.5.10^3$ (IC50)	180 min	
		CN^-		$> 3.10^3$ (IC50)	30 min	
		Chlortoluron		100 (IC60)	30 min	
		Atrazine		100 (IC27)	30 min	
		Cu^{2+}		$1.5.10^3$ (IC70)	420 min	
	Synechococcus species	Atrazine		< 10	10 min	Hansen *et al.*, 1989
		Linuron		100		
		Diuron		200		
		Metoxuron		50		
		Lindane		100		
	Rhodococcus species	Phenol	4		5 min	Riedel *et al.*, 1993
		3-chlorophenol	4			
		3-chlorophenol	4			
		4-chlorophenol	4			
	Chloroplastes and thylakoïdes	Atrazine	1			Purcell *et al.*, 1990
		Diuron	0.07			
		Atrazine		12	5 min	Rouillon *et al.*, 1995 b
Fluorescence and O_2	*Chlorella reinhardii* "Fluox-algae"	Atrazine		1		Schmitz *et al.*, 1993
Fluorescence	*Chlorella vulgaris*	Simazine		12	5 min	Weston and Robinson, 1991
	Chlorella reinhardii "Biosens-algae-toximeter"	Atrazine		2		Schmitz *et al.*, 1993
	Chlorella reinhardii "IFW-fluorometer"			5	5 min	
	Scenedesmus subspicatus "Fluotox"			1	5 min	
				10	60 min	
Delayed fluorescence	*Chlorella reinhardii* "DF-algae-toximeter"	Atrazine		0.4		Schmitz *et al.*, 1993
	Synechococcus	DCMU		223	10 min	Rawson *et al.*, 1987
		Chlortoluron		2.10^3		
		Linuron		17		
		Atrazine		33		Schmitz *et al.*, 1993
		Atrazine		467 (IC50)	5 à 10 min	Van Hoof *et al.*, 1992
		Linuron		626 (IC50)		

Table 3 : Characteristics of the main biosensors using photosynthetic organisms as biocatalysts (part 2).

Electronic transport		Atrazine	10	2 min	Hansen, 1994
		Chlortoluron	10		
		Metamitron	200		
		Diuron	2.5		
		Linuron	50		
		Terbutylatrazine	25		
		Simazine	10		
		Crimidine	25		
		Isoproturon	25		
	thylakoïdes	Diuron	0.45 (IC50)		Carpentier *et al.*, 1989
		Atrazine	7 (IC50)		
		$NaNO_2$	550 (IC50)	3 à 5 min	Purcell et Carpentier, 1990
		$NaSO_3$	2.10^3 (IC50)		
		$CuCl_2$	720 (IC50)		
		$PbCl_2$	10^3 (IC50)		
		$HgCl_2$	2.10^3 (IC50)		
		$ZnCl_2$	2.10^3 (IC50)		
		$CdCl_2$	4.10^3 (IC50)		
		Diuron	2.10^{-2}	5 min	Rouillon *et al.*, 1995 c
		Atrazine	0.2		
		$HgCl_2$	10^3	5 min	Rouillon *et al.*, 1995 a
		$PbCl_2$	10^3		
		$CdCl_2$	10^3		
		$NiCl_2$	10^2		
		$ZnCl_2$	10^2		
		$CuCl_2$	10^2		
Specific electrodes	H_2 *Chromatium species*	S^{2-}	400	5-10 min	Matsunaga *et al.*, 1984 b
	chloride and bromide *Rhodococcus species*	2-Ethylenebromide	40	5 min	Peter *et al.*, 1996
		1-Chlorobutane	220		

* Lowest concentration inducing a response (LOEC). The concentrations of inorganic elements are expressed as $\mu g/l$ or μM of the corresponding cation or anion.

Table 4 : Characteristics of the main biosensors using yeast as biocatalysts.

RESPONSE / SPECIES	YEAST	SUBSTANCES	LOEC* µM	LOEC* µg/l	RESPONSE TIME	REFERENCES
O_2						
Trichosporon cutaneum	Immobilized PVA gel	BOD		4.10^3 to 10^5	30 s	Riedel *et al.*, 1988
Trichosporon beigelii (cutaneum)	Immobilized PVA gel	Phenol	2		5 min	Riedel *et al.*, 1995
		2-chlorophenol	2			
		3-chlorophenol	2			
		4-chlorophenol	2			
		2,3-dichlorophenol	2			
		2,4-dichlorophenol	2			
		2,5-dichlorophenol	2			
Saccharomyces cerevisae	Immobilized "Chitopearl"	Cyanide	0 to 15		10 min	Ikebukoro *et al.*, 1996
Saccharomyces cerevisae	Immobilized Nitrate cellulose membrane	CN^-	0.3 to 150		2 min	Nakanishi *et al.*, 1996

* Lowest concentration inducing a response (LOEC). The concentrations of inorganic elements are expressed as µg/l or µM of the corresponding cation or anion.

4. Requirements for microbiological biosensors used in biomonitoring

As underlined above, specificity and sensitivity are important to determine the field of application of a biosensor and its use. These two properties are the main characteristics studied to judge the interest of a biocatalyst and its associated transducer. These studies are conducted on laboratory pilots, and generally over a short period of time.

A large number of microbiological biosensors have been proposed on the basis of such results. Yet, this first step must be followed by in situ studies, allowing other criteria to be evaluated :

- longevity,
- storage of the biocatalyst,
- reliability,
- practicability,
- servicing time,
- computer equipment,
- absence of interference leading to false alarms,...

These criteria are crucial in determining the interest of the instrument. Unfortunately, most of the investigations on microbial biosensors are still performed at a laboratory level. The validation step in the open environment has yet to be carried out.

A study initiated by several German institutions was carried out on the River Rhine, from 1990 to 1992, in order to test and compare automated biomonitors on the basis of the above criteria. This study is exemplary of what needs to be done to optimize the implementation of automated biotests. Results highlighted the interest of algal biosensors with fluorimetric transducers (Schmitz et al., 1993).

5. Needs in biosensor technology development

The in situ validation is a necessary step in the development of biosensors. This step takes time, needs much work and is also costly. The difficulty of the operation is expressed by the discrepancy between the large number of biosensors described after feasibility studies in the lab, and the rarity of automated biological systems in use in biomonitoring at the moment. This problem is not restricted to microbial sensors, but general to all automated biomonitors.

In fact, what we call a biosensor is not simply an efficient association between biocatalyst and transducer, but a device with systems which must avoid failure and interference, and including : thermostatisation, addition of nutritive solutions, adjustment of pH and perhaps salinity, exposure of the photosynthetic catalysts to light, elimination of suspended solids if necessary,... All the parameters known to influence the response of the biological reagent need to be controlled. The efficiency of these controls will insure the reliability of the alarm system.

Other hydraulics-related problems may also arise in the device. From our experience, dynamics problems are frequent and often take a long time to be identified because they are generally unexpected. The biocatalyst is always incriminated in the case of failure of the systems : yet, most of the time, problems arise from other causes : mechanics, physics, dynamics.

This enumeration of traps which obstruct the implementation of alarm systems, aims to underline the fact that we should concentrate on studying the devices under field conditions.
We have now to focus our efforts on the validation of biosensors in field situations. This is a priority if there is to be a biomonitoring of waters for an improved protection of the aquatic environment. Otherwise, biosensors will remain attractive tools for scientists.

Another area which deserves to be explored is the search for non specific microbial biosensors with a higher sensitivity than the present systems. Lower detection thresholds of toxicants will allow applications which biomonitor the quality of rivers, surface waters and underground waters, characterized by a low level of pollution, but with possible long term toxic effects. At the moment, only specific microbial sensors present such a level of sensitivity. The association of multiple specific biosensors could extend their array of detection. This kind of system would certainly deserve to be implemented. However, an in situ validation will still have to be carried out in order to guarantee the system's reliability for environmental biomonitoring.

The systems, which are fast, simple and cheap, are likely to have the most commercial success and the most wide-spread use in environmental biomonitoring, provided their reliability has been proved in the environment. This enthusiastic goal cannot be reached without the cooperation of biologists, engineers, statisticians, electronicians, this close collaboration being absolutely necessary to ensure success. But above all, the sponsoring of European and governmental institutions is needed, as well as that of industries and commercial societies interested in the development of this new technology. Success will require a team effort, but the interests at stake are great, both environmentally and commercially.

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