Algal biosensors for aquatic ecosystems monitoring

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Abstract. The harmful effect of toxic chemicals on natural ecosystems has led to an increasing demand for early-warning systems to detect those toxicants at very low concentrations levels. Whole-cell biosensors based either on chlorophyll fluorescence or enzyme (phosphatase and esterase) inhibition are constructed for real-time detection and on-line monitoring. Results show that these devices are sensitive to heavy metals and pesticides. The system allows the cells to operate in their natural environment which favours long term stability and reflects the toxic action mechanism providing therefore an ecological interest.

1 Introduction

Aquatic ecosystems monitoring has currently referred to sensitive and reliable methods based on spectrometric and electrochemical techniques (ICP-MS, GC-MS, SAA…) that ensures detection of specific chemicals at low concentration levels [1,2]. However, those techniques are costly, time-consuming and limited to a restricted number of species. Additionally, they have to face long delays after sampling to produce expected results. Therefore, continuous detection and on-site monitoring are in great demand in aquatic ecosystems management. This could be achieved with biological sensors permanently settled in the areas under control. Whole-cell biosensors presented hereafter are based on metabolic perturbation of immobilized algal cells in the presence of toxicants. Algal cells are chosen for their high sensitivity and their place in the ecosystem: being at the very beginning of the trophic chain, they represent a good biological marker of ecosystem pollution and an early-warning system to prevent irreversible effects [3,4,5,6]. The work presented here concerns the construction of a biosensor from unicellular green algae, not having undergone any genetic modification. This tool can thus be placed *in situ* and makes it possible to evaluate the response of the algae in their natural environment. In this paper, the objective is to use two different enzymes to screen two different families of toxicants and mainly to detect pesticides and heavy metals at the same time.

2 General principle of biosensors

A biosensor can be considered as a combination of a *bioreceptor*, the biological component, and a *transducer*, the detection method. The total effect of a biosensor is to transform a biological event into an electrical signal. The first link of a biosensor is the *bioreceptor*, which has a particularly selective site that identifies the analyte. The bioreceptor ensures molecular

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recognition, and may transform the analyte in some way [7]. This localized modification is generally made via an immobilized enzyme, which transforms the analyte into a product that is detectable by the transducer [8,9]. This is the case for enzyme sensors. Sometimes, however, the enzyme is only stable in its natural environment, which cannot be modified, and the whole cell or microorganism is immobilized on the biosensor [10].

The other component of a biosensor is the *transducer*, which exploits the biochemical modification of the substrate by the bioreceptor by transforming it into an electrical signal (Figure 1). We could say that the transducer converts one type of energy into another. The choice of transducer depends on the type of biochemical modification, it should make optimal use of the product of the bioreceptor and give a signal that is sensitive, easily monitored, and has minimal background noise. Low background noise reduces the detection limit and improves the biosensor performance.

The combination of any bioreceptor such as enzymes [11, 12, 13], immuno-agents [14], tissues [15] or cells [16, 17, 18] with any transducer leads to a large number of biosensors (Table 1). Electrochemical transducers couple relatively easily with enzymes, and so such biosensors were first reported [19].

3- Biosensors applications

Biosensors have many commercial applications in a large range of activities. The most important applications are in medicine (in hospitals or in the home) and in the food produce industry for the control of manufacturing processes. More recently, many biosensors have been applied to environmental control: the bioreceptor being purified enzymes or whole cells directly immobilized on a transducer.

3.1 Biosensors based on enzyme inhibition

The biosensor makes use of an enzyme layer and a transducer, in close contact with each other, to form a single unit for detection of heavy metals, organophosphorous or organochlorinated pesticides [12,20,21]. Enzymes are commonly used in their purified form to achieve specific detection of a toxic analyte by enzyme inhibition. The percentage of inhibition is directly correlated to analyte concentration [22, 23]. For pesticides determination, cholinesterase and urease have been used in conjunction with electrochemical transducers. Recently, a conductometric acetylcholinesterase biosensor was constructed for assessment of toxicity of methyl parathion and its photodegradation products in water [24]. Since a wide range of enzymes (urease, glucose oxydase, invertase) are inhibited by heavy metal ions at low concentrations, they were immobilized onto different transducers for metal ions determination [25, 26]. However, those enzyme sensors have the inherent drawbacks of purified enzymes whose easy denaturation hinders their use in pollution control.

3.2 Biosensors based on whole cells activity

Microbial species can provide a valuable source of biocatalyst material for whole cell biosensor design and have proven to be amenable to immobilization in biosensor configuration. Microbes have a number of advantages as biological sensing materials in the biosensor design : they are present ubiquitously and are able to metabolise a wide range of chemical compounds. Microorganims have a great capacity to adapt to adverse conditions and to develop the ability to degrade new molecules with time. Biosensor electrodes have been developed that incorporate a range of bacterial, yeast and algal cell types [16,27]. Compared

to biosensors using purified enzyme , whole-cell biosensors are more resistant to the activity loss because their enzymes and cofactors are hosted in an environment optimized by nature. Therefore, these biosensors are more suitable to meet all the requirements for environmental surveillance [18, 28]: they can identify *in situ* the presence of a toxic compound as soon as it is released in waste water or aquatic environment.
Other whole cell biosensors were constructed from genetically modified cells [21]. Those techniques may improve the biosensor sensitivity and selectivity but are no longer able to reflect the ecosystem operating conditions. In the present work, only native cells have been used to preserve the ecological aspect of the media under study.

4 Algal biosensors

First algal biosensors made use of an oxygen electrode to detect the oxygen production [16] that results from the photosynthetic activity commonly observed in all plants. However, this corresponds to an unspecific sensing since many pollutants are more or less inhibitors of the oxygen emission.
Algal biosensors developed in this study have the ability to detect a group of pollutants provided they affect a particular alga metabolic pathway. This is the case of pesticides and heavy metals which are strong inhibitors of acetylcholinesterase and alkaline phosphatase, both are located in *Chlorella vulgaris.* This green alga belongs to the *Chlorophycea* group and it was selected for its greater stability in producing biological signals. The chlorophyll fluorescence emitted from its photosynthetic activity enables pesticides detection [18] while inhibition of its alkaline phosphatase and esterase allows determination of heavy metals [29] and organophosphorous insecticides [30] respectively. The corresponding signals are obtained with optical and conductometric transducers. This tool has been designed to monitor simultaneously several metabolic activities of immobilized algal cells (chlorophyll fluorescence and alkaline phosphatase / esterase activities). Since they are not genetically modified, they can be used for real-time screening of the various families of pollutants coexisting on the same site. The response of algae under chemical stress can then be analyzed directly from their original medium.

4.1 Construction of an optical algal biosensor

The algal strain used in this study was *Chlorella vulgaris* (CCAP 211/12) purchased from The Culture Collection of Algae and Protozoa at Cumbria, United Kindom. The axenic algal strain was grown in the culture medium and under conditions described by the International Organization for Standardization (ISO,1989). The active membrane was obtained by physical entrapment of the algal cells onto a porous matrix. Immobilization was achieved by simple filtration of an algal suspension on a glass fiber or quartz membrane. The optical biosensor was constructed with the tip of an optical fiber bundle placed in front of the algal membrane at a few millimeters distance in order to allow the sample solution to circulate between them (Figure 2).
The various biological signal results from:
- chlorophyll fluorescence produced by the photosynthetic activity
- esterase activity involving in photosynthesis
- phosphatase activity essential to phosphorous metabolism in algal growth

Optical signals are obtained directly with chlorophyll fluorescence measurement [18] or after injection of fluorescent substrates with enzyme activity measurement [28]. Toxicant concentrations are determined from the variation of fluorescence amplitudes. Alkaline

phosphatase activity in *Chlorella vulgaris* exhibits a good stability during 30 days, whereas a drift of esterase activity and chlorophyll fluorescence was observed after 4 to 5 days (unpublished results). In this case, the membrane needs to be changed after 5 days for the biosensor to keep its optimal response.
An optical biosensor associated with a fluorimeter may result in a rather cumbersome equipment. Conversely, a conductometric transducer connected to its measuring system can easily be miniaturized and the reduced cost will allow this biosensor to be placed on several sites under monitoring.

4.2 Construction of a micro-conductometric algal biosensor

This biosensor is based on the local changes in conductivity of the "bio-membrane" resulting from alkaline phosphatase and esterase activities which produce ionic species. Algal cells are immobilized on a pair of interdigitated platinum electrodes printed on a Si/SiO_2 substrate of 1 mm thickness (dimensions 5 mm x 30 mm) which were fabricated at the Institute of Chemo- and Biosensorics (Muenster, Germany) [31]. Each finger of the electrode was 10 µm wide and 1 mm long, with 10 µm spacing between fingers (Fig. 3). Another similar pair of electrodes is used as a reference. The sensitive area of each electrode pair was about 1 mm x 1.5 mm. The in-phase differential signal between the pair of electrodes was registered by a "home made" conductometric laboratory amplifier in which a small-amplitude alternative voltage of 10 mV with a high frequency of 100 kHz were applied. Under these conditions, it has been demonstrated [32] that the transfer resistance as well as the Warburg impedance can be neglected so that the output signal is directly proportional to the resistance (or conductance) within the "bio-membrane". In this paper, the results were obtained under the same conditions.
The algal cells are entrapped in bovine serum albumin (BSA) crosslinked with glutaraldehyde [7, 17]. This micro-biosensor is so small and inexpensive (the miniaturization allows a mass production at low cost) that it can easily work on the field with a portable conductimeter.

5. Results

Figure 4 shows various responses of a conductometric alkaline phosphatase biosensor to p-nitrophenyl phosphate (pNPP) used as a substrate. Similar curves are obtained with an optical algal biosensor. The results agree with those carried out in test tubes with non-immobilized algae, which confirms the possibility to use algal biosensors to monitor their metabolic activities.
Determination of alkaline phosphatase activity (APA) can also be carried out with methylumbelliferyl phosphate (MUP) as a substrate. The reaction product methylumbelliferone (MUF) is fluorescent. APA can easily be measured from the MUF fluorescence emission (460 nm) under excitation light (350 nm) when the MUP solution is brought into contact with the enzyme. Figure 5 gives an example of the algal APA inhibition measured with an optical algal biosensor before (**A**) and after (**B**) injection of a municipal solid waste effluent in a flow system. MUP is injected in the flow as a substrate to produce the optical response.
Chemical analysis of these effluents showed high concentrations of heavy metals (more than 1mg/L), which can explain the inhibition effect observed on algae phosphatase activity
Tableau 2 shows results obtained with the optical biosensor for herbicides detection. The biosensor was tested in the absence of toxic compounds and in the presence of herbicides

(atrazine, simazine, isoproturon and diuron) to compare their effects on fluorescence induction. The presence of these herbicides increases the fluorescence emission. The detection limit, the toxic concentration EC 50 that affects 50% of the response, and the reversibility of action of these pollutants are listed in Table 2. Detection of herbicides was always achieved with concentration level down to 1µg/L The fluorescence based-biosensor using algae cells seems to be particularly suitable for detection of herbicides. Detection limits of this biosensor are compatible for aquatic environment quality monitoring. Compared to other previous paper [17, 28, 30], the results obtained here show that a single system can be used to assess the level of pollution with the possibility to identify the corresponding family of toxicants since the optical or conductometric specific responses of these biosensors enables to target the corresponding pesticides or heavy metals present in aquatic environment.

6 Conclusion

Whole-cell algal biosensors have proven to be successful as early warning systems to identify the presence of a pollutant before it causes damage to the ecosystem. These devices can be adopted by industry to monitor effluents or sewage treatment plants and by decision-makers in charge of aquatic ecosystems surveillance. The two types of biosensors presented in this study exhibit similar detection limits. An advantage of miniaturization of conductometric biosensors is to produce a large number of low-cost field devices to be placed in the areas under monitoring.

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Figures and table captions

Fig. 1. The various stages of determination with a biosensor [7]

Fig. 2. Optical algal biosensor

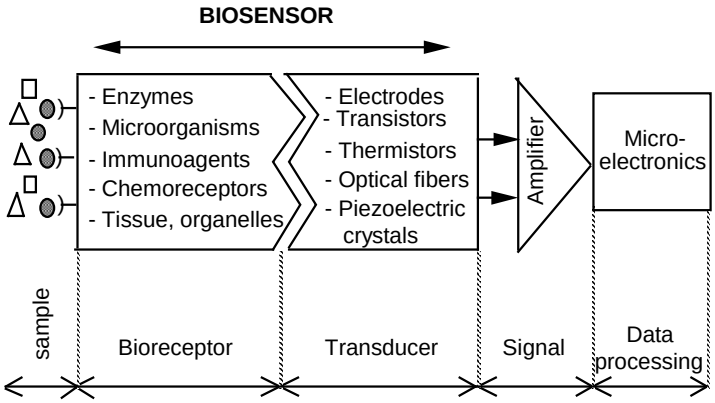
Fig. 3. Schematic representation of a conductometric algal biosensor

Fig. 4. Conductometric biosensor response to pNPP as a substrate

Fig. 5. Alkaline Phosphatase detection with optical biosensor on algae sample before (A) and after (B) exposure to munipal solid waste effluent.

Table 1. Typical combinations of bioreceptors and transducers in environmental monitoring

Table 2. Characteristics of a biosensor response to herbicides based on chlorophyll fluorescence



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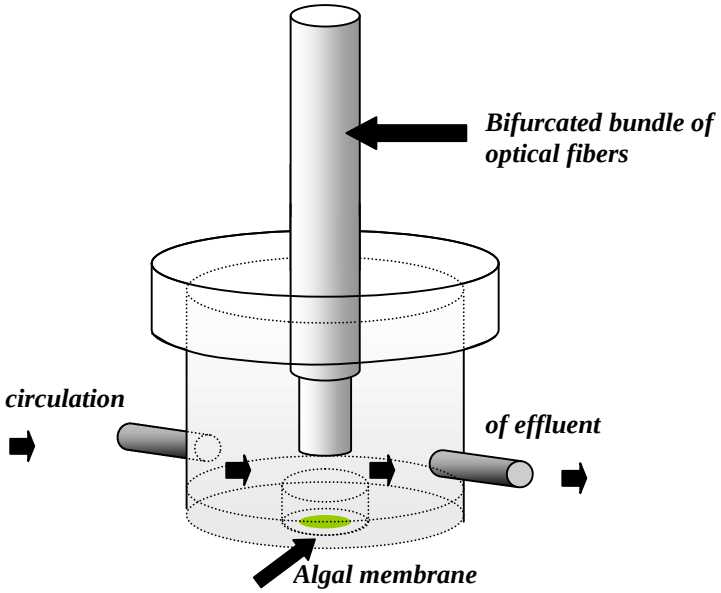

Fig. 2. Optical algal biosensor

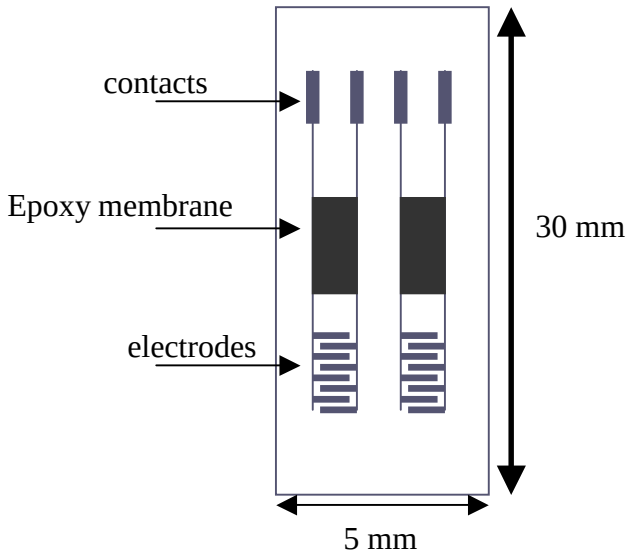

Fig. 3. Schematic representation of a conductometric algal biosensor

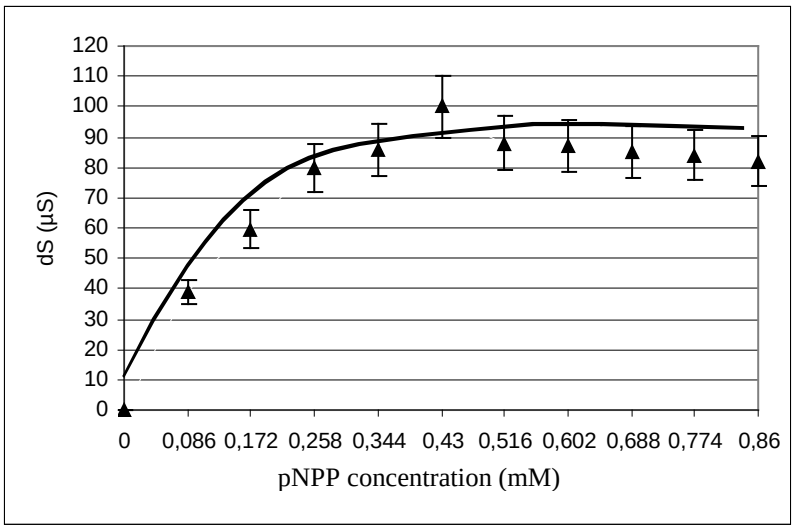

Fig. 4. Conductometric biosensor response to pNPP as a substrate

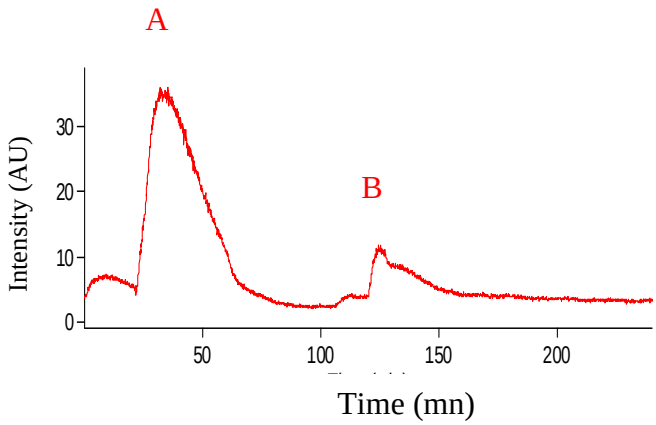

Fig. 5. Alkaline Phosphatase detection with algal optical biosensor in the absence (A) and in the presence (B) of a municipal solid waste effluent in the carrier flow. Substrate is injected in the flow to produce optical response.

Table 1. Typical combinations of bioreceptors and transducers in environmental monitoring

Bioreceptors	Transducers	Signals	References
Enzymes	Conductometric	Enzyme activity	[11]
	Potentiometric	Tyrosinase, glucose oxydase, urease, phosphatase activities	[12]
	Amperometric		[13]
Immuno-agents	Potentiometric	Antigen/antibody recognition	[14]
Tissus	Potentiometric Amperometric	Dosage de cystéine, glutamine	[15]
Cells	Potentiometric	Cell respiration	[16]
	ISFET	Esterases, phosphatase activity	[17]
	Optical fibre	Fluorescence	[18]

Table 2. Characteristics of a biosensor response to herbicides based on chlorophyll fluorescence

Herbicides	detection limits (µg/l)	EC 50 (µg/l)	Reversibility
Atrazine	0,25	96 ± 4	Yes
Simazine	0,5	127 ± 4	Yes
Isoproturon	0,025	35 ± 2	Yes
Diuron	0,025	39 ± 3	Yes