Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biosensors and Bioelectronics 19 (2004) 1089–1096
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Development of novel conductometric biosensors based on immobilised whole cell *Chlorella vulgaris* microalgae

Celine Chouteau [a,b], Sergei Dzyadevych [c], Jean-Marc Chovelon [b,*], Claude Durrieu [a]

[a] *Laboratoire des Sciences de l'Environnement, Ecole Nationale des Travaux Publics de l'Etat, rue Maurice Audin, 69518 Vaulx-en-Velin Cedex, France*
[b] *Laboratoire d'Application de la Chimie à l'Environnement, Faculty of Chemistry, Université Claude Bernard Lyon 1, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France*
[c] *Laboratory of Biomolecular Electronics, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, 150 Zabolotnogo Street, Kiev 03143, Ukraine*

Received 25 April 2003; received in revised form 10 October 2003; accepted 14 October 2003

Abstract

A novel biosensor based on immobilised whole cell *Chlorella vulgaris* microalgae as a bioreceptor and interdigitated conductometric electrodes as a transducer has been developed and tested for alkaline phosphatase activity (APA) analysis. These sensors were also used for the detection of toxic compounds, namely cadmium ions, in aquatic habitats. Algae were immobilised inside bovine serum albumin (BSA) membranes cross-linked with glutaraldehyde vapours. The detection of the local conductivity variations caused by algae enzymatic reactions could be achieved. The inhibition of *C. vulgaris* microalgae Alkaline phosphatase activities in presence of cadmium ions was measured. These results were compared with measurements in bioassays. It finally appeared that conductometric biosensors using algae seemed more sensitive than bioassays to detect low levels of cadmium ions (the detection limit for the first experiments was 1 ppb of Cd^{2+}).

The main advantages of these alkaline phosphatase biosensors consist of their high specificity in regard to the toxic compounds they enable to detect, but also on their high stability since contrary to enzymatic biosensors, they use whole algae cells with APs on their walls.

Keywords: Biosensor; *Chlorella vulgaris* microalgae; Cadmium; Alkaline phosphatase activity; Toxicity assays

1. Introduction

The aquatic ecosystem health is a key element in environmental issues. Indeed water is an essential resource needed in most human activities. Protecting water quality requires early warning systems for on line and in situ pollution monitoring.

Phosphorus is needed for microphyte growth of which it is often the limiting factor. Some microphyte species can survive under strongly deficient phosphorous conditions. These micro-organisms can indeed stock large amounts of phosphorous. Phosphatase enzymes can hydrolyse phosphate containing molecules in this purpose. Depending on the species and the pH, different types of phosphatases can be found namely alkaline, acid and neutral phosphatases.

Recently, research concerning biosensors has led to the development of enzyme sensors using immobilised single enzymes (Dzadevych et al., 1994; Shul'Ga et al., 1994; Wan et al., 1999; Dennisson and Turner, 1995; Turner et al., 1987). These sensors have been tested successfully for the detection of toxic compounds in environmental controls as well as food and medicine analysis.

Enzyme-based sensors are highly selective systems for the detection of toxic compounds because of their high specificity of groups of inhibitors towards a particular enzyme. For instance, alkaline phosphatase activity (APA) is known to be mainly inhibited by heavy metal ions. From this statement, developing a multi-enzymatic biosensor will allow the detection of different groups of pollutants by the same device (Arkhypova et al., 2001). However, immobilising different enzymes on a multisensor arrays is not so easy, because all enzymes must be working under the same operational conditions simultaneously. Several problems namely the enzyme stability, their high price must be overcome.

* Corresponding author. Tel.: +33-4-7243-2638; fax: +33-4-7244-8438.
E-mail address: chovelon@univ-lyon1.fr (J.-M. Chovelon).

0956-5663/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2003.10.012

The use of micro-organisms for multi-enzymatic biosensor design can be a good solution, each algal cell containing a large number of enzymes. Moreover using a living organism gives information concerning the ecotoxicological effects of pollutants on these organisms.

This paper describes the development of a conductometric biosensor using immobilised whole cell algae, *Chlorella vulgaris*, for APA analysis and heavy metal detection (for example, cadmium ions). Using whole cell algae is very interesting since these cells are the primary producers of aquatic food webs (Khoshmanesh et al., 1996): any disturbances may have consequences on the upper levels. Bioassays have already been developed for the detection of *C. vulgaris* alkaline phosphatase activity and the influence of heavy metal ions (Durrieu et al., 2003).

Some enzymatic reactions involve either consumption or production of charged species and, therefore, lead to a global change in the ionic composition of the tested sample that can be detected with conductometric biosensors. These sensors present a number of advantages (a) thin-film electrode are suitable for miniaturisation and large-scale production using inexpensive technology; (b) they do not require any reference electrode; (c) transducers are not light sensitive; (d) the driving voltage can be sufficiently low to decrease significantly the power consumption and (e) large spectrum of compounds of different nature can be determined on the basis of various reactions and mechanisms, but this can also be considered as a disadvantage since it lowers the selectivity of the sensor.

The first steps in the development of these biosensors are presented in this paper such as the possibility to follow APA analysis with conductometric biosensors as well as the first optimisations and toxicity assays. These results are compared with measurements by bioassays.

2. Experimental

2.1. Materials

The *C. vulgaris* strain (CCAP 211/12) was purchased from the culture collection of algae and protozoa at Cumbria, UK. The axenic algal strain was grown in the culture medium and under conditions described by the International Organisation for Standardisation (ISO, 8692, 1989). The APA measurements require a 21-day-long starvation period in the culture medium without phosphate (Fitzgerald and Nelson, 1966). The concentration in algae cultures was $\approx 3 \times 10^7$ to 5×10^7 cells/ml.

Bovine serum albumin (BSA) and 25% aqueous solution of glutaraldehyde (GA) were purchased from Sigma–Aldrich. Calcium alginate and $CaCl_2$ was purchased from Fluka.

Two substrates were used to determine alkaline phosphatase activity: methylumbellifery phosphate (MUP) and paranitrophenyl phosphate (pNPP) from Sigma–Aldrich.

$Cd(NO_3)_2$ salt (of analytical grade) was used to inactivate alkaline phosphatase activity. All other reagents were of analytical grade.

2.2. Sensor design

The conductometric transducers were fabricated at the Institute of Chemo- and Bio-sensorics (Munster, Germany) (Trebbe et al., 2001). Two pairs of Pt (150 nm thick) interdigitated electrodes were made by the lift-off process on the pyrex glass substrate. The Ti intermediate layer of a 50-nm thick was used to improve adhesion of Pt to substrate. Central part of the sensor chip was passivated by Si_3N_4 layer to define the electrodes working area. Both the digits width and interdigital distance were 10 μm, and their length was about 1 mm. Thus, the sensitive part of each electrode was about 1 mm^2.

Measurements based on the detection of solution conductance variations inside membrane. Alkaline phosphatase, as many other enzymes, induces catalytic reactions consuming and producing different ionic species resulting in measurable conductivity changes.

2.3. Algae immobilisation

Previous studies have shown that pure enzymes can be immobilised on electrodes using bovine serum albumin and glutaraldehyde as a crosslinker (Zhylyak et al., 1995; Dzyadevych et al., 2002). Moreover Babu and Panda (1991) have also used a similar technique to immobilise *Escherichia coli* cells. This is why this technique has been tested in this study. On the other hand, as literature reports on the use of calcium alginate beads for algae immobilisation, the latter has also been tested (Dainty et al., 1986; Bozeman et al., 1989).

2.3.1. BSA membranes

The active membrane deposited on the sensitive area of the electrode was formed by cross-linking algae with bovine serum albumin in saturated GA vapours. This protocol was adapted from pure enzyme membranes used with some conductometric and ISFET biosensors (Dzadevych et al., 1994; Shul'Ga et al., 1994).

Two mixtures were prepared. The first one contained 100 μl algae solution and 10% (w/v) BSA was deposited on one of the electrode's sensitive surfaces using a drop method. The second mixture contained 100 μl heated algae (at 90 °C for 15 min; Dainty et al., 1986) and 10% (w/v) BSA was deposited on the electrode reference surface. Measurements for heated algae by bioassays show that heat inhibits the APA (unpublished results).

Finally, sensors were placed in saturated GA vapours. After exposure, membranes were dried at room temperature from 15 to 30 min.

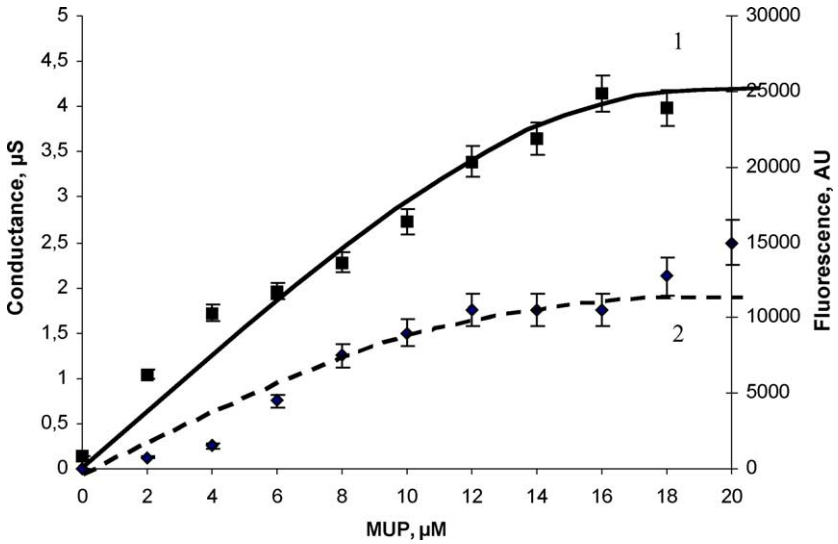

Fig. 1. Calibration curves for MUP determination obtained by bioassays (1) and conductometric biosensors with BSA membranes (2). APA measurements were conducted in Tris–HCl buffer (pH 8.5, 10 mM for biosensors and 100 mM for bioassays) and 1 mM $MgCl_2$.

2.3.2. Calcium alginate membranes

To improve adhesion a drop of $K_2Cr_2O_7/H_2SO_4$ solution was deposited on the sensitive areas of the transducers for 10 min and washed afterwards with distilled water.

Two mixtures were prepared. The first mixture was prepared by allowing 100 μl algae solution to be mixed with 5% (w/v) calcium alginate. The second mixture was prepared with heated algae and 5% (w/v) calcium alginate and used for reference.

A first layer of the different mixtures was deposited on each sensitive area. Biosensors were placed in the fridge for 30 min. After drying, a second layer was dropped and immediately after, biosensors were immersed in $CaCl_2$ (0.1 M) for 8 min to cross-link membranes.

2.4. Measurements

2.4.1. APAs measurements

For biosensors, measurements were carried out in daylight at room temperature in a 5 ml glass cell filled with a Tris–HCl buffer (10 mM, pH 8.5) and $MgCl_2$ (1 mM) solution. Biosensors were immersed in a vigorously stirred sample solution.

Note: Concentration of Tris–HCl buffer is 10 times smaller than for bioassays because of the high conductivity of this solution that would create an important high background signal. Moreover, the small concentration used did not disturb the pH of the solution.

After stabilisation of the output signal, different aliquots of stock solution of substrates (MUP (2 mM) or pNPP

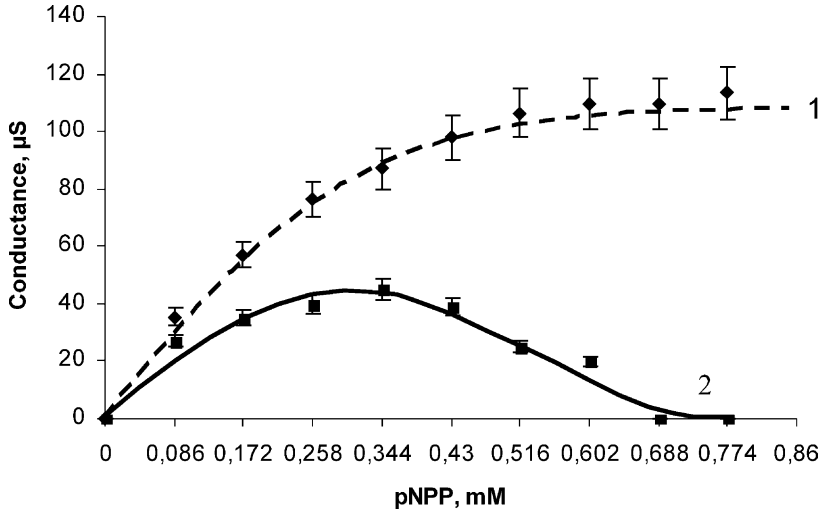

Fig. 2. Calibration curves for pNPP determination obtained by conductometric biosensor with BSA (1) and calcium alginate (2) membranes. APA measurements were conducted in 10 mM Tris–HCl (pH 8.5) and 1 mM $MgCl_2$.

(43 mM)) were added into the vessel. The differential output signal was registered using a 'home made' conductometric laboratory apparatus (Patskovsky and Volotovsky, 1996) and the responses of the biosensor were recorded as a function of substrate concentration.

For bioassays, measurements were based on fluorescence detected by a spectrofluorimeter (Fluostar, BMG). The enzymatic reaction using MUP (2 mM) as a substrate gives a fluorescent product, the MUF (methylumbelliferone), that can be easily detected using an optic fibre at an excitation and emission wavelengths of 365 and 460 nm, respectively (Durrieu and Tran Minh, 2002).

The pNPP substrate gives a coloured product that can be detected using colorimetry. Moreover, as it has been shown that the MUP susbtrate gives a larger and quicker signal. As a result, the MUP substrate has been chosen for bioassays (Lubian et al., 1992).

Assays were carried out in 96- or 48-well plates. For each substrate concentration, eight replicates were carried out. In every well, Tris (pH 8.5, 0.1 M), $MgCl_2$ (1 mM) and algae solution (80 μl) were mixed. Substrate was added at the very last moment.

2.4.2. Toxicity measurements

2.4.2.1. Cadmium toxicity. For biosensors, the decrease of the pNPP substrate response caused by short (≈1 min) and long exposures to Cd^{2+} solutions (concentrations from 0.1 to 10 ppm) was used to estimate the enzyme inhibition.

Bioassays were carried out in 48-well plates. The APA inhibition could be estimated using the decrease of fluorescence of the MUF product for algae exposed to Cd^{2+} solutions (concentrations from 0.1 to 10 ppm) from 0 to 4 h.

Table 1
Comparison between pNPP and MUP as substrates for APA detection measured by conductometric biosensor

	Concentration range	Sensitivity	Response time (min)	R.S.D. (%)
pNPP	0–1 mM	100 μS/mM	<5	8
MUP	0–20 μM	0.1 μS/μM	<5	10

APA measurements were conducted in 10 mM Tris–HCl (pH 8.5) and 1 mM $MgCl_2$.

2.5. Storage

Biosensors were prepared each day and stored at 4 °C between experiments. For storage, biosensors were kept in culture medium without phosphate for 30 days.

3. Results and discussion

3.1. APA measurements

The first step of this work was to confirm the possibility to detect APA with algae conductometric biosensors. The principle of operation of such biosensor is based on the following reaction:

$$\text{substrate} \xrightarrow{\text{phosphatase}} \text{product} + PO_4^{3-}$$

The two most widely used substrates for assays are *p*-nitrophenyl phosphate and 4-methylumbelliferyl phosphate, leading to the release of *p*-nitrophenol and 4-methylumbelliferone, respectively. As APA can be measured by bioassays using MUP, comparisons with biosensors could be done readily. As shown in Fig. 1, the curves have the same shape and the same substrate concentration range for the

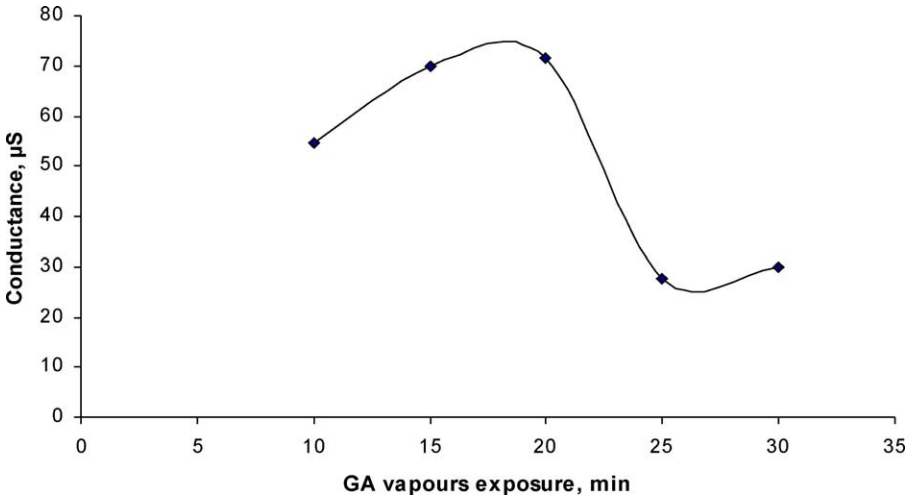

Fig. 3. Dependence of conductometric biosensor response on different GA vapours exposure. APA measurements were conducted in 10 mM Tris–HCl (pH 8.5) and 1 mM $MgCl_2$ with 43 mM pNPP solution as substrate.

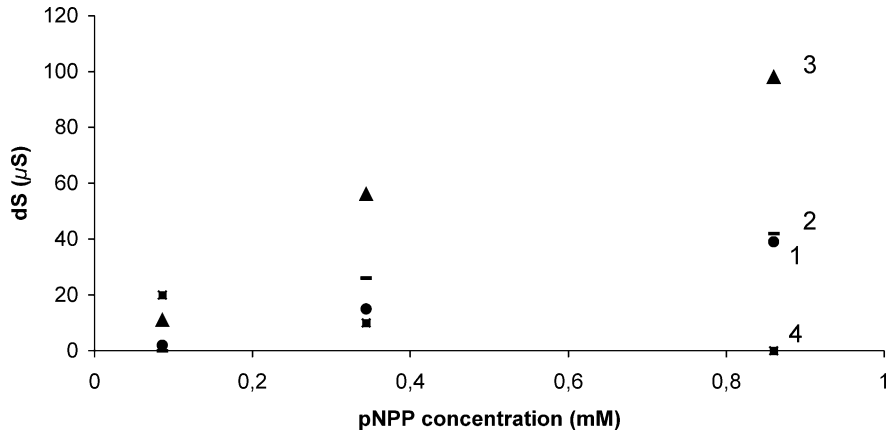

Fig. 4. Alkaline phosphatase activity measured with conductometric biosensors for different algal concentrations in BSA membranes (1) $<10^7$ cells/ml, (2) 1×10^7 to 4×10^7 cells/ml, (3) 4×10^7 to 5×10^7 cells/ml and (4) $>10^8$ cells/ml.

two different transduction modes: the signal increases up to a saturating substrate concentration.

Further measurements with biosensors were carried out with both substrates, MUP and pNPP (see Figs. 1 and 2). After comparing the results with both substrates (Table 1), pNPP looked as more suitable as it gave the highest signal amplitudes and allowed to get a typical Mickaelis–Menten kinetic, as shown in Fig. 2 (Rawn, 1989). Moreover, pNPP is prepared from tablets dissolved in distilled water contrary to MUP for which a solvent is required that created a background noise when injected. Therefore, the following experiments were done with pNPP as the most appropriate substrate.

Two membranes were studied for biosensors: BSA membranes and calcium alginate membranes. As shown in Fig. 2, the signal measured using calcium alginate membranes decreases for high pNPP concentrations (>0.3 mM pNPP) contrary to the signal for BSA membranes that is clearly a Mickaelis kinetic with a saturation level from 0.5 mM pNPP.

Dainty et al. (1986) have shown that cation chelating agents such as phosphate can cause disruption or dissolution of alginate beads . The alkaline phosphatase enzymatic reaction produces phosphate ions. Moreover, Dainty et al. have also shown that calcium alginate beads were stable for pH up to 5.5 and low phosphate concentrations but for increasing pH and phosphate concentrations, bead dissolution increased. The APA measurements were carried out at pH 8.5, which is clearly out of the optimal stability pH range for alginate gels. Therefore, calcium alginate membranes were not appropriate for this work.

Considering BSA membranes, the toxicity of GA vapours for algae was checked. Before proceeding to experiments with biosensors, 96-well plates filled with algae solutions (at different concentrations in the range 6×10^7 to $\approx 1.2 \times 10^7$ cells/ml) were placed in saturated GA vapour for 20 min. For a 30 min long exposure, APA was inhibited of 15% at the most. Since biosensors were placed for less than 30 min in GA vapours, this inhibition was not considered as significant. BSA membranes were finally been chosen for future experiments.

For these membranes, GA vapour exposure is an important parameter since GA as a cross-linker gives its resistance to the membranes. As shown in Fig. 3 an optimal signal can be observed for a 20 min long exposure, the curve through the points was inserted to guide the eye and represents no model. In addition, APA was measured every 10 min for 2 h using BSA membranes exposed to 10, 14, 18 and 20 min to GA vapours to follow the signal stability. For GA exposure lower than 14 min, the APA signal decreased to at least 30% after 2 h. For 18 and 20 min no significant decrease was observed. These results confirm the operational stability for a 20 min-long exposure to GA vapours.

Concerning algae concentrations in membranes, it exists an optimal concentration, namely 4×10^7 to 5×10^7 cells/ml as shown on Fig. 4 (set of points number 3). It was observed that the signal amplitude decreased at algae concentrations above 10^8 cells/ml (see set of points number 4). In this case enzymatic reactions can only occur on the border of the membranes (see Fig. 5) preventing substrate molecules from diffusing inside and reacting with algae situated near

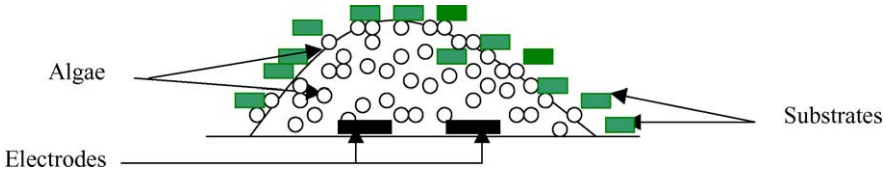

Fig. 5. For high algae concentration, the substrate is transformed on the membrane border where lots of enzymes are available. Yet, the local conductivity variation resulting of the enzymatic reaction can not be detected by the sensitive area.

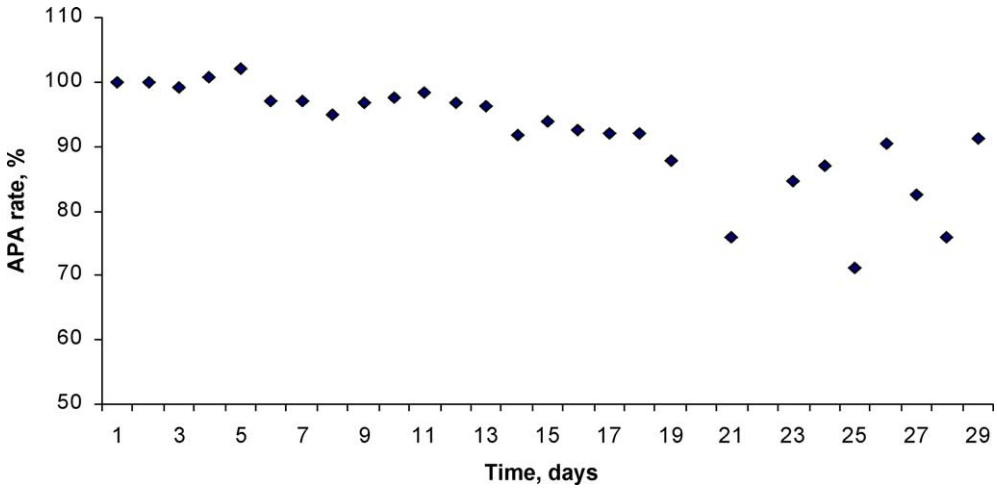

Fig. 6. Evolution of APA rate for 30 days measured with conductometric biosensor with BSA membranes. APA measurements were conducted in 10 mM Tris–HCl (pH 8.5) and 1 mM $MgCl_2$ with 43 mM pNPP solution as substrate.

the sensitive areas. As a consequence, a low signal was observed. Moreover, very low algae concentrations in membranes ($<4 \times 10^7$ cells/ml) also gives slight conductivity variations since only a few substrate molecules can be transformed (sets of points numbers 1 and 2). It is interesting to note that for pure enzyme membranes the same conclusion has already been done (Mai Anh et al., 2002).

The relative standard deviation of the sensor did not exceed 8% for the same sensor. Comparisons between different sensors for toxicity assays will be achieved using inhibition rates:

$$100 \times \left(1 - \frac{dS_{\text{after exposure}}}{dS_{\text{before exposure}}}\right) (\%)$$

Under storage conditions described in Section 2.5, the sensor response appeared to be stable for 20 days (see Fig. 6). This result compares well with what can be obtained with different sorts of biosensors (Dzyadevych et al., 2002; Gil et al., 2002).

3.2. Toxicity measurement assays

The conductometric biosensors developed are supposed to be used for the detection of pollutants, especially cadmium ions.

Note: Inhibition rates will be considered as significant when they exceed 10–15%.

Two different exposures were tested to study alkaline phosphatase inhibitions caused by these ions. First, for short exposure times, a comparison between curves obtained with bioassays and biosensors was done (see Fig. 7). In both cases, for smaller cadmium concentrations (<10 ppb), a slight increase can be observed, whereas APA is inhibited by high cadmium concentrations. The activation of some enzymes by low cadmium concentrations has already been studied for long exposures (>1 h) it could be explained by cellular stress: indeed to prevent the cell from cadmium damages, stress promoters are produced inducing an increase of some enzymatic activities (Olabarrieta et al., 2001;

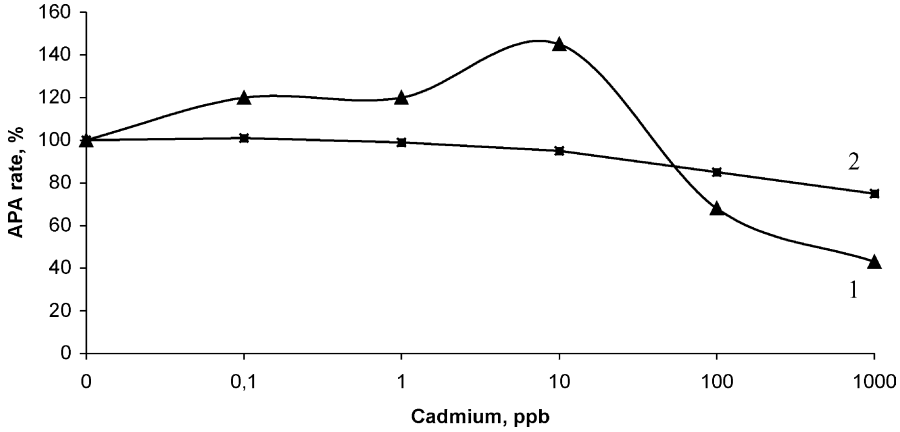

Fig. 7. Evolution of the APA rate measured with algae conductometric biosensors (1) and biossays (2) after an immediate contact with different cadmium concentrations. APA measurements were conducted in Tris–HCl (pH 8.5, 1 mM for biosensors and 10 mM for bioassays) and 1 mM $MgCl_2$ with a 43 mM pNPP solution as substrate.

Table 2
Comparisons between bioassays and biosensors concerning APA rate for prior contacts with cadmium

Exposure time to cadmium (h)	Bioassays (%)		Conductometric biosensors (%)	
	10 ppb	100 ppb	10 ppb	100 ppb
1	100	100	>70	0
2	95	90	30–50	0
4	80	65	0	0

Mazorra et al., 2002). Moreover, in the case presented here exposure is not long enough. But it is possible, at small concentrations, to compare cadmium to oligo-elements usually activating the enzymatic activity.

For long exposures (Table 2), from 0 to 2 h, we obtained higher inhibition rates with biosensors than with bioassays.

Inhibition (or activation for short exposures) rates are higher using biosensors than bioassays. It can be explained by the different ratios 'number of alga cells/cadmium' in both cases. Indeed in membranes, low amounts of algae were immobilised compared to bioassays using free algae: for biosensors, the ratio algae/cadmium is lower than for bioassays. As inhibitions rates are inversely proportional to these ratios, as a result, biosensors give higher inhibition rates and they seem to be more sensitive to detect the APA modifications.

4. Conclusion

The conductometric biosensor presented in this paper uses a new type of membranes containing immobilised whole cells.

In aquatic ecosystems, algae are the first trophic level: any disturbances could be reported to upper levels. This is one of the main interest of this work since it gave the opportunity to follow the response of a living organism to a pollutant, namely cadmium ions. An organism possesses different self-defence mechanisms which can reduce the theoretical effects of pollutants. Contrary to chemical analysis used to detect determined pollutants, whole cell biosensors can detect any stresses disturbing the organism metabolism.

For environmental management, the small and quick answering biosensors presented in this paper can be considered as competitive in situ tools as soon as the effects of pollutants on APA can be reversed, which means reusable membranes. A study led on phosphatase enzyme showed the efficiency of EDTA (Zhylyak et al., 1995; Corbisier et al., 1999; Kim et al., 2000) to reverse the action of some heavy metal ions. This is one of the main issue that will be studied with these biosensors.

Interferences of real matrices with these biosensors will have to be considered in future works. It is important to point out that these algal sensors can only be used to analyse aqueous samples taken from rivers, lakes, underground waters or from soils (leachates).

Acknowledgements

Part of this work was supported by France-Ukraine Bilateral Program 'Dnipro'.

References

Arkhypova, V.N., Dzyadevych, S.V., Soldatkin, A.P., El'skaya, A.V., Jaffrezic-Renault, N., Jaffrezic, H., Martelet, C., 2001. Multibiosensor based on enzyme inhibition analysis for determination of different toxic substances. Talanta 55, 919–927.

Babu, P.S.R., Panda, T., 1991. Studies on improved techniques for immobilizing and stabilizing penicillin amidase associated with *E. coli* cells. Enzyme Microb. Technol. 13 (8), 676–682.

Bozeman, J., Koopman, B., Bitton, G., 1989. Toxicity testing using immobilized algae. Aquat. Toxicol. 14, 345–352.

Corbisier, P., Van Der Lelie, D., Borremans, B., Provoost, A., De Lorenzo, V., Brown, N.L., Lloyd, J.R., Csoregi, E., Johansson, G., Mattiasson, B., 1999. Whole-cell- and protein-based biosensors for the detection of bioavailable heavy metals in environmental samples. Anal. Chim. Acta 387 (3), 235–244.

Dainty, A.L., Goulding, K.H., Robinson, P.K., Simpkins, I., Trevan, M.D., 1986. Stability of alginate-immobilized algal cells. Biotechnol. Bioeng. XXVIII, 210–216.

Dennisson, M.J., Turner, A.P.F., 1995. Biosensors for environmental monitoring. Biotechnol. Adv. 13, 1–12.

Durrieu C., Baddredine I., Daix C., 2003. A dialysis system with phytoplankton for monitoring chemical pollution in freshwater ecosystems by alkaline phophatase assay, J. Appl. Phycol., in press.

Durrieu, C., Tran Minh, C., 2002. Optical algal biosensor using alkaline phosphatase for determination of heavy metals. Ecotoxicol. Environ. Safety 51 (3), 206–209.

Dzadevych, S.V., Shul'Ga, A.A., Patskovsky, S.V., Arkhipova, V.N., Soldatkin, A.P., Strikha, V.I., 1994. Thin-film conductometric sensors for enzyme biotransducers. Russ. J. Electrochem. 30 (8), 987–991.

Dzyadevych, S.V., Soldatkin, A.P., Chovelon, J.M., 2002. Assessment of the toxicity of methyl parathion and its photodegradation products in water samples using conductometric enzyme biosensors. Anal. Chim. Acta 459, 33–41.

Fitzgerald, G.P., Nelson, T.C., 1966. Extractive and enzymatic analyses for limiting or surplus phosphorous in algae. J. Phycol. 2, 32–37.

Gil, G.C., Kim, Y.J., Gu, M.B., 2002. Enhancement in the sensitivity of gas biosensor by using an advanced immobilization of a recombinant bioluminescent bacterium. Biosens. Bioelectron. 17, 427–432.

Kim, J.-H., Cho, H.J., Ryu, S.-E., Choi, M.-U., 2000. Effects of metal ions on the activity of protein tyrosine phosphatase VHR: highly potent and reversible oxidative inactivation by Cu^{2+} ion. Arch. Biochem. Biophys. 382 (1), 72–80.

Khoshmanesh, A., Lawson, F., Prince, I.G., 1996. Cadmium uptake by unicellular green microalgae. Biochem. Eng. J. 62, 81–88.

Lubian, L.M., Blasco, J., Establier, R., 1992. A comparative study of acid and alkaline phosphatase activties in several strains of Nannochloris (Chlorophyceae) and Nannochloropsis (eustigmatophyceae). Br. Phycol. J. 27, 119–130.

Mai Anh, T., Dzyadevych, S.V., Soldatkin, A.P., Duc Chien, N., Jaffrezic-Renault, N., Chovelon, J.M., 2002. Development of tyrosinase biosensor based on pH sensitive effect transistor for phenol determination in water solutions. Talanta 56, 627–634.

Mazorra, M.T., Rubio, J.A., Blasco, J., 2002. Acid and alkaline phosphatase activities in the clam Scrobicularia plana: kinetics characteristics and effects of heavy metals. Comp. Biochem. Physiol. B 131, 241–249.

Olabarrieta, I., L'Azou, B., Yuric, S., Cambar, J., Cajaraville, M.P., 2001. In Vitro effects of cadmium on two different animal cell models. Toxicol. In Vitro 15 (4/5), 511–517.

Patskovsky, S.V., Volotovsky, V.V., 1996. Device for conductometric measurements of thin-films planar electrodes. Devices Exp. Tech. 4, 168.

Rawn J.D., 1989. Enzymatic Kinetics, Biochemistry International Edition, vol. 1. Neil Patterson Publishers, Burlington, NC, p. 1105.

Shul'Ga A., Dzyadevych S.V., Soldatkin A.P., Patskovsky S.V., Strikha V.I., El'Skaya A.V., 1994. Thin-film conductometric biosensors for glucose and urea determination, Biosens. Bioelectron. 9

Trebbe, U., Niggemann, M., Cammann, K., Fiaccabrino, G.C., Koudelka-Hep, M., Dzyadevich, S., Shulga, O., 2001. A new calcium-sensor based on ion-selective conductometric microsensors—membranes and features. Fresenius J. Anal. Chem. 371, 734–739.

Turner A.P.F., Karube I., Wilson G.S., 1987. Biosensors, Fundamentals and Applications. Oxford University Press, Oxford.

Wan, K., Chovelon, J.M., Jaffrezic-Renault, N., Soldatkin, A.P., 1999. Sensitive detection of pesticides using ENFET with enzymes immobilized by cross linking and entrapment method. Sens. Actuators B 58, 399–408.

Zhylyak G.A., Dzyadevych S.V., Korpan Y.I., Soldatkin A.P., El'Skaya A.V., 1995. Application of urease conductometric biosensor for heavy-metal ion determination, Sens. Actuators B (24/25), 145–148.