Whole cell algal biosensors for urban waters monitoring

Biocapteurs à cellules algales entières pour la surveillance des eaux urbaines

Durrieu Claude [1], Guedri Houssemeddine [1], Berezhetskyy Andriy [1, 2], Chovelon Jean-Marc [2]

1 ENTPE, LSE, rue Maurice Audin, 69518 Vaulx-en-velin cédex, France

2 Université Lyon I, LACE, Bd du 11 novembre 1918, 69100 Villeubanne, France

RESUME

La gestion durable des eaux urbaines nécessite des outils d'alarme précoce permettant de détecter la présence de composés toxiques de manière à réagir rapidement en cas de pollution. Les biocapteurs peuvent remplir ce rôle dans la détection des polluants grâce à leur sensibilité, leur faible coût et leur adaptation aisée à un mode de surveillance in situ et en continu. Le fonctionnement des biocapteurs à cellules algales présentés ici est basé sur la perturbation métabolique de cellules immobilisées après exposition à des polluants. Dans ce travail , des mesures ont été réalisées au moyen d'un biocapteur conductimétrique sur des eaux pluviales urbaines. Les résultats ont été comparés à ceux obtenus avec des bio-essais sur algues libres et ont permis de montrer la sensibilité des biocapteurs et leur intérêt pour le monitoring in situ.

ABSTRACT

Sustainable urban water management leads to the requirement of early warning system (EWS) to detect toxic compounds in order to react quickly in case of pollution. Biosensors for pollutants determination can act as EWS thanks to their unique characteristics with include their sensitivity, they low cost and their easy adaptation for continuous detection and on-site monitoring. Algal cells biosensors presented hereafter are based on metabolic perturbations of immobilized cells in the presence of toxicants. In this study, measurements were carried out with conductometric biosensors on urban stormwater. Results were compared to those obtained with bioassays on free algae and showed the high sensitivity of biosensors and their interest for on-site monitoring.

MOTS CLES

Biocapteurs, algues, pollution, métaux lourds, eaux pluviales urbaines.

1 INTRODUCTION

The environmental impact of urban waters (waste and storm) poses a great challenge to environmental protection and the pursuit of sustainable development of urban areas. Their harmfull effects on ecosystems were shown in numerous studies (Marsalek *et al.*, 1999; Baun *et al*., 2006).The necessity of a sustainable management of urban waters leads to the requirement of early warning system (EWS) to detect different chemicals in situ at very low concentrations in order to react quickly for limiting impact on natural surface and ground waters. Biosensors for pollutants determination can act as EWS thanks for their unique characteristics which include their sensitivity , their low cost and their easy adaptation for continuous detection on-site monitoring (Rogers 1995).

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 electric signal. The first link of a biosensor is the bioreceptor, which has a particularly selective site that identifies the analyte and ensures molecular recognition. In this work, bioreceptor is *Chlorella vulgaris* chosen among unicellular algal species because they are present ubiquitously in natural waters and are able to metabolise a wide range of chemical compounds (Tran-Minh, 2001).

These whole-cell algal biosensors are based on metabolic perturbations of immobilized cells in the presence of toxicants and have the ability to detect different 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 acethylcholinesterase and alkaline phosphatase, both are located on *chlorella vulgaris* membranes.

Compared to biosensors using purified enzyme, whole cell biosensor 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 (Vedrine *et al*., 2003). They can identify in situ the presence of a toxic compound as soon as it released in waste water or aquatic environment. Other whole cell biosensors were constructed from genetically modified cells (Corbisier *et al*.1999). 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.

Previous studies showed a good correlation between measurements carried out on river waters, polluted by heavy metals and pesticides, and results from chemical analysis (Chouteau *et al*. 2004).

The aim of this study is to assess the potentiality of a conductometric biosensor to monitor urban water toxicity. Experiments were conducted on urban stormwater brought to the labolatory. Results were compared with measurements by bioassays.

2 METHODS

2.1 Cells culture

The *Chlorella vulgaris* strain (CCAP 211 / 12) was purchased from the culture collection of Algae and Protozoa at Cumbria, United Kingdom. The axenic algal strain was grown in the culture medium and under conditions described by the international Organization for Standardization (ISO 8692).

2.2 Biosensor design

The conductometric transducers were fabricated at the Institute of Chemo-and Biosensors (Munster Germany) (Trebbe et al. 2001). Two airs f Pt(150 nm thick) interdigitated electrodes were made by the lift-off process on the Pyrex glass substrate. The Ti intermediate layer of 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 nm and their length was about 1 mm. Thus, the sensitive part of each electrode was about 1 mm^2.

Measurements are based on the detection of solution conductivity variation inside algal cells immobilized. Alkaline phosphatase induces catalytic reaction consuming / producing different ionic species resulting in measurable conductivity changes.

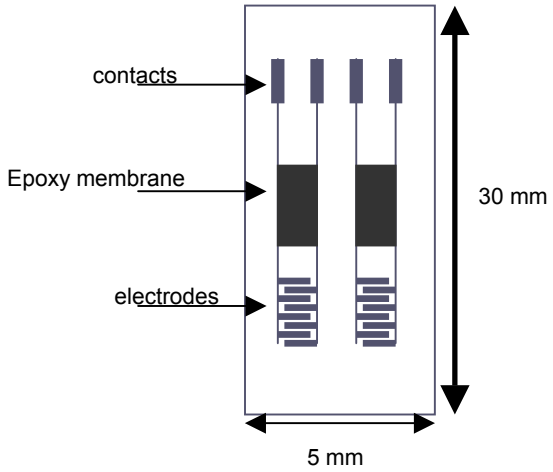

Figure 1 : conductometric biosensor

2.3 Algae immobilization

Sodium silicate (0,4 M, 4 ml) and LUDOX (8,5 M, 4 ml) were thoroughly mixed to obtain a homogeneous silica solution. An HCl 4 M solution was then added drop by drop until an appropriate pH is reached to induce the gelation process. Immediately an algal solution containing 1.3 x 108 cells / ml and 10 % (w/w) glycerol was introduced and the mixture was deposited on the sensitive area of the electrode.

2.4 Measurments

2.4.1 Enzymatic reaction measurements

For biosensors, measurements were carried out in daylight at room temperature in a 5 ml glass cell filled with Tris-HCl (10 mM, pH 8.4)

Biosensors were immersed in this vigorously stirred solution. After stabilisation of the output signal, different aliquots of the substrate stock solution were added into the vessel. The differential output signal (dS) was registered using a “home made” conductometric laboratory apparatus and the steady state response of the biosensor was plotted against the substrate concentration

For bioassays, free algae are used and measurements in microplates are based on fluorescence detected by a spectrofluorometer (Fluostar BMG). The alkaline phosphatase enzymatic reaction using MethylUmbelliferylPhosphate (MUP, Sigma) as substrate gives fluorescent product MethylUmbelliferone (MUF). Essays were carried out in 96 wells microplates.

2.4.2 Toxicity measurements

For biosensors, dS was measured for a definite substrate concentration. The biosensor was then preincubated in a test solution for 15 mn . After washing dS before and after exposure to the test solution were compared and the residual activity rate was calculated.

For bioassays, 48 wells microplate were filled with algal solution. After sedimentation, culture medium could be removed and replaced by the test solution. Exposure last two hours. After removing the test solution and resuspending algae in distilled water, enzymatic activity was measured in 96 wells microplate as above-mentioned.

3 RESULTS AND DISCUSSION

3.1.1 Enzymatic activity detection using conductometric biosensors

In a previous work, it has been proved that phosphatase alkaline activity can be monitored for immobilized *Chlorella vulgaris* using conductometric biosensors (Chouteau et al., 2004). As shown in fig 2, the enzymatic activity follows a classical Michaelis-Menten behaviour. The relative standard deviation of the sensor did not exceed 8 %.

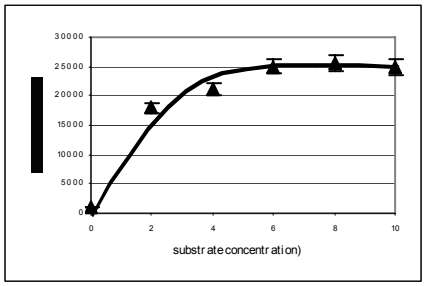

figure 2 : Alkaline phosphatase activity measured with a conductometric biosensor

3.1.2 Biosensor calibration for heavy metal detection

Alkaline phosphatase measurements were carried out before and after exposure to heavy metals ions. Fig 3 shows phosphatase activity (expressed as percent of control before exposure).

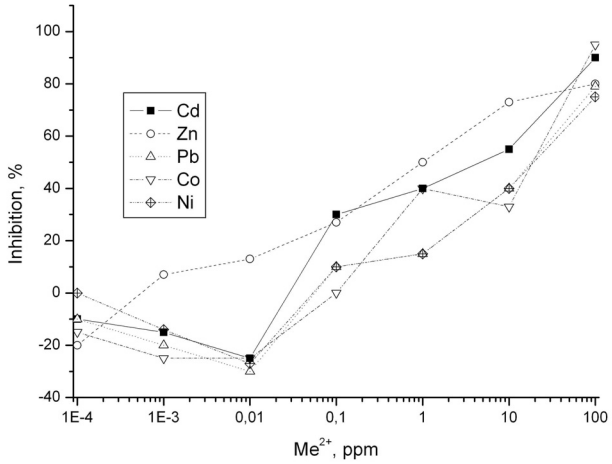

Figure 3 : evolution of phosphatase activity rate after exposure to different metal ions

Inhibition can reach 80 % of control for 100 mg/ L heavy metal. At low concentrations (0.1 - 10 µg/l) metal ions as Cd, Pb, Co, Ni enhance phosphatase activity. The activation of some enzymes by low metal concentrations has already been studied and could be explained by cellular stress : indeed to prevent the cell from metal damages, stress promoters are produced inducing an increase of some enzymatic activities (Mazora et al. 2002).

3.1.3 Biosensor responses after exposure to urban stormwaters

The biosensor developed is supposed to be used for the detection of pollutants especially heavy metal ions.

Measurements were carried out after exposures to different concentrations of urban stormwater in the mixture assay. Stormwater was sterilized before experiment at 130°C, 1.5 bar for suppressing bacterial phosphatase activity. In fig 4, biosensor response can be compared with results of fig 3. The lowest concentrations of stormwater enhance phosphates activity as higher concentrations inhibit this activity.

Heavy metal contain of stormwater tested was determinated by chemical analysis (resuts showed in table 1). A mixture of metal ions with a concentration between ppb and ppm, can explain results obtained.

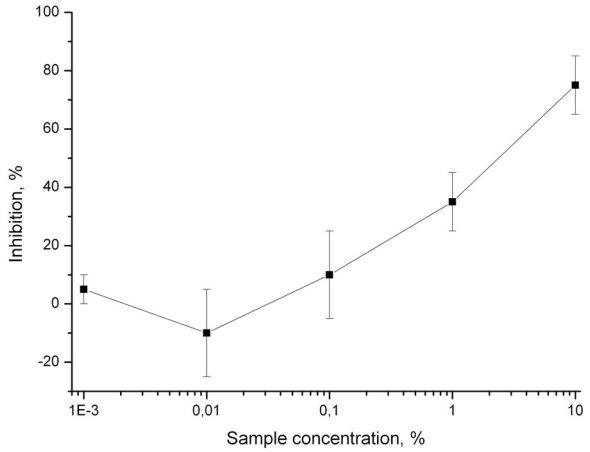

Fig ure 4 : Evolution of algal phosphatase activity rate after exposure at different concentrations of urban storwater detected with conductometric biosensor

Zn (µg:l)	Pb (µg/l)	Cu (µg/l)	Cr (µg/l)	Ni (µg/l)	Cd (µg/l)	Fe (mg/l)	Al (mg/l)
740	46.6	146	8.8	3.30	0.23	1.81	0.94

Table 1 : heavy metal contain of urban stormwater tested

For the lowest concentrations of stormwater tested an activation of phosphatase activity was obtained due to the low concentration of metal ions. For higher concentrations a good correlation was obtained between concentration of sample and inhibition rate.

3.1.4 Bioassay responses after exposure to urban stormwater

Bioassays on microplate were carried out on free algae. Fig 5 shows phosphatase activity rate after 2 hours exposure of algal cells to different concentrations of stormwater samples.

A comparison between figures 4 and 5 shows the same behaviour between phosphatase activity of free cells and cells immobilized on conductometric biosensor. (Activation for lowest concentrations and inhibition for the highest).

However, inhibition (or activation for low concentrations) rates are higher using biosensors than bioassays. It can be explained by the different ratios 'number of algal cells/toxicant elements' in both cases. Indeed on biosensors, low amounts of algae were immobilized compared to bioassays using free algae : for biosensors the ratio algae : toxicants is lower than for bioassays. As inhibition rate are inversely proportional to these ratios as a result, biosensors give higher inhibition rates and they seem to be more sensitive to detect the enzymatic activity modification.

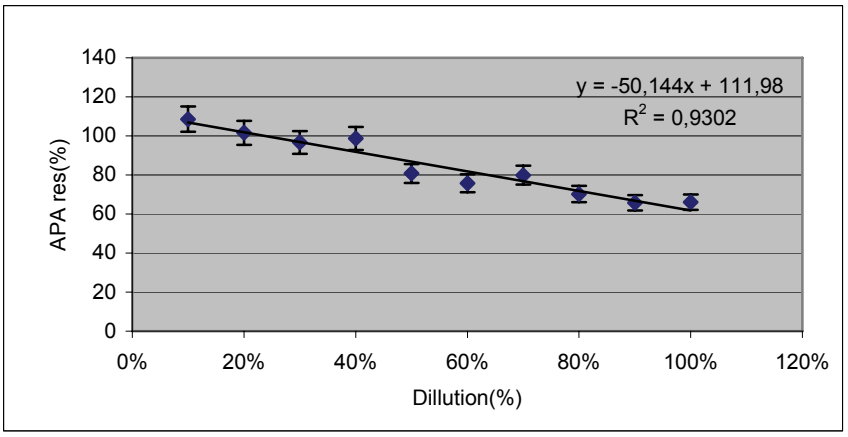

Figure 5 : evolution of phosphatase activity rate detected on free algae after exposure to urban strormwater

Algal growth inhibition test was carried out on *Pseudokirchneriella sucapitata* according to AFNOR (NF T90-375 1998). Results showed very low toxicity of stormwater tested on algal growth (CE 50 was not reached with 80 % of stormwater in assay).

Then, changes in phosphatase activity reflect specific interactions between pollutants and algae and not a total attack of the cells.

4 CONCLUSIONS

The conductometric algal biosensor presented in this study seems to be successful as early warning system to identify the presence of pollutants as metal compounds.

In aquatic environment, 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 pollutants. Contrary to chemical analysis, used to detect determined pollutants, algal cell biosensors can detect any stresses disturbing the organism metabolism.

Interferences of real matrices with these biosensors will have to be considered in the next step. After that, biosensors will be placed in the area under monitoring.

5 REFERENCES

Baun A., Eriksson E., Ledin A. and Mikkelsen P.S. (2006). A methodology of ranking and hazard identification of xenobiotic organic compounds in urban stormwater. Science of total environment, 370 (1), 29-38.

Chouteau C., Dzyadevych S., Chovelon J.M. and Durrieu C. (2004). Development of novel conductometric biosensors based on immobilized whole cell Chlorella vulgaris microalgae, Biosensors Bioelectorn. 19 (9), 1089-1096.

Corbisier P., Vander lelie D., Borremans B., Provoots A., de Lorenzo V., Brown N.L., Lloyd J.R., Hobman J.L., Csöregi E., Johansson G. and Mattiasson B. (1999). Whole cell and protein based biosensors for the detection of bioavailable heavy metals in environmental samples. Anal. Chim. Acta, 387, 235-244.

ISO 8692, international Organisation of Standardization (1989), Water quality : fresh water growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*.

Marsalek J., Rochfort Q., Mayer T., Servos M., Dutka B. and Brownlee B. (1999). Toxicity testing for controlling urban wet-weather pollution : advantages and limitations. Urban water, 1(1), 91-103.

Mazora; M.T., Rubio J.A. and Blasco J. Acid and alkaline phosphatase activities in the clam Scrobicularia plana : kinetics characteristics and effect of heavy metals. Comp. Biochem. Physiol.B 131, 241-249.

Rogers K.R. (1995). Biosensors for environmental applications. Biosensors bioelectron., 10, 533-541.

Tran-Minh C.(2001). Les biocapteurs, principe, construction et applications. Paris : Masson, 158 p.

Trebbe U., Niggemann M., Cammann K., Fiaccabrino C., Koudelka-Hep M., Dzyadevych S. and Shluga O. (2001). A new calcium-sensor based on ion-seective conductomtric microsensors-membranes and features. Fresen J. Anal. Chem 371, 734-739.

Vedrine C., Leclerc J.C. Durrieu C. and Tran-Minh C. (2003). Optical whole cell biosensor using Chlorella vulgaris designed for monitoring herbicides. Biosensors Bioelectron. 18 (4) 457-463.