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Ultra-sensitive conductometric detection of pesticides based on inhibition of esterase activity in *Arthrospira platensis*

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ABSTRACT

Enzymatic conductometric biosensor, using immobilized *Arthrospira platensis* cells on gold interdigitated electrodes, for the detection of pesticides in water, was elaborated. Cholinesterase activity (AChE) was inhibited by pesticides and a variation of the local conductivity was measured after addition of the substrate acetylthiocholine chloride (AChCl). The Michaelis–Menten constant (Km) was evaluated to be 1.8 mM through a calibration curve of AChCl. Inhibition of AChE was observed with paraoxon-methyl, parathion-methyl, triazine and diuron with a detection limit of 10^{-18} M, 10^{-20} M, 10^{-20} M and 10^{-12} M, respectively and the half maximal inhibitory concentration (IC50) was determined at 10^{-16} M, 10^{-20} M, 10^{-18} M and 10^{-06} M, respectively. An important decrease of response time $\tau_{90\%}$ was recorded for AChE response towards AChCl after 30 min cell exposure to pesticides. Scanning electron microscopy images revealed a degradation of the cell surface in presence of pesticides at 10^{-06} M.

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1. Introduction

Organophosphorus and carbamate pesticides are widely used in agriculture because they are less persistent in the environment than organochlorates (DDT, aldrin, lindane, etc.) (Martorell et al., 1997). Nevertheless, their toxicity is a serious problem, affecting the equilibrium of aquatic systems and farm workers health. Moreover, they contaminate farm products and groundwater. These pesticides are toxic because they act as inhibitors of acetylcholinesterase activity that is responsible for the hydrolysis of acetylcholine (ACh) at cholinergic synapses, which is necessary for the control of the neurotransmission (Tourmaa, 1995; Cabello et al., 2001). They are liposoluble and are capable to penetrate the skin, the placenta, and the foetus (Kramer et al., 2002; Villeneuve et al., 1972).

Traditional analytical techniques for determination of pesticide residue are gas chromatography (GC), liquid chromatography (LC) (Hall et al., 1997), or hyphenations (GC–MS or LC–MS/MS) and others (Yao et al., 1991; Sherma, 1993, 1995). These methods provide a fine and complete response, nonetheless, they need heavy and expensive equipment, highly skilled labours, complex procedure and time consuming. However, environmental quality control requires early warning systems for on line and in situ monitoring electrochemical platform detection (Sbartai et al., 2012; Dennison and Turner, 1995, Tekaya et al., 2013, 2012) and biosensors can constitute such devices. They are sensitive, portable, easy to use, and capable to provide reliable analytical information. Various inhibition biosensor devices based on the immobilization of acetylcholinesterase organophosphorus hydrolase onto various electrochemical, piezoelectric or optical transducers, have been used for the continuous assessment of pesticides (Halamek et al., 2005; Marty et al., 1995; La Rosa et al., 1995). Electrochemical biosensors for the measurement of pesticides are based on the enzymatic inhibition of AChE (Campanella et al., 1991; Martorell et al., 1994).

During years 2007–2009, Namour et al., 2010 counted two dozens of papers on detection of pesticides, mainly on organophosphates, of some carbamates, as presented in Table 1 extracted from their recent review. Among these, twelve articles present electrochemical methods on modified electrodes, among which seven enzymatic biosensors based on choline esterase activity, leading to the lowest detection limits. Other micro-sensors are

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Table 1
Micro-sensors for organophosphate, carbamates and pesticides determination in, water (Namour et al., 2010).

Method	Compound	LoD[a]
Colourimetry on gold nanoparticles	Chlorpyrifos	20 μg/L
	Malathion	100 μg/L
Guided shear horizontal surface acoustic wave devices on $LiTaO_3$	Phosmet	5 mg/L
	Parathion	2 mg/L
Voltammetry on clay modified electrodes containing $Ni_2Al{-}NO_3$	Glyphosate	169 μg/L
	Glufosinate	905 μg/L
Amperometric activity measurement of acetylcholinesterase immobilized on screen-printed graphite electrode	Chlorpyrifos	2 μg/L
Voltammetry on glassy carbon electrode modified with a poly-L-cysteine film	Methyl parathion	1.7 μg/L
Amperometric activity measurement of acetylcholinesterase immobilized on gold nanoparticles and silk fibroin modified platinum electrode	Methyl paraoxon	6 ng/L
	Carbofuran	7 ng/L
	Phoxim	0.6 μg/L
Amperometric activity measurement of acetylcholinesterase immobilized on calcium carbonate-chitosan composite film	Methyl parathion	1 μg/L
Amperometric activity measurement of acetylcholinesterase and choline oxidase within separate hybrid mesoporous silica membranes	Diazinon-oxon	0.37 μg/L
Amperometric activity measurement of acetylcholinesterase on gold-platinum bimetallic nanoparticles onto 3-aminopropyltriethoxy silane modified glassy carbon electrode	Paraoxon ethyl	9.5 μg/L
	Sarin	6 μg/L
	Aldicarb	48 μg/L
Amperometric activity measurement of acetylcholinesterase on poly(dimethylsiloxane)–poly (diallyldimethylammonium)/gold nanoparticles composite film	Paraoxon	0.5 ng/L
	Parathion	1.0 ng/L
Voltammetry on nano-alumine film modified electrode	Parathion	0.3 μg/L
Amperometric reactivation measurement of cholinesterase from organophosphorus-inhibited rat saliva on carbon nanotube modified screen printed carbon electrode	Paraoxon	0.14 μg/L
Voltammetry on conducting MIP deposited on a platinum electrode	Atrazine	4.5 μg/L
Surface plasmon resonance (SPR) on monoclonal anti-bodies immobilized on a gold sensor surface	Atrazine	20 ng/L
Oxygen production and fluorescence measures on the algae *Elodea canadensis* culture	Atrazine	10 μg/L
Surface plasmon resonance (SPR) on monoclonal anti-bodies immobilized on sensor chip surface	Isoproturon	0.1 μg/L
Resonance characteristics hepatoma cultured on an indium tin oxide surface of quartz crystal	Paraquat	2.7 mg/L
Surface plasmon resonance (SPR) on monoclonal anti-bodies on gold-thin layer	DDT	15 ng/L

[a] LoD: Limit of Detection.

devoted to measure atrazine (Pardieu et al., 2009; Farré et al., 2007; Vervllet-Scheebaurn et al., 2008), isoproturon (Gouzy et al., 2009), paraquat (Lee et al., 2009) or DDT-like compounds (Mauriz et al., 2007).

Recently, the development of whole-cell biosensors has raised an increasing interest. Microalgae such as *Chlorella vulgaris*, were used in various studies to develop whole-cell biosensors for the control of toxic pollutants in aquatic environments (Durrieu and Tran-Minh, 2002; Pandard et al., 1993; Chouteau et al., 2005; Guedri and Durrieu, 2008). Gupta et al. (1998) have shown that AChE could be detected in algae (Gupta et al., 1998). Enzymatic conductometric biosensor using immobilized *Chlorella vulgaris* for the detection of organophosphorous compounds in water samples was elaborated by Chouteau et al. (2005). They reported that paraoxon-methyl inhibits *Chlorella vulgaris* AChE contrary to parathion-methyl and carbofuran.

Arthrospira platensis is a Gram negative bacterium. It is also considered as a blue-green microalga and called Spirulina. Salvi et al. (1994) studied the cloning and the characterization of the gene encoding an esterase from *Arthrospira platensis*.

In this work, kinetic proprieties of AChE were directly determined using conductometric transduction which has been extensively used for probing enzymatic reactions for biomedical applications, such as urea (Nouira et al., 2012) and glucose (Dzyadevych et al., 1998) detection or for environmental applications (Jaffrezic-Renault and Dzyadevych, 2008; Korpan et al., 2000; Wang et al., 2006).

In conductometric enzymatic biosensors, enzymatic reaction is confined close to the interdigitated electrode surface, because enzyme is cross-linked in contact with this surface, in presence or in absence of gold nanoparticles. The interdigitated electrodes allow the measurement of the change of conductivity in the region defined by field lines. The thickness probed by field lines is of the order of the interdigit distance (few tens of mm) (Pänke et al., 2008). As it has been modelled (Sheppard et al., 1996; Temple-Boyer et al., 2008), the observed steady-state response of the conductometric biosensor is the result of the reaction rate limited kinetics of the enzymatic reaction and the diffusive flux of urea hydrolysis products away from the transducer surface, in the boundary layer. The most representative result of enzymatic reaction is pH variation; a pH limit value is reached at the transducer surface (Temple-Boyer et al., 2008).

In order to improve storage stability of enzyme based biosensors, different immobilizations and electrodes have been essayed, such as carbon paste electrodes (CPEs), solid graphite electrodes (Choi et al., 2008), surface-modified electrodes (Nistor and Emneus, 1999). In this study, interdigitated transducers (IDTs) were used in the conductometric device, and their geometry takes advantage of the changed conditions for the current flow which mainly occurs close to the surface and, thus, shows a much higher sensitivity towards surface change compared to the conventional flat design of electrodes. Gold nanoparticles were used, in this work, for the immobilization of Spirulina cells. Gold nanoparticles have been considered to have a wide range of potential applications including biomarkers (Liu et al., 2008), biosensors (Bai et al., 2007; Pandey et al., 2008) molecular recognition systems (Liu et al., 1998; Labande and Astruc, 2000) and nanoscale electronics etc. (Sato et al., 1997). The functionalization of gold nanoparticles may provide desired functional groups to facilitate biomolecule immobilization, such as Liu et al. (2010) who describe the layer-by-layer assembly of ferrocene poly(ethylenimine) and gold nanoparticles (Liu et al., 2010).

In this study, Spirulina cells were coated with PAH-coated gold nanoparticles through co-reticulation with Bovine serum albumin (BSA), by glutaraldehyde (GA) vapours cross-linking and they were observed using Scanning Electron Microscopy (SEM).

2. Experimental

2.1. Chemical and biological reagents

Acetylthiocholine chloride (AChCl) purchased from Sigma–Aldrich was used as the substrate for measuring esterase activity. It was dissolved in the buffer solution to prevent a change to the total conductivity of the reaction when adding the substrate. The buffer was Sodium phosphate (Na_2 HPO_4), purchased from Sigma–Aldrich. Na_2 HPO_4 was diluted in Milli-Q water to obtain a concentration of 5 mM and the pH was 5.2.

The stock solutions of AChE inhibitors (Paraoxon-methyl, Parathion-methyl, Triazine and Diuron), were prepared from product purchased from Sigma–Aldrich. Stock solutions were stored at 4 °C and fresh dilutions were prepared, in the solution buffer (Na_2 HPO_4), 15 min before each series of analysis.

Monodispersed gold nanoparticles were purchased from Sigma–Aldrich where the mean particle size was 20 nm.

Bovine serum albumin (BSA), glutaraldehyde (GA) (grade II, 25% aqueous solution) and the polyelectrolyte used in this study, Poly(allylamine Hydrochloride) (PAH), were purchased from Sigma–Aldrich.

Arthrospira platensis (Compere 1968/3786 strain) called Spirulina, was cultivated under sterile conditions in Zarrouk liquid medium containing: (g/L) $NaNO_3$, 2.50; K_2HPO_4, 0.50; $NaHCO_3$, 10.00; NaCl, 1.00; $MgSO_4\,7H_2O$, 0.2; $CaCl_2\,2H_2O$, 0.02; $FeSO_4\,7H_2O$, 0.01. All salts were of analytical grade and were purchased from Acros Organics. The medium was adjusted to pH 9.0 using NaOH solution. Cultivation was conducted in 5 L Erlenmeyer flasks. Cultures were maintained at 26 ± 1 °C under air bubbling and continuously exposed to fluorescent lamps (100 μmol photon/m^2 s). After, the biomass was recovered by filtration, washed with physiological water for the removal of nutrient salts, and then dried at 40 °C for 48 h. Then, it was lyophilized and the resulting powder was protected from moisture by storage in a closed vessel at 4 °C. The Spirulina was dissolved in $Na_2\,HPO_4$ and it was filtered (0.8 μm) before analysis. As Spirulina is made of transparent cells stacked end-to-end and entrapped with a sheath forming a spiral filament, the sheath was broken through filtration in order to separate individual cells. This step was very crucial in our studies.

2.2. Scanning electronic microscopy

The SEM images were captured using a Quanta™ 250 microscope (FEI) in degraded pression mode. A pretreatment of cells was required before imaging. Cells were fixed with covalent links using Glutaraldehyde solution (3%, v/v) purchased from Sigma–Aldrich, specially purified for use as an electron microscopy fixative. Spirulina-based biosensors were immerged in this solution for 45 min. After, a series of dehydration in successive absolute ethanol solutions were applied for 10 min using increased concentrations (20%, 40%, 60%, 80%, and 100% ethanol).

2.3. Sensor design

The conductometric transducers (Fig. 1a) were fabricated at the V.Ye. Lashkaryov Institute of Semiconductor Physics (Kyiv, Ukraine). Each of them consisted of two identical pairs of interdigitated thin film electrodes (150 nm thick), fabricated by gold vapour deposition onto a non-conducting pyroceramic substrate (5 × 30 mm). The technique of sensor manufacturing has been reported previously (Arkhypova et al., 2005; Dzyadevych et al., 2009). A 50 nm thick intermediate Cr layer was used to improve the adhesion of Au to the substrate. Both the digit width and interdigital distance were 10 μm, and their length was ~1.5 mm. As a result, the sensitive area of each pair of electrodes was ~2.9 mm^2 (Fig. 1a).

2.4. Conductometric device

A biosensor analyzer consisted of a sensor block and an electronic measuring block (portable four-channel biosensor analyzer) was developed in collaboration with Institute of Electrodynamics of National Academy of Sciences of Ukraine and it was reported by Dzyadevych et al. (2009). The sensor block consisted of a stand with a fixed block of holders; each holder was connected to the fingers of an appropriate conductometric biosensor. An electronic measuring block consisted of the following modules: a secondary transducer and a basic measurement-control module. A personal computer with a specific software support was an integral part of the measuring block. Using a generator, a sinusoidal excitation signal with a frequency of 30 kHz and amplitude of 10 mV was applied to the two exciting electrodes. These conditions avoided faradic processes, double-layer charging, and polarization of the microelectrodes. The signals measured were processed using the lock-in amplifier method, i.e. they were filtered using a low bandwidth centred on the excitation frequency and then transmitted to the synchronous detection which provided the difference of conductivity between the working electrode and the reference electrode. In fact, this system transformed the local variations in conductivity to a change of the active conductivity which can be recovered as information at the output of the system, such as variations in the output signal versus time.

Measurements were performed at room temperature in a glass cell of 4 mL. The biosensor was immersed gently into the buffer ($Na_2\,HPO_4$, 5 mM). A magnetic stirrer ensured solution homogeneity throughout experimentations. After the signal stabilization, different quantities of the substrate were added in the measurement cell. A rinsing step was performed between two successive measurements due to their reversible character.

2.5. Electrochemical impedance spectroscopy (EIS) measurements

The measurements were performed with an electrochemical impedance analyzer "Voltalab PGZ 402" (Hach Lnage, France) in a two electrode configuration. Data acquisition and processing via 'Voltamaster' software was provided by the same company. All EIS measurements were taken at a frequency range of 100 mHz to 100 kHz at room temperature. The same glass cell (4 mL) was filled with $Na_2\,HPO_4$ (5 mM) under vigorous magnetic stirring.

2.6. Preparation of PAH-coated gold nanoparticles

A suspension of gold nanoparticles (1% w/w) was firstly centrifuged at 20 °C using a centrifugator 2–6 K SIGMA from Fisher Bioblock Scientific at 9000 g for 20 min. The rich supernatant phase was then eliminated and the gold nanoparticles were immersed in 100 mL of aqueous solutions of 5 mg mL^{-1} of PAH (positive

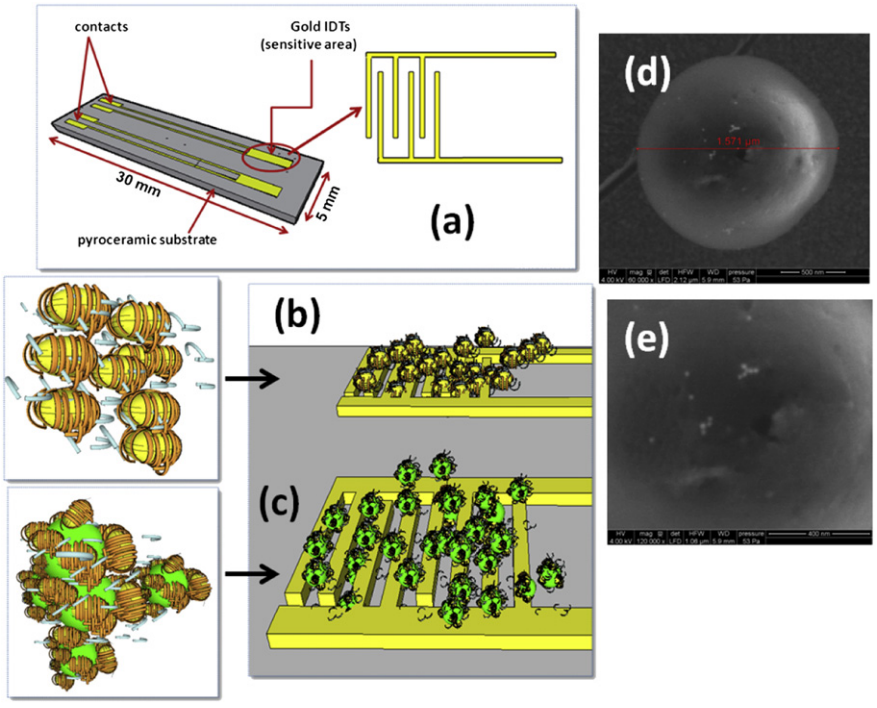

Fig. 1. (**a**) Scheme of microelectrodes with gold IDTs, (**b**) PAH-coated gold nanoparticles through co-reticulation with BSA (20%) by glutaraldehyde vapours cross-linking, immobilized on the reference electrode, (**c**) Spirulina cells coated with PAH-coated gold nanoparticles through co-reticulation with BSA (20%) by glutaraldehyde vapours cross-linking, immobilized on the working electrode, (**d**) SEM image of Spirulina cell, (**e**) An enlargement of the cell image that shows the attachment of PAH-coated gold nanoparticles on the cell surface. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

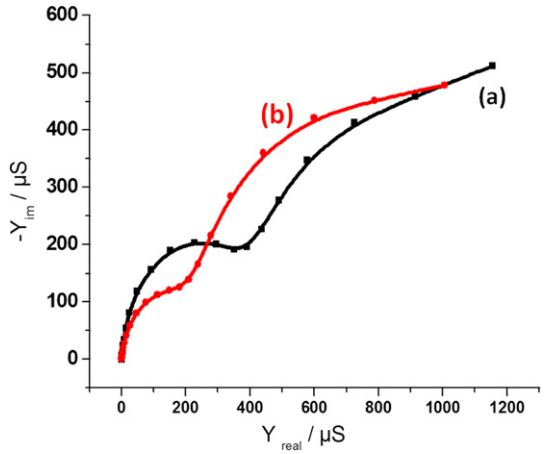

Fig. 2. Complex admittance spectra of (**a**) bare electrode and (**b**) modified electrode, where: frequency range = 100 mHz–100 kHz, amplitude potential = 10 mV, with measurements made in Na_2 HPO_4 (5 mmol/L at pH 5.2).

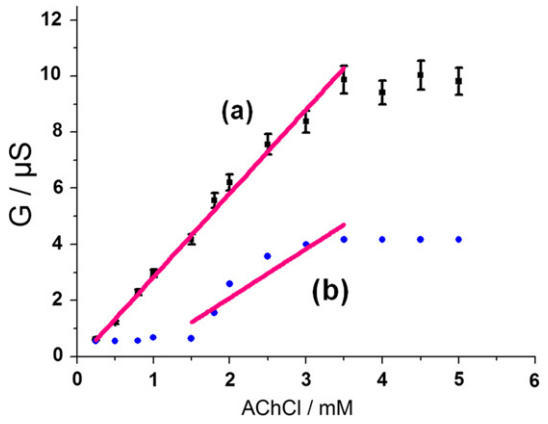

Fig. 4. Calibration curve of the AChE with AChCl concentration: (a) with gold nanoparticles (b) without gold nanoparticles. The mean values and error bars have been calculated from 3 experimentations with different biosensors, under the same experimental conditions (Na_2 HPO_4, 5 mmol/L at pH 5.2).

charge), under mechanical stirring for 15 min. After the adsorption process, PAH-coated gold nanoparticles were removed by similar centrifugation and redispersion in 20 mM of phosphate buffer at pH 7.3.

3. Results and discussion

3.1. Biosensor functionalization

IDT electrodes were cleaned by sonication for 10 min, followed by chemical treatment in piranha solution (H_2SO_4/H_2O_2, 3:1 v/v) for 5 min. The electrodes were then rinsed with absolute ethanol and finally dried under a flow of nitrogen.

The PAH-coated gold nanoparticles were dispersed in 20 mM phosphate buffer (pH 7.3) containing 5% of BSA and 5% of Spirulina solution. After 1 h of mixing, this solution was centrifuged at the same conditions described recently and redispersed in 20 mM phosphate buffer. A volume of 0.7 µL of this solution was deposited onto the sensitive area of the first pair of IDTs representing the work electrode (Fig. 1c). The cyanobacteria concentration was 0.3 g/L throughout this study. On the second pair of IDTs representing the reference electrode (Fig. 1b), PAH-coated gold nanoparticles were dispersed in 20 mM phosphate buffer (pH 7.3) containing 20% of BSA. 0.7 µL of this solution was deposited onto the sensitive area of the reference sensor. This configuration allowed differential mode measurements. The sensors were then placed in saturated GA vapours for 30 min. After exposure, the biosensors were dried at room temperature for 30 min and stored at 4 °C before the experiments.

Electrochemical admittance spectra were recorded for a bare microelectrode and after 12 h immobilization of Spirulina cells. Fig. 2 shows an evolution of complex admittance spectra, validating the immobilization process.

SEM images revealed the coexistence of several populations of Spirulina cells with 1.2 µm a mean size. An enlargement of the cell image in Fig. 1e shows the attachment of PAH-coated gold nanoparticles on the cell surface.

3.2. Characteristics of kinetics for ChE activity

The modified electrodes were tested for their sensitivity to the substrate AChCl. The response time $\tau_{90\%}$ was deduced at 83 min (Fig. 3a). Typical real time response occurs in two steps. A first very rapid one and a slower one (Fig. 3b). The enzyme and the substrate first combine to form the Michaelis enzyme–substrate complex. The acetyl group then transfers to the serine group in the active site of the esterase molecule to form the acetylated enzyme and the first product (choline chloride). After that, there is a hydrolysis of the acetylated enzyme to produce the free enzyme and the second hydrolytic product (acetic acid) (Roberto-Andres and Narayanaswamy, 1997).

The calibration curve of the enzyme during exposure to various concentrations of AChCl was investigated in triplicate and it is depicted in Fig. 4a where the corresponding equation is:

$$G/\mu S = (2.98)[\text{AChCl}]/\text{mM} + 0.16, \quad (1)$$

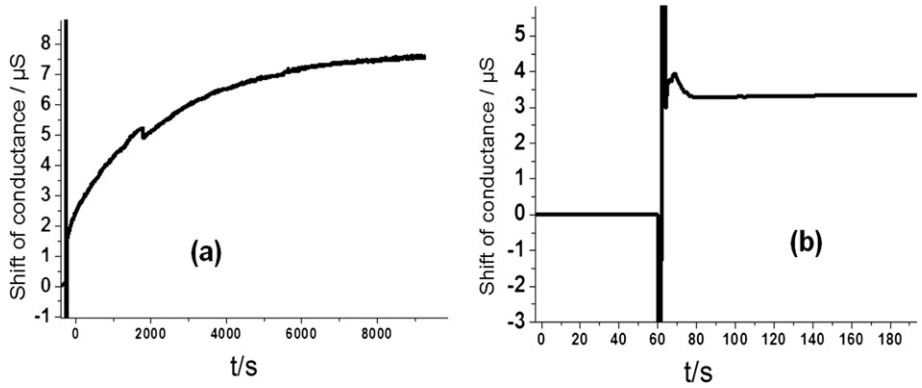

Fig. 3. Typical real time response of the conductometric transducer, to the substrate AChCl and gold nanoparticles effect: (a) before incubation in paraoxon-methyl (10^{-18} mol/L), (b) after 30 min of incubation in paraoxon-methyl (10^{-18} mol/L) (AChCl, 3.5 mM and Na_2 HPO_4, 5 mmol/L at pH 5.2).

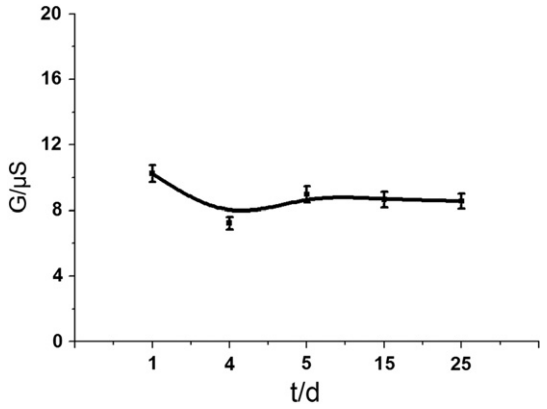

Fig. 5. Evolution of the signal of conductometric biosensors stored at 4 °C in dry conditions. The mean values and error bars have been calculated from 3 experimentation with different biosensors, under the same experimental conditions (AChCl, 3.5 mM and Na_2 HPO_4, 5 mmol/L at pH 5.2).

As shown in Fig. 4a, the enzymatic activity follows a classical Michaelis–Menten behaviour and reaches a level corresponding to enzyme saturation at concentrations greater or equal to 3.5 mM AChCl. Mean values and error bars were calculated from three measurements using different biosensors and the relative standard deviation of the sensor did not exceed 8%.

The Michaelis–Menten constant (Km) was calculated at 1.8 mM. This value is close to Km (1.9 mM) determined by Masson et al. (2001) Km (1.49 mM) (Dave et al., 2000) and Km (1.5 mM) (Rush et al., 1980) where ChE was extracted from *Homo sapiens*, *Rattus norvegicus* and *Oryctolagus cuniculus*, respectively.

Fig. 4b presents the calibration curve of AChE performed without gold nanoparticles functionalization and the corresponding equation is.

$$G/\mu S = (1.74)[\mathrm{AChCl}]/\mathrm{mM} + 1.4, \quad (2)$$

The overlay of both curves shows that the presence of nanoparticles amplifies the shift of conductance (Fig. 4). Therefore, due to their electrical conductivity, gold nanoparticles can behave as nanoelectrodes, as already been shown by Nouira et al. (2012) in case of another hydrolase enzyme.

Storage stability of Spirulina biosensor was monitored for more than 1 month. The biosensor responses as a function of a storage time are shown in Fig. 5. Each point of the curve corresponded to an arithmetic average of between 3 measurements with 3 different biosensors stored in dry conditions at 4 °C (each measurement corresponded to a response to 3.5 mM of AChCl). Experiments show a stability of the conductivity during 25 days. Therefore, the lifetime of the biosensor can be estimated to be more than 25 days.

3.3. Inhibition measurements

To evaluate the effects of pesticides on the enzymatic activity of the Spirulina, measurements were performed before (dS_{before}) and after (dS_{after}) incubation in 7 concentrations of paraoxon-methyl, parathion-methyl, triazine and diuron: 10^{-20} M, 10^{-18} M, 10^{-16} M, 10^{-14} M, 10^{-12} M, 10^{-09} M and 10^{-06} M. This requirement arises from the fact that the detection process involves the comparison of the enzyme activity before and after the exposure to the inhibitor. The change in sensor response should therefore be attributed to the inhibitor and not to the poor or non-reproducible sensor performance.

Results were expressed as a percentage of residual activity (A_{res}) given by the following expression:

$$A_{res}(\%) = \frac{dS_{after}}{dS_{before}} \times 100 \quad (3)$$

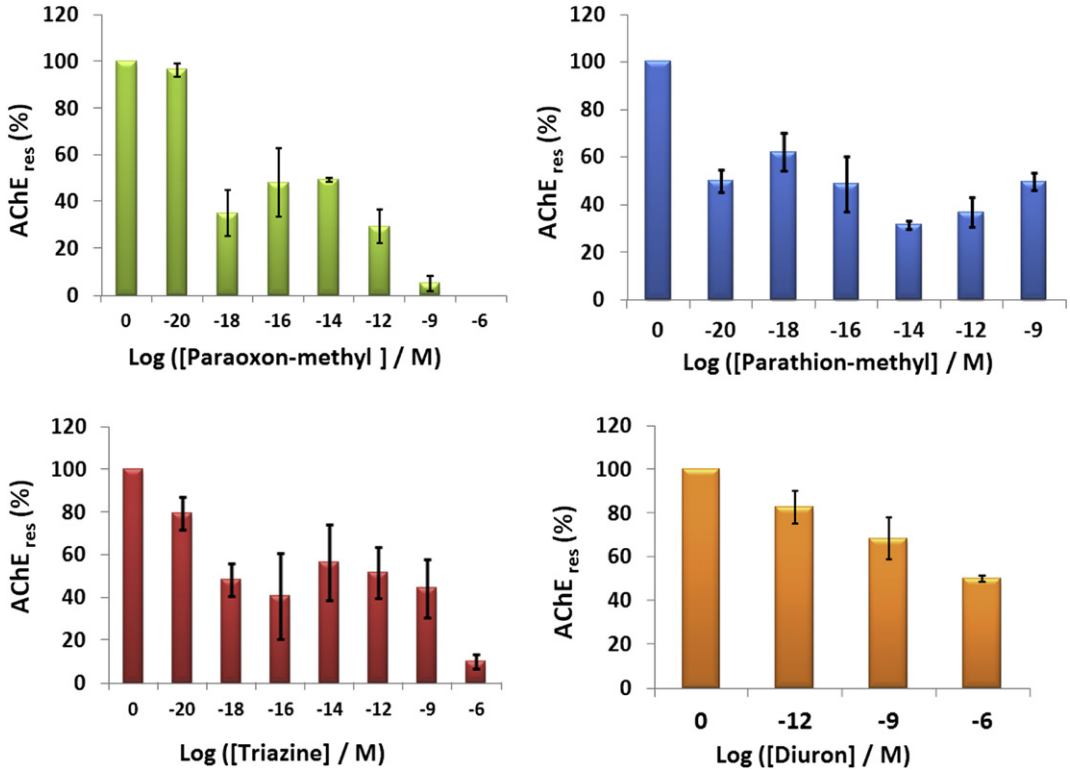

Fig. 6. $AChE_{res}$ (residual) for 30 min exposure to paraoxon-methyl, parathion-methyl, triazine and diuron. The mean values and error bars have been calculated from 3 experimentations with different biosensors under the same experimental conditions (AChCl, 3.5 mM, and Na_2 HPO_4, 5 mmol/L at pH 5.2).

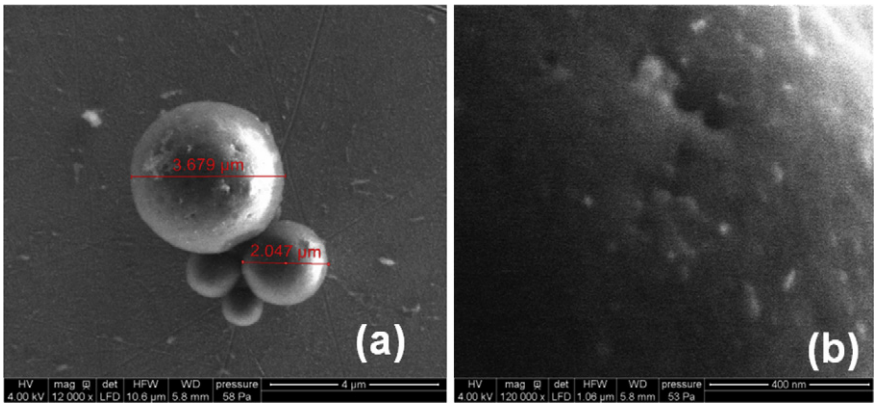

Fig. 7. SEM images of: (a) cell exposed to parathion-methyl (10^{-06} mol/L), (b) an enlargement of the cell image that shows the surface perforation.

Inhibition measurements and pesticides detection experiments were performed at a substrate saturation concentration of 3.5 mM. The irreversible phosphorylation of the AChE active site when biosensors are incubated with pesticides leads to a remarkable decrease in the electrode response time $\tau_{90\%}$ going from 80 min to few seconds (Fig. 3b).

The inhibition of AChE is normally attributed to the acylation of the serine –OH in the active site of the enzyme by these compounds (O'Brien, 1976; Wilson, 1963). The reaction with acetylcholinesterase is analogous to the enzyme–substrate reaction, where by a Michaelis enzyme–pesticide complex is first formed, followed by the transfer of the pesticide acyl groups to the serine –OH of the enzyme, and the concomitant release of a side product (Roberto-Andres and Narayanaswamy, 1997).

Residual AChE activities are presented in Fig. 6. These histograms show that the AChE can be inhibited in presence of paraoxon-methyl, parathion-methyl, triazine and diuron with a detection limit of 10^{-18} M, 10^{-20} M, 10^{-20} M and 10^{-12} M, respectively and the half maximal inhibitory concentration (IC50) was determined at 10^{-16} M, 10^{-20} M, 10^{-18} M and 10^{-06} M, respectively. Here, the detection limits were lower in comparison with previous studies performed with conductometric biosensors based on *Chlorella*, where the detection limit was 4.10^{-07} M of paraoxon-methyl (Chouteau et al., 2005). It is also low compared to pure acetylcholinesterase biosensors (Dzyadevych et al., 2002; Villatte et al., 1998).

SEM images were performed on cyanobacteria exposed to pesticides after conductometric measurements. No modifications on cell, exposed to low concentrations of pesticides, have been observed, but there is a perforation on cells exposed to 10^{-06} M of pesticides (Fig. 7a and b).

4. Conclusion

This study describes an enzymatic conductometric biosensor using immobilized whole cells of *Arthrospira platensis* for the detection of pesticides. Inhibition of AChE was observed with paraoxon-methyl, parathion-methyl, triazine and diuron with a detection limit of 10^{-18} M, 10^{-20} M, 10^{-20} M and 10^{-12} M, respectively and the half maximal inhibitory concentration (IC50) was determined at 10^{-16} M, 10^{-20} M, 10^{-18} M and 10^{-06} M, respectively. This biosensor performance is sensitive enough regarding UE drinking water standards that require 0.1 µg/L for one pesticide or metabolite and 0.5 µg/L for all pesticides and metabolites found in the sample.

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