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Design of a macroalgae amperometric biosensor; application to the rapid monitoring of organophosphate insecticides in an agroecosystem

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HIGHLIGHTS

- A macroalgae based biosensor based on tetracyanoquinodimethane (TCNQ) mediator was designed.
- The designed biosensor was used to analyze methyl parathion OP insecticide in water samples.
- The novel immobilization design led to enhanced stability and sensitivity of the biosensor.
- Macroalgae-biosensor could be used as a low-cost and sensitive screening method to detect target analyte.

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ABSTRACT

The immobilization of enzymes onto transducer support is a mature technology and has been successfully implemented to improve biocatalytic processes for diverse applications. However, there exists still need to design more sophisticated and specialized strategies to enhance the functional properties of the biosensors. In this work, a biosensor platform based on innovative fabrication strategy was designed, and employed for the detection of organophosphate (OP) in natural waters. The biosensor was prepared by incorporating acetylcholinesterase enzyme (AChE) to the graphite paste modified with tetracyanoquinodimethane (TCNQ) mediator, along with the use of a macroalgae (*Cladaphropsis* membranous) as a functional immobilization support. The novel immobilization design resulted in a synergic effect, and led to enhanced stability and sensitivity of the biosensor. The designed biosensor was used to analyze methyl parathion OP insecticide in water samples collected from a demonstrably contaminated lake of São Luis Island, Maranhão, Northeast of Brazil. Water analysis revealed that the aquatic ecosystem was polluted by sub-ppm concentrations of the OP insecticide, and a good correlation was found between values obtained through biosensor and GC–MS techniques. Our results demonstrated that macroalgae-biosensor could be used as a low-cost and sensitive screening method to detect target analyte.

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1. Introduction

Biosorption process has gained much attention in recent years for the removal of pollutants or recovery of metal ions by binding to biomass from aqueous solution. Various biomasses including bacteria, yeast, fungi, algae and mosses have reported for the cost effective removal of heavy metals (Low and Lee, 1991; Arica et al., 2004). For example, Algae biomasses present a suitable biosorption platform due to presence of biopolymers in their cell walls (Chojnacka et al., 2005). These functional groups results in metal ion binding by adsorption, ion exchange or covalent binding (Kalyani et al., 2004). The integration of biosorption process in biosensors could be an attractive alternative to the existing methods (Alpat et al., 2008). However, there is no study implying the algae biomass as immobilization support in biosensors.

Alginate is one of the basic component of the algae biomass, and is composed of chains of alternating α-L-guluronic acid and β-D-mannuronic acid residues(Taqieddin and Amiji, 2004). Based on above observation, macroalgae species *Cladophoropsis membranacea* due to its alginate content was used as enzyme immobilization support in the present work. Traditionally, different strategies to

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improve enzymatic assays performance have been reported as almost independent platforms. However, the use of different immobilization methods have shown synergic effect to improve the analytical performance of the biosensors (Cowan and Fernandez-Lafuente, 2011; Rodrigues et al., 2011). Despite of the potential advantages, there are only few studies on the coupled use of different tools to improve assays performance. The present study was aimed to explore the novel properties of macroalgae as immobilization support, along with use of coupled tools to enhance the assay performance. To best of our knowledge, such a study based on macroalgae as independent or in combination with other immobilization support has not been reported in the literature. The design platform was demonstrated by immobilizing acetylcholinesterase enzyme (AChE) to detect organophosphate in ecosystem.

Since the early twentieth century, the synthetic organic pesticides are widely used in pest control with the aim of increasing agricultural productivity. They are source of water contamination with serious risks to human and animal health. Additionally, they cause changes in ecosystems with detrimental consequences for the environment and agriculture domains (Menezes et al., 2010). The emergence of organophosphorus (OP) pesticides gradually replaced the chlorinated ones. But although less persistent than the chlorinated hydrocarbons, carbamate and organophosphate insecticides have a peculiar harmful characteristic to living organisms. They act directly on the nervous system by inhibiting the action of acetylcholinesterase enzymes, causing nerve dysfunction which can lead to death (Van Dyk and Pletschke, 2011). Over the past 20 years, the use of agrochemicals in Brazil has grown substantially, increasing the poisoning cases and contamination in soil, water and air. Because of this, some governmental programs have been developed in order to monitor and to control residues of such substances in the environment (Nunes et al., 2004).

Enzymatic biosensors are promising tools for the monitoring of pesticides due to their high sensitivity and selectivity, very short response time and minimal sample treatment (Andreescu and Marty, 2006; Dondoi et al., 2006; Sinha et al., 2010).

Among the enzymes employed for the construction of biosensors devoted to pesticide analysis, the acetylcholinesterase enzyme (AChE) is the most commonly used one. These devices are designed to supplement or replace the existing reference methods of analysis (i.e. chromatographic methods), simplifying or eliminating sample preparation and reducing the analysis time and the cost(Andreescu et al., 2002). Our results demonstrated that the present macroalgae-biosensor is sensitive and cost effective screening method to detect organophosphate in ecosystem.

2. Experimental

2.1. Materials

Chromatographic grade methanol and ultrapure sodium sulfate were obtained from Merck. Acetylcholinesterase (AChE 3.1.1.7 type-VI-S/1.5 mg, electric eel source, 500 U/1.5 mg) and acetylthiocholine chloride (ATChCl) were purchased from Sigma–Aldrich Chemicals Co., USA. AChE stock solutions were prepared directly in 0.9% NaCl (w/v) and stored at −18 °C. Methyl parathion standard (99% purity) was obtained from Riedel-de-Haen, Fluka, USA. Dithio-bis 5,5-(2-nitrobenzoic acid) (DTNB), hydroxyethylcellulose (HEC) and bovine serumalbumine (BSA) were purchased from Sigma–Aldrich Chemicals Co., USA. The graphite fine powder was procured from Merck, USA. All bio-reagents were prepared in phosphate buffer solution (PBS, pH = 7.2).

2.2. Macroalgae collection and hydrolysis

Macroalgae species *C. membranacea* has been collected by scrapping gently the mangrove root from the swamp, during the rainy season at the Anil River estuary, near the neighborhood Jaracati (São Luís, MA, Brazil), and stored in a plastic container at 4 °C. Afterwards, the macroalgae was air dried for 48 h. Then it was chopped and converted to the powder form. Papain solution, prepared in sodium acetate buffer (pH = 5.0), EDTA and cysteine was used to get a solution mixture of it. This mixture was exposed to a nitrogen gas flux for 10 min, and then the hydrolysis of the macroalgae was completed into a water bath at 50 °C for 3 h. After this, the material was filtered and the final residue was air-dried for a period of 5 h, and finely pulverized. The obtained powder was stored at 10 °C.

2.3. Instrumentation

Electrochemical measurements were performed using the biosensor connected to a potentiostat/galvanostat (Microautolab Type III), while spectrophotometric measurements were carried out with a UV/Vis spectrophotometer (Biospectro SSP-220). Neon lamp was used in the cold stage for enzyme polymerization onto the electrode surface.

Chromatographic analyses were performed with a gas chromatography model 3900 equipment containing a divider type split/splitless and a mass spectrometric detector model Saturn 2100T (both from Varian, Palo Alto, California). Chromatographic data were treated by comparing the mass spectra obtained with the NIST Library to those presented by the MS Workstation, version 6.9, from Varian. For the identification of the methyl parathion pesticide, the MS has selected the following ions: [M], [M-138] and [M-154] in the full scan mode in a 200–360 m/z range. The main chromatographic conditions were: capillary column VF-5-MS Factor Four (30 m × 0.25 mm id), helium as carrier gas (1 mL min^{-1} flow rate), injector temperature of 250 °C; temperature in the column programmed to 100 °C held for 1 min and then increasing to 250 °C, 30 °C min^{-1}; total run time of 8.5 min; retention time of 6.9 min. The SPME procedure is summarized as follow: The PDMS fiber was fixed to the extraction GC module. In a headspace vial of 20 mL capacity of 10 mL standard solution (prepared in methanol), was added or 10 mL of water sample plus Na_2SO_4 10% (*w*/*v*) solution. The extraction process occurred in a time of 40 min at 80 °C. After desorption, the compounds were analyzed by GC/MS according above.

2.4. Enzyme selection and biosensor preparation

Several tests were performed with three types of acetylcholinesterases, extracted from human erythrocyte, bovine erythrocyte and electric eel. The enzyme activity was measured before each immobilization by spectrometry. A two-steps immobilization method was employed for the biosensor preparation. Firstly, macroalgae powder was incorporated in a proportion of 7.5% onto the enzymatic paste prior to apply it at the working electrode surface. Thus, the modifying macroalgae paste was prepared by mixing macroalgae powder to 30% PVA–SBQ, 2% BSA and 1% HEC. Secondly, the AChE solution (pH 7.2) was added to this mixture, the final sensitive paste homogenized by vortexing at 5000 rpm during 10 s and following 2 μL of this paste was manually deposed on TCNQ-modified graphite working electrode prepared as previously described (Andreescu et al., 2002; Andreescu and Marty, 2006). At the end of this immobilization method, the enzymatic load was of 2 mU per sensor. The obtained AChE-biosensors were kept in the refrigerator at −4 °C for at least 2 days prior to use.

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