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Development of a lab-on-chip electrochemical biosensor for water quality analysis based on microalgal photosynthesis

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ABSTRACT

The present work was dedicated to the development of a lab-on-chip device for water toxicity analysis and more particularly herbicide detection in water. It consists in a portable system for on-site detection composed of three-electrode electrochemical microcells, integrated on a fluidic platform constructed on a glass substrate. The final goal is to yield a system that gives the possibility of conducting double, complementary detection: electrochemical and optical and therefore all materials used for the fabrication of the lab-on-chip platform were selected in order to obtain a device compatible with optical technology. The basic detection principle consisted in electrochemically monitoring disturbances in metabolic photosynthetic activities of algae induced by the presence of Diuron herbicide. Algal response, evaluated through oxygen (O_2) monitoring through photosynthesis was different for each herbicide concentration in the examined sample. A concentration-dependent inhibition effect of the herbicide on photosynthesis was demonstrated. Herbicide detection was achieved through a range (blank – 1 μM Diuron herbicide solution) covering the limit of maximum acceptable concentration imposed by Canadian government (0.64 μM), using a halogen white light source for the stimulation of algal photosynthetic apparatus. Superior sensitivity results (limit of detection of around 0.1 μM) were obtained with an organic light emitting diode (OLED), having an emission spectrum adapted to algal absorption spectrum and assembled on the final system.

1. Introduction

Assessment of water quality has been generating major interest over the past few years as there is an essential need to preserve freshwater sources such as lakes, rivers, water reservoirs and ground waters. Several factors can be responsible for water quality degradation such as the presence of heavy metals, organic contaminants, pathogenic micro-organisms as well as an excess in nutrients leading to eutrophication. A particular interest has been placed on the detection of pesticides due to their ever-growing use but also the lack of instructions for their proper application and control of the post-application phase.

Herbicides represent a category of pesticides that are used to protect crops and non-crop areas and prevent growth of undesired weeds. Herbicides can easily penetrate the soil, be transported to rivers through groundwater paths and be often detected in different water bodies such as lakes and rivers. Diuron, or 3-(3,4-dichlorophenyl)-1,1-dimethylurea is an urea-based herbicide, widely employed for total, non-selective vegetation control (Fedtke and Duke, 2004). It is mainly used upon non-crop areas, in irrigation or drainage canals but has also found applications in paints to protect from fouling. According to conducted surveys, Diuron has been found in 70% of European rivers and is also ranked high upon water contaminants for Australian, Canadian and U.S agencies, posing considerable threats to aquatic microorganisms.

Herbicide determination and detection are most commonly performed in laboratories. Conventional methods include advanced instrumental techniques such as gas and liquid chromatography coupled with different detection techniques as mass spectrometry, chemiluminescence or electrochemical detection. These techniques are highly sensitive, selective and they include controlled and validated protocols. However, it is still essential to meet the ever-growing need for systems appropriate for rapid, on

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site analysis. Biosensors are analytical detection devices that convert a biochemical phenomenon into a detectable and measurable signal, which can be amplified and treated. These devices meet the requirements of an application that demands a low-cost portable system for on-site detection, providing an early indication by sorting the samples needed to be further analyzed by conventional techniques. Biosensors consist of two principal parts, the biological sensing element, the so-called bioreceptor, and the physical transducer.

In order to determine the biological detection element and physical transducer to be used for the detection of herbicides, it is essential to study the mode of action of each herbicide on the targeted vegetation. They can inhibit cell growth, fluorescence and photosynthesis depending on their molecular structure and site of action (Ross and Childs, 1996). More particularly, Diuron, similarly to 50% of herbicides used today, inhibits photosynthesis, acting at vital systems of the photosynthetic apparatus. As stated by Davison, given the fact that algal physiology resembles to the one of the targeted vegetation, microalgae are directly affected by herbicides (Davison, 1991). They can thus be successfully used as biological recognition elements among herbicide biosensors (Brayner et al., 2011). Furthermore, they integrate several other advantages related to the use of whole cells such as their robustness, stability as well as the simple procedures related to their cultivation, isolation and manipulation(Giardi and Piletska, 2006). Based on previously reported ecotoxicological studies on monitoring the effect of herbicides on living organisms (Schubnell et al., 1999), *Chlamydomonas reinhardtii*, wild type microalgae were selected as biological recognition elements as they are extensively studied and characterized.

As a matter of fact, the presence of Diuron herbicide has a visible impact on the photosynthetic oxygen production and the emitted algal fluorescence (see Supplementary Information S1). The majority of microalgal biosensors that aim at detecting Diuron are therefore either based on fluorescence or photosynthetic oxygen production monitoring (Brayner et al., 2011). These two approaches are effective alternatives to the conventional method which is the standard growth test, where the inhibition of algal growth is measured (Ma et al., 2002). As a matter of fact, although this method yields good results in terms of limit of detection and sensitivity, long assay duration is an important issue when rapid results are desired. Concerning fluorescence biosensors, they are based on optical transduction system in order to detect the photons emitted by algae, while the transduction for oxygen monitoring is performed through electrochemical measurements. It is demonstrated in literature that fluorescence-based biosensors employed for the detection of Diuron have often high performances with low limits of detection (Naessens et al., 2000). However, they often demand high stabilization times and can only be effective when optically clear, not turbid samples are examined (Haigh-Flórez et al., 2014). Consequently, a complementary electrochemical biosensor can be beneficial to the determination of pollution level as this type of sensor can yield solid and stable systems that are easily miniaturized and simple to use.

Among previous experimental works based on algal photosynthetic activity as an indicator of the presence of Diuron, amperometric monitoring is reported several times as the detection technique (Shitanda et al., 2009; Koblízek et al., 2002). The inhibiting effect on photosynthetic activity of algae is evaluated by monitoring electrochemically the photosynthetically produced oxygen and the concentration inhibiting 50% of the activity (IC_{50}) is estimated.

The aim of this study is to develop a lab-on-chip system with integrated electrochemical and fluorescence sensors enabling double complementary detection. The present work therefore reports the development of the electrochemical detection system integrated on a fluidic platform for the detection of toxicants based on algal physiology. The system uses small sample quantities due to the incorporation of microfluidic structures, is easily implemented and simple to use for on-site measurements. The three-electrode electrochemical system, integrating an ultra-microelectrode (UME) array of platinum black (Pt-Bl) could effectively follow modifications in photosynthetic oxygen production rates due to pollutants. The design of the electrochemical device is compatible with optical technology in order to further integrate light source and fluorescence detection in the same substrate. To study the effects of illumination on algae photosynthesis two light sources will be evaluated and compared: a classical halogen light and an organic light emitting diode (OLED) with a specifically selected wavelength. The development of the second option has been considered in order to obtain a final sensing lab-on-chip with integrated light source.

2. Materials and methods

2.1. Fabrication procedure

The lab-on-chip platform was comprised of the electrochemical sensors (Tsopela et al., 2014) as well as the fluidic structure with channels and measurement chambers for sample testing through the bioassays (see Supplementary Figs. S1 and S2). Six independent detection chambers were designed on each platform enabling the simultaneous processing of different assays. The complete electrochemical cells were integrated on the three chambers while the other ones were dedicated to the further work involving fluorescence-based optical detection. In this way, it is possible to increase analysis frequency by conducting parallel analysis of several samples in order to reduce false alarms. Moreover, this matrix configuration gives the possibility of calibrating the sensor by using one of the chambers for control measurement with a non-polluted sample and compare with the values obtained for the polluted samples. It also enables future integration of different algal species in order to increase sensor selectivity as each algae species will be sensitive to different pollutant giving the possibility of conducting multi-analysis. Concerning the light source for algal excitation, the fabrication of a blue OLED was considered.

The entire fabrication procedure (lab-on-chip platform and OLED) is detailed in Supplementary Information, S2.

2.2. Bioassays

2.2.1. O_2 measurement in control algal solutions

Green algal cells were used through the bioassays and the cultivation procedure is explicated in Supplementary Information S3. The response of the sensor was firstly evaluated in control algal solutions that do not contain any herbicide. Given the fact that the detection principle is based on following the algal photosynthetic activity, the electroactive species to generate the electrical signal was oxygen (O_2) which is electrochemically reduced on the PtBl working electrode surface. The recorded reduction current was proportional to the concentration of dissolved O_2 in algal solution. O_2 evolution was followed through photosynthesis and respiration process during light and dark cycles. Experiments were carried out in a dark Faraday cage using an external, halogen white light source or a blue OLED as excitation sources for algal photosynthesis. The potentiostat used was Bio-Logic SP-200 equipped with a low current option. The centrifuged algal cells, re-suspended either in HSM medium or lake water samples was injected in the detection chamber by simply using a syringe. Chronoamperometry was conducted and the potential applied corresponds to the O_2