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Silicon-based microfabricated microbial fuel cell toxicity sensor

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ABSTRACT

Microbial fuel cells (MFCs) have been used for several years as biosensors for measuring environmental parameters such as biochemical oxygen demand and water toxicity. The present study is focused on the detection of toxic matter using a novel silicon-based MFC. Like other existing toxicity sensors based on MFCs, this device is capable of detecting the variation on the current produced by the cell when toxic compounds are present in the medium. The MFC approach presented in this work aims to obtain a simple, compact and planar device for its further application as a biosensor in the design and fabrication of equipment for toxicity monitoring. It consists on a proton exchange membrane placed between two microfabricated silicon plates that act as current collectors. An array of square 80 μm × 80 μm vertical channels, 300 μm deep, have been defined trough the plates over an area of 6 mm × 6 mm. The final testing assembly incorporates two perspex pieces positioned onto the plates as reservoirs with a working volume of 144 μL per compartment.

The operation of the microdevice as a direct electron transfer MFC has been validated by comparing its performance against a larger scale MFC, run under the same conditions. The device has been tested as a toxicity sensor by setting it at a fixed current while monitoring changes in the output power. A drop in the power production is observed when a toxic compound is added to the anode compartment. The compact design of the device makes it suitable for its incorporation into measurement equipment either as an individual device or as an array of sensors for high throughput processing.

1. Introduction

As a consequence of human activity, large amounts of toxic spills with different toxic levels are generated, which directly or indirectly could contaminate aquifers and superficial waters, preventing their later exploitation.

The polluting agents susceptible to generate these problems are of very diverse nature and go from pesticides, coming from the agrarian activity, to chemical compounds from different industrial sectors that could deliberately or accidentally contaminate continental waters. The consequences of these spills are catastrophic for the environment.

Biological assays for toxicity have been implemented to detect acute toxicity as well as chronic toxicity in environmental samples as well as in different types of effluents. Chronic toxicity assays are usually based on the observation of alterations in the normal function of complex organisms. The type of organism used as a rule in chronic toxicity assays are invertebrates such as mussels or crustaceans, or even vertebrates like fish. The duration of the assays ranges from a couple of days to as long as several weeks. Chronic toxicity assays attempt to detect long time effects of toxics on the endocrine, nervous or immune system as well as the possible effect on the reproductive capacity of the organisms and the emergence of mutations in their offspring. On the other hand, acute toxicity assays usually detect the existence of massive and often lethal effects on less complex life forms. Acute toxicity analyses detect the decrease or termination of measurable biological activities when subject organisms are exposed to toxic-containing samples. The biological activities detected in this assays are diverse: autofluorescence in algae, bioluminescence in selected bacteria, oxygen respiration by activated sludge samples. Average type required to perform some of these assays is about 96 h.

The use of microbial fuel cells for detection of toxic compounds can minimize the time, the cost and the personnel required for measuring toxicity in water.

Although there are several proved methodologies for toxicity measurements, this is the first time that a microfabricated toxicity sensor based on microbial fuel cells has been developed.

A microbial fuel cell (MFC) is a device capable of converting the chemical energy from organic compounds, such as simple carbo-

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hydrates or organic waste matter, into electricity using bacteria as biocatalysts (Bennetto, 1990; Bond and Lovley, 2003; Davila et al., 2008; Logan and Regan, 2006; Lovley, 2006; Rabaey et al., 2003; Shukla et al., 2004). The design of a microbial fuel cell usually consists of two electrodes, anode and cathode, placed in independent compartments divided by a proton exchange membrane. In the anodic compartment, organic compounds (e.g., glucose, acetate) are oxidized by microorganisms, generating electrons and protons in the process. Under normal aerobic microbial growth, the electrons generated in this process would be transferred to oxygen. However, in the anode compartment and in the absence of oxygen, bacteria are forced to transfer electrons to the anode that acts, in this case as an external acceptor (Bennetto, 1990). The electrons flow from the anode to the cathode powering an external circuit. At the same time, protons generated in the anode compartment cross the membrane to the cathodic compartment to recombine with electrons reducing the oxygen in the medium to form water molecules. Transfer of electrons to the anode can occur, either directly, in the case of bacteria able to use insoluble metal oxides, such as Fe(III) and Mn(IV), or indirectly, via redox mediators (Logan and Regan, 2006; Schröder, 2007).

Microbial fuel cells have been proposed in the past as biosensors for measuring environmental parameters such as biochemical oxygen demand and water toxicity (Chang et al., 2004; Gil et al., 2003; Kim et al., 2003, 2007; Lei et al., 2006; Tront et al., 2008a,b). They have also been miniaturized in some other studies (Biffinger et al., 2007; Ringeisen et al., 2006, 2007) using graphite felt or reticulated vitreous carbon electrodes. Regarding the micro-scale, where silicon-based electrodes are employed, only Chiao et al. (2006) have reported a study where a maximum power density of 2.3 $\mu W\,cm^{-2}$ was obtained.

Because microfabricated MFCs cannot compete with micromachined electrochemical fuel cells to power microsystems, the present study is focused on the detection of toxic compounds by applying the operation principle of a miniaturized microbial fuel cell to develop a silicon-based toxicity biosensor. The biosensor is based on a microbial fuel cell device. The sensor detects the presence of toxic materials using an electrochemically active microorganism as it has been previously studied by Kim et al. (2007). When toxic materials are present in the anodic compartment, generation of electricity by the electrically active bacteria in the cell is decreased remarkably.

The main advantage of the sensor approach presented in this work is to obtain a simple, compact and planar device for its further application in the design and fabrication of equipment for toxicity monitoring. The simple design and easy setup of the device makes it suitable for its incorporation into measurement equipment either as an individual device or as an array of sensors for high throughput processing.

2. Materials and methods

2.1. Microorganisms and growth conditions

Geobacter sulfurreducens (DSM 12127) was used throughout the experimental work. A *G. sulfurreducens* strain was growth in a solution containing the following (per liter): 1.5 g of NH_4Cl, 0.6 of NaH_2PO_4, 0.1 g of KCl, 2.5 g of $NaHCO_3$, 0.82 g of sodium acetate, 8 g of sodium fumarate, 10 mL of vitamin mix and 10 mL of mineral mix (Bond and Lovley, 2003; Lovley and Phillips, 1988). The medium was adjusted to pH 6.8. Sodium acetate served as the electron donor. All incubations were done at 30 °C under anaerobic conditions. Culture growth time varied from 4 to 7 days.

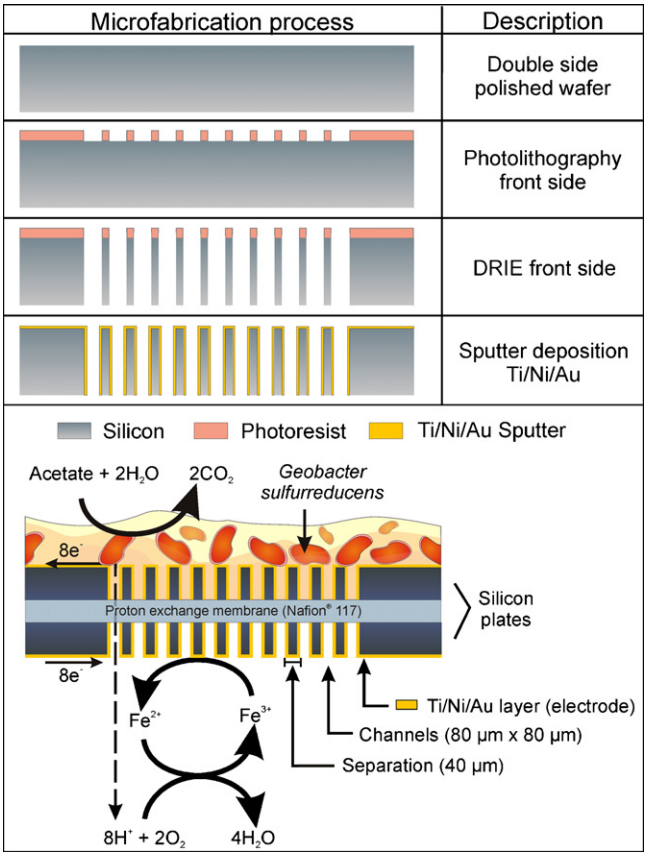

Fig. 1. Main steps of the microfabrication and assembly process. The bottom part of the figure shows a cross-section of the toxicity biosensor using *G. sulfurreducens* as the biological sensing element.

2.2. Electrodes microfabrication

The electrode elements were micromachined using a combination of standard photolithography and deep reactive ion etching (DRIE) on double side polished silicon wafers. Fig. 1 summarizes the main steps of the microfabrication process. In order to act as current collectors, the electrodes were covered with a 150 nm Ti/Ni/Au sputtered tri-layer to provide electrical conductivity. The first Ti layer is used to improve the adhesion of metals to the silicon substrate. Then the Ni layer provides an appropriate electrical conductivity of the electrodes, which is then covered by the Au layer to prevent oxidation. The resulting electrodes consist of several arrays of square 40 $\mu m \times 40\,\mu m$ vertical channels with a depth of 300 μm, defining a surface area of 5 mm × 5 mm on a 10 mm × 14 mm silicon chip. These channels allowed the diffusion of the protons liberated during acetate oxidation towards the ion selective membrane. A SEM image of the top-view of a microchannel array is shown in Fig. 5A. Similar structures to the electrodes of the device employed in this study had been previously described for the development of a silicon-based passive direct methanol fuel cell (Esquivel et al., 2008).

2.3. Assembly and system set-up

The device consists on a proton exchange membrane placed between two of the above described microfabricated silicon plates that act as current collectors. The final testing assembly incorporates two perspex pieces positioned onto the plates as reservoirs with a working volume of 144 μL per compartment with 2 mm inlets and outlets at the sides of the compartments, which helped to have a good and constant flux with no leakages (Fig. 2).

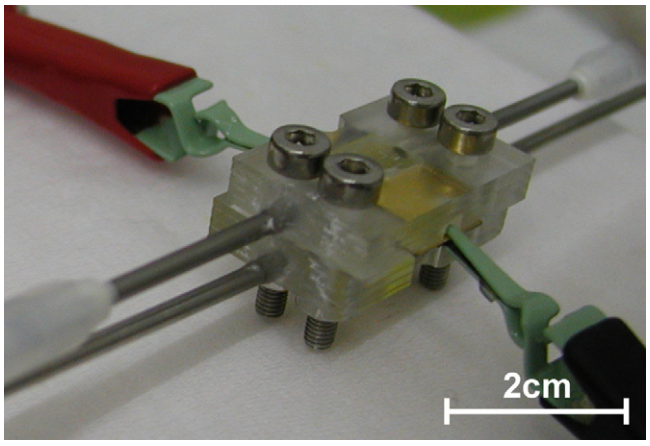

Fig. 2. Final testing assembly of the developed toxicity biosensor based on MFC.

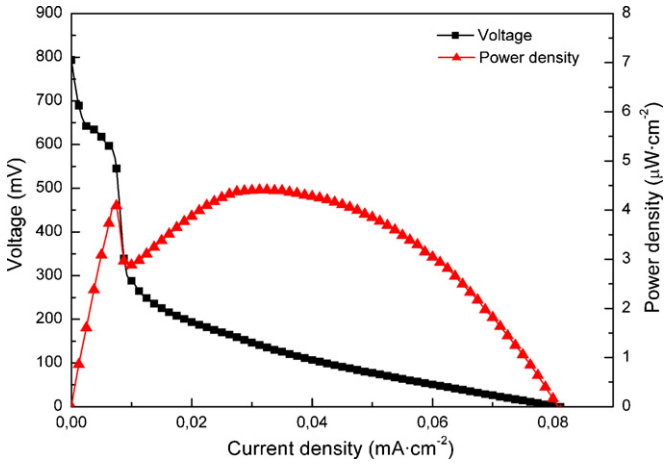

Fig. 3. Response obtained with a large scale direct electron transfer MFC.

The system was set-up by passing a flow of a *G. sulfurreducens* culture with 10 mM acetate trough the anode compartment for several days until the system achieved its maximum performance as a fuel cell, that is, until a biofilm was formed on the surface of the electrodes. At the cathode compartment, an oxygenated solution containing 100 mM phosphate buffer and 50 mM potassium ferricyanide was used. The later helps to accelerate the reduction of oxygen in water.

In this cell configuration, *G. sulfurreducens* works as a biocatalyst using a direct electron transfer mechanism to deliver electrons to the electrode. This type of bacteria forms biofilms on surfaces being its ability to generate energy related to the thickness and density of the biofilm.

Since bacteria must be in physical contact with the electrode, the Au-side of the anode was placed facing the culture compartment. The Au-side of the cathode was placed facing the potassium ferricyanide solution (both silicon sides of the electrodes where facing the membrane). This is shown in Fig. 1.

3. Results and discussion

The operation of the microdevice as a MFC has been validated by comparing its performance against a larger scale MFC, run under the same conditions and following the methods used in previous studies (Davila et al., 2008). Both MFCs were inoculated with the same culture in order to compare power densities obtained. The larger MFC had carbon paper electrodes with a surface area of 40 cm^2 and 84 mL compartments.

A maximum power density of 4.4 $\mu W\,cm^{-2}$ was obtained with the large scale MFC (Fig. 3) while the microdevice yielded a maximum power density of 6.5 $\mu W\,cm^{-2}$ (Fig. 4). These results corroborate the correct operation of the micro-MFC. Despite the fact that our microdevice reached maximum current densities (0.014 $mA\,cm^{-2}$) about four times lower than those found in a similarly operated large volume fuel cell (0.08 $mA\,cm^{-2}$), maximum power densities were significantly higher in the micro fuel cell. This better performance is a direct consequence of the fact that in the micro fuel cell voltage drop occurred at higher current densities. The ability to sustain high voltages up to high current densities reflects the fact that in our microdevice the distance between all the elements (biofilm, anode, proton exchange membrane and anode) are minimal, virtually zero. As a result, the internal resistance of the cell due to mass transport limitations between the different elements is minimal and ohmic losses usually responsible for voltage drop in the range of maximum power output, have been reduced to a minimum. Compared to the power densities reported by Kim et al. (2007), our device is capable to deliver 3000 times more power density at the same current density ranges.

Once the operation of the micro-cell as a MFC was validated, the culture was removed and replaced by fresh medium without bacteria. Because the operation conditions employed allowed for growth of both a microbial biofilm attached to the anode and free living cells suspended in the bulk of the anolyte, it was necessary to check which the contribution of each element was. Thus, Fig. 4 shows the polarization curves of the fuel cell obtained before and after replacing the anolyte suspension with fresh culture medium. After eliminating the bacteria present in the medium, the maximum power density dropped from 6.53 to 5.17 $\mu W\,cm^{-2}$, showing that the contribution of suspended bacteria was of about 20% of the power generated in the cell. SEM images of *G. sulfurreducens* attached to the electrode surface and to the electrode channel walls are shown in Fig. 5, showing the extent and the geometry of the attachment of the *Geobacter* biofilm to the Au electrode surface.

The operation of the device as toxicity sensor involves setting the cell at a fixed current while monitoring the changes in the output voltage caused by the addition of the toxic compound. In order to ensure a stable measurement without compromising fuel cell performance due to mass transport limitations, the current was fixed to 1 μA – which corresponded to a current density of 0.004 $mA\,cm^{-2}$. Prior to the toxicity measurement, the cells were operated in a fixed current mode in order to test the stability of

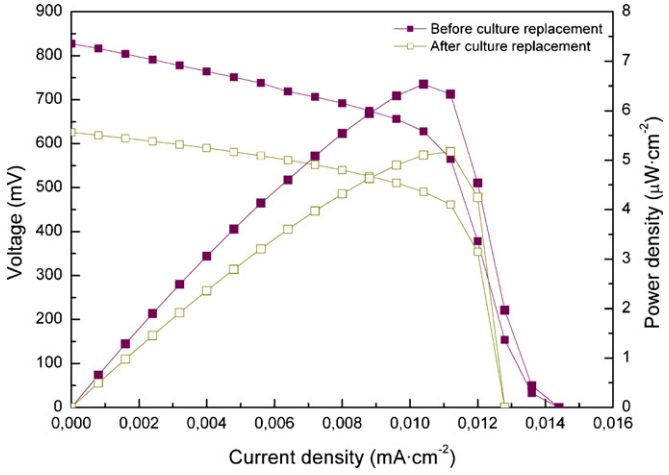

Fig. 4. Maximum power densities obtained with the microdevice operating as a MFC before and after replacing the bacterial culture by fresh medium without bacteria.

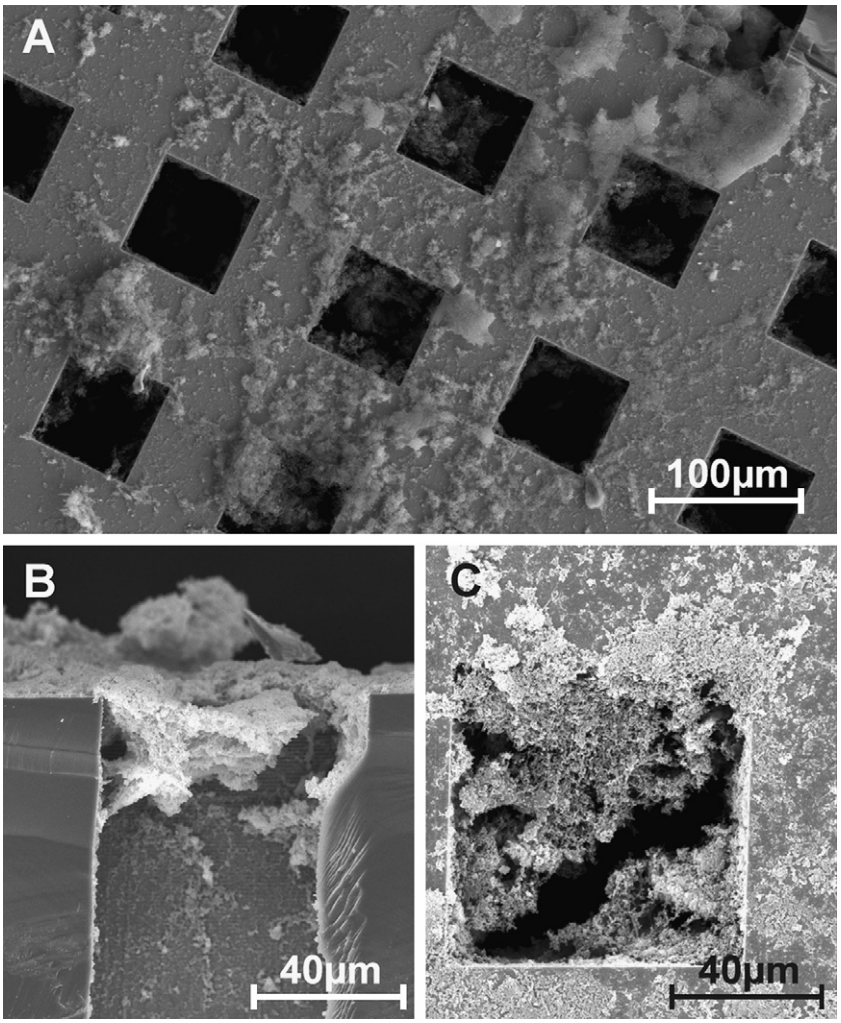

Fig. 5. SEM images showing the top view of the biofilm growth on a silicon micromachined current collector (A), *Geobacter sulfurreducens* attached to the channel walls (B) and to the electrode surface (C).

the output voltage in time. These tests were carried out in different cells and a variation less than 5% in the output voltage within periods of at least 24 h was observed. These measurements were considered as control experiments in the absence of toxic.

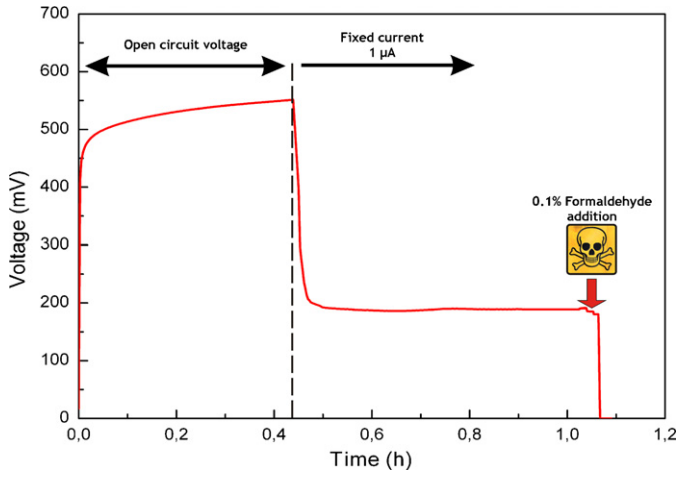

Fig. 6. Voltage evolution of the MFC biosensor: toxicity measurement response obtained with the microfabricated device when 0.1% formaldehyde was added to the anodic compartment of the cell.

Formaldehyde was chosen as the toxic to be added to the MFC for toxicity experiments since it is a commonly used disinfectant and biocide in microbiology, which allowed observing the inhibition of bacterial metabolic activity.

In a first trial, once the signal of the MFC set to a fixed current stabilized, formaldehyde was added to the MFC anode compartment at a final concentration of 4%. An immediate drop in the power production was observed (Fig. 6). Formaldehyde concentration was lowered stepwise in subsequent experiments in order to measure the minimum formaldehyde concentration detected by our device. The lowest tested concentration was 0.1% and in all cases the sensor was able to detect the presence of the toxic compound showing a steep drop in the output voltage. All tested concentrations irreversibly inactivated the biofilm (activity was not recovered when going back to a clean substrate solution).

4. Conclusions

The application of a silicon-based microbial fuel cell (MFC) as toxicity biosensor has been validated. The developed microfabricated device was able to give an efficient performance when tested as a microbial fuel cell due to an efficient biofilm formation on the surface and on the channel walls of the electrodes. This first approach constitutes a proof of concept that shows how the microdevice can be operated as a toxicity biosensor able to provide

an instantaneous response when exposed to the presence of a toxic compound.

Performance and sensitivity of a MFC-based toxicity sensor depends to a large extent on the type of organism used in the cell. Recent work by Patil et al. (2010) shows that *Geobacter* is not inhibited by a range o compounds from antibiotics to heavy metals. Even though our current implementation of the sensor requires the use of electrogenic organisms (*Geobacter* or *Shewanella*), we are currently exploring the possibility of engineering our system to use organisms that although unable of direct electron transfer to the anode might provide enhanced sensitivity to different compounds and concentrations.

Further work has to be done in order to determine the sensitivity and response of the sensor to different toxic compounds. Moreover, the development of an efficient cathode that allows the elimination of potassium ferrycianide is needed to achieve a simpler design.

Lately, different ideas of using bacteria in the cathodic compartment are being developed, which opens a wide range of possibilities for biosensors. The bacteria diversity that can be employed in microbial fuel cells, the different electron transfer mechanisms and all the parameters that affect their performance give us an estimation of the complexity of this type of devices. However, this diversity of parameters allows selecting and screening for microorganisms with specific activity for certain chemical compounds in order to design more complex, sensitive and high selectivity devices with fast responses.

The compact design of the microdevice fabricated along this study makes it suitable for its incorporation into measurement equipment either as an individual device or as an array of sensors for high throughput processing.

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