

Microfabricated Biosensors for the Determination of Biochemical Oxygen Demand (BOD) in Water

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Abstract

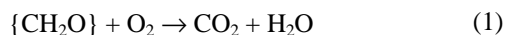
In this work, the design, manufacture and characterization of electrochemical biosensors based on thick film (screen printing) and thin film (lift-off) technologies is presented for the determination of biochemical oxygen demand in water.

Keywords: Sputtering; Photolithography; Lift-off; Screen Printing; Biochemical Oxygen Demand (BOD).

Introduction

The increase of the world population and its impact in the use of energy and natural resources bring as a result, a great demand of freshwater and a high production of wastewater. For this reason the environmental biosensors are a growing area of application. Moreover, the discharge of raw municipal and industrial wastewater into rivers, lakes and marine environments is an extended practice all over the world. Consequently, natural ecosystems (freshwater ones are especially sensitive) are being overexploited and suffering strong damage, sometimes close to their natural homeostatic capacity.

There are several standard methods used to evaluate the degree of organic pollution in freshwater, the biochemical oxygen demand (BOD) being one of the most useful. This assay is usually performed in BOD bottles, where the water samples, previously fully oxygenated, are incubated during 5 days (BOD₅) under specific conditions.^[1] A representative stoichiometric equation for the aerobic oxidation of an organic substrate is the following one:



Despite its widespread use, there are many inherent problems associated with the BOD₅ method. Clearly, the 5-day time is the most significant limitation where a rapid feedback is required for environmental monitoring and/or processes control. An important factor in this time delay is the low solubility of oxygen, which quickly becomes the rate-limiting reagent in the catabolism of organic matter^[2]. Other problems associated with the BOD₅ assay include a limited linear working range (also due to the low solubility of oxygen), complicated and time consuming procedures, and questionable accuracy and reproducibility.^[1] In this case, O₂ was replaced by the ferricyanide ion, which served as an alternative electron acceptor in the biochemical reaction. The advantage of using an alternative electron acceptor such as ferricyanide is its high solubility compared to oxygen. This allows the use of much higher microbial populations without rapid depletion of the electron acceptor. Besides, the whole process can

be followed by electrochemical methods. As a result, the long incubation times required to microbially oxidize significant amounts of organic substrate are greatly decreased. Specifically, degradation levels comparable to the 5-day BOD₅ assay for the glucose–glutamic acid (GGA) standard BOD solution can be achieved in 1 h or less under appropriate conditions^[3].

In this work, the design, fabrication, and characterization of an electrochemical miniature transducer for measuring BOD in water, is presented. Microfabrication processes, basically thick and thin film deposition and photolithography techniques were used to obtain a wide range of meso- and microelectrodes.

Two different technologies were employed to develop the electrodes. In the first one, a biosensor prototype using thick film technology was developed in order to study the feasibility of the method. In the second case, PVD-sputtering, photolithography and lift-off techniques were employed to obtain an improved device, which will permit the integration of electrochemical cells into MEMS.

Electrodes Design & Manufacture

The sensor design, made up of three electrodes, was developed on the basis of a previous one carried on Pt electrodes deposited on Si and glass substrates [4,8]. Screen printing and sputtering techniques have been employed to manufacture the electrodes of BOD biosensor.

Thick film electrodes were printed by conventional screen printing technology. Commercial Au organometallic paste (ESL D8083) and 96 % α -Al₂O₃ substrates were employed. The three-electrode layout was transferred by means of photolithography to a stainless steel mesh (400 wires per inch) with a photosensitive film (Ulano CDF-2). Au ink printing was carried with an EKRA Microtronic-II Printer and dried at 125°C during 15 min.

Thin film Au electrodes were fabricated by physical vapor deposition (PVD) with sputtering, BOC Edwards Auto 500 model. The design was transferred through lift-off, using a TI-35E Microchemicals photoresist reverse mode and a Mask Aligner EVG 620.

An example of the resulting three-electrode arrangement is shown in Fig. 1.

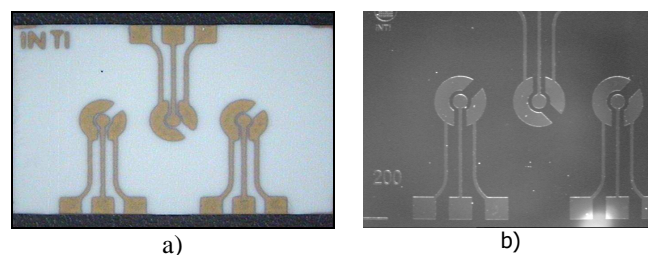


Fig. 1. a) Thick film electrodes; b) thin film electrodes.

The electrodes were integrated in an acrylic cell provided an entrance channel and an exit channel. This configuration gives the possibility of analyzing both quiescent and flowing solutions. The cells are shown in the Fig. 2.

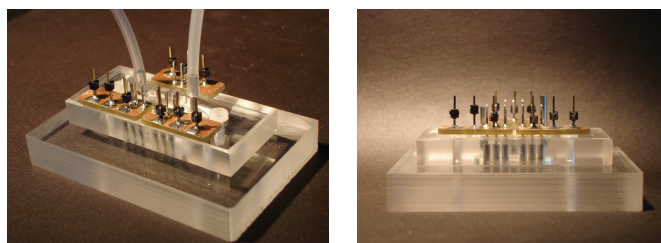


Fig. 2. Acrylic electrochemical cells with connections for the electrodes and catheters for the solutions transport.

Electrochemical Measurements

Measurements in standard ferricyanide/ferrocyanide solutions

As the BOD determination in the sensors presented in this work consists ultimately in an electrochemical measurement, the electrochemical behavior of the electrodes and cells was firstly tested in ferricyanide/ferrocyanide solutions by two different electrochemical techniques: cyclic voltammetry (CV) and amperometry. In the case of CV, a current versus potential plot, called cyclic voltammogram, is obtained by applying potential sweeps between the electrodes. In the case of the amperometric technique, a potential step is applied to the sensing electrode and the resulting current transients are recorded. The measured current is proportional to the concentration of the electroactive substance.

Test solutions of x mM $K_3Fe(CN)_6$ (potassium ferricyanide) + x mM $K_4Fe(CN)_6$ (potassium ferrocyanide) + 0.1 M KCl, with $x = 10, 25, \text{ and } 50$, were prepared from analytical grade reagents and distilled water. Potassium chloride (KCl) was employed as supporting electrolyte to provide a high conductivity.

The electrochemical measurements in the miniature cell were carried out employing the three-electrode configuration shown in Fig. 1, with a working electrode ($\varnothing = 1\text{ mm}$), a pseudo-reference electrode, and a counterelectrode.

With the aim of validating the electrochemical performance of the film electrodes and the miniature cell, electrochemical measurements were also performed in a three-electrode cell of conventional characteristics, consisting of a bulk Au working electrode ($\varnothing = 5\text{ mm}$), a reference electrode (standard calomel electrode), and a Pt wire as counterelectrode.

The voltammetric curves were scanned between -500 mV and $+500\text{ mV}$ with a rate of 50 mV s^{-1} . Amperometric curves were obtained applying potential steps of $+500\text{ mV}$ for both electrodes. The potential of the working electrode was controlled with a potentiostat EG&G PAR 273A.

Figs. 3 and 4 show the electrochemical response at several concentrations of $\text{Fe(CN)}_6^{3-/4-}$ for each device. The voltammograms and the current transients show similar features for bulk and film electrodes. Moreover, a linear relationship between current and the electroactive species has been found, as expected under these conditions.^[7]

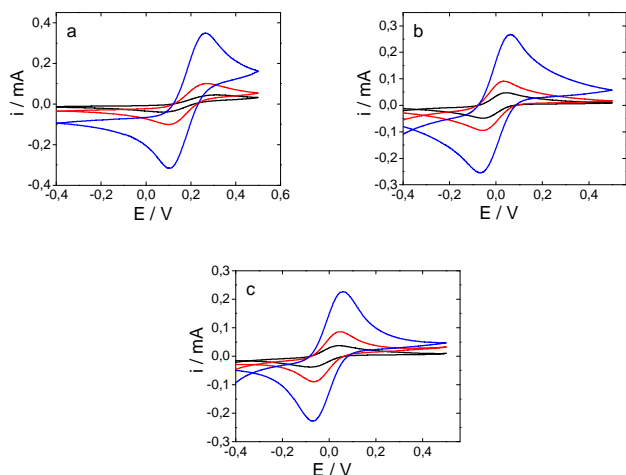


Fig. 3. Cyclic voltammograms obtained in solutions of $x\text{ mM K}_3\text{Fe(CN)}_6 + x\text{ mM K}_4\text{Fe(CN)}_6$ ($x = 10, 25, 50$) + 0.1 M KCl for bulk electrodes (a), thick film electrodes (b) and thin film electrodes (c).

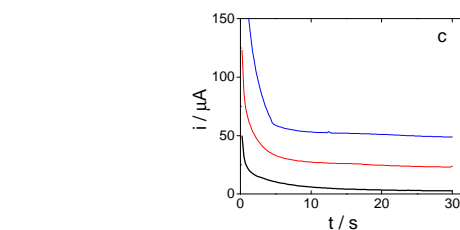
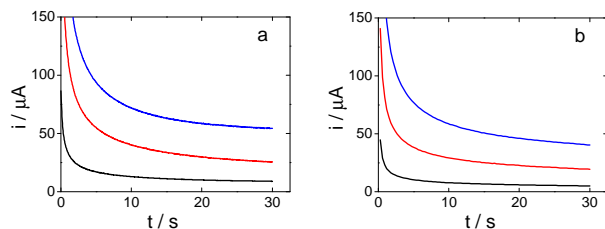


Fig. 4. Amperometric curves obtained in solutions of $x\text{ mM K}_3\text{Fe(CN)}_6 + x\text{ mM K}_4\text{Fe(CN)}_6$ ($x = 10, 25, 50$) + 0.1 M KCl for bulk electrodes (a), thick film electrodes (b) and thin film electrodes (c).

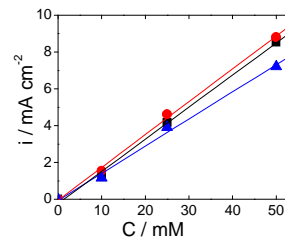
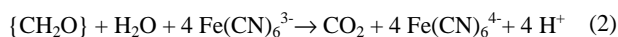


Fig. 5. Current from amperometric curves obtained at 6.25 s with solutions of different concentration of $\text{K}_4\text{Fe(CN)}_6 / \text{K}_3\text{Fe(CN)}_6$ in bulk electrodes (squares), thick film electrodes (circles) and thin film electrodes (triangles).

Measurements of biochemical oxygen demand

The method employed in this work to determine BOD is based on the degradation of organic matter by microorganisms^[5] using potassium ferricyanide as an electron acceptor, a process which can be expressed as:



Commercially available capsules employed in the determination of BOD contain a variety of lyophilized, nonpathogenic bacteria which degrade organic matter present in wastewater. In this work, a bacterial strain, able to degrade glucose/glutamic acid, isolated and characterized in the laboratory isolated from Bodseed capsules (BIOsystems International), was employed according to the following protocol:

- 1) A bacterial strain BO365 was inoculated in an environment defined.
- 2) It was incubated up to a value of absorbance of approximately 1.
- 3) An aliquot of 20 ml was taken, centrifuged, and suspended in 2 ml half defined environment without glucose.
- 4) The following systems were prepared: $1.8\text{ ml K}_3\text{Fe(CN)}_6\ 50\text{ mM}$ in $0.1\text{ M KCl} + 0.1\text{ ml}$ buffer media suspension + $X\text{ ml}$ glucose solution to a final

concentration of glucose/glutamic acid (GGA) 0, 0.2, 0.4, and 0.8 g l⁻¹.

5) The samples were incubated at 37°C for 2 h, and then 0.1 ml of ethanol was added to stop further reaction.

Amperometric measurements were carried out with the resulting solutions. The current transients obtained for bulk and film electrodes are presented in Fig. 6. The value of the current $i(\tau)$ at a given time elapsed τ from the potential step application is proportional to the initial glucose concentration, as can be seen in Fig. 7.

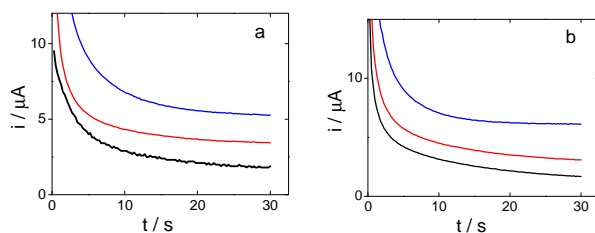


Fig. 6. Amperometric curves obtained with solution of γ (g/l GGA) for electrodes ($\gamma = 0.2, 0.4, 0.8$) for a) thick film electrodes and b) thin film electrodes.

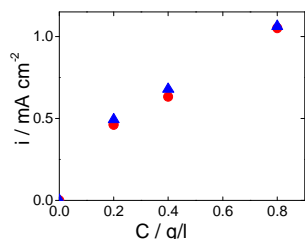


Fig. 7. Current from amperometric curves obtained at 6.25 s with different initial concentrations of GGA in water employing thick film electrodes (circles) and thin film electrodes (triangles).

Moreover, those values of current are proportional to the ferricyanide ion concentration, which can be calculated with the value of the slope $\partial C_{\text{Fe(III)}}/\partial i(\tau)$ from the calibrations curves such as those shown in Fig. 5. Finally, the amount of degraded organic matter is related to the ferricyanide ion production through the stoichiometric relationship. In sum, the biochemical oxygen demand can be obtained from the following equation:

$$\text{BOD (g l}^{-1}\text{)} = k \partial C_{\text{Fe(III)}}/\partial i(\tau) i(\tau) \quad (3)$$

where k is a factor which depends on the stoichiometry and yield of the reaction. Thus, a rapid determination of BOD can be achieved with a compact electrochemical device.

Conclusions

Miniaturized electrochemical biosensors were manufactured using different technologies of microfabrication.

The electrochemical response of these microcells was studied by potentiostatic and potentiodynamic methods, in solutions containing $\text{Fe(CN)}_6^{3-/4-}$ and glucose/glutamic acid. Measurements in a conventional electrochemical macro-cell were performed in order to assess the influence of the scaling-down in the response of the devices.

Linear relationships between current and concentration in the 10-50 mM range were found for both macro and micro-cells.

It is possible to determine the BOD in water using microfabricated electrochemical cells; using ferrocyanide as a mediator and microbial cells as biocatalysts.

These results open the possibility for the design and fabrication of fully integrated biosensors for monitoring and control the quality of waters.

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