

ELECTROCHEMICAL DETECTION OF BIOFILMS

M. I. Montenegro*, I. A. Pinho**, M. J. Vieira**

Departamento de Química*/Centro de Engenharia Biológica** – IBFQ, Universidade do Minho, 4700-320 Braga, Portugal

Biofilms are the result of adhesion and growth of microorganisms, creating microenvironments – a polymeric matrix – where several microbial reactions take place [1]. Usually, biofilms are divided in two groups: the ones that are beneficial, as in wastewater treatment or production of specific products, and the detrimental biofilms such as the ones that appear in drinking water pipes and heat exchangers. In any case it is very important to detect the biofilm as soon as possible, to increase its growth or to avoid the risks associated with its presence.

The ideal detector must allow the easy detection of biofilms in the early stages of formation and on line.

Electrochemical techniques are well known for their role in analytical chemistry, allowing the determination and quantification of a large number of organic, inorganic and biological compounds. These techniques have largely proved to provide an efficient means for detection *in situ* and on line of a variety of compounds [2].

In the present work, the development of a detector to function *in situ* in flow systems is described. The technique used is repetitive cyclic voltammetry applied to a platinum planar electrode of small area introduced in the system, which together with an auxiliary electrode and a reference electrode constitute an electrochemical cell. When the solution where the electrode is immersed is air-free aqueous sulfuric acid, and the platinum electrode surface is clean, the current plotted *versus* electrode potential is a cyclic voltammogram depicted in figure 1 [3]. This is a very well-known pattern and

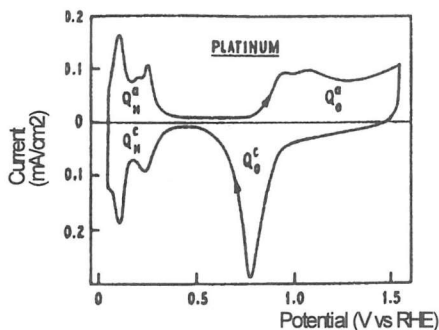


Figure 1 – Voltammogram at a platinum electrode in 1 M sulfuric acid at 25 °C; continuous triangular potential sweeps at 40 mVs⁻¹. Q_H^c and Q_H^a correspond, respectively, to the adsorption and desorption of hydrogen, and Q_O^a and Q_O^c to the adsorption and desorption of oxygen (reprinted from Woods, R., 1976).

details on this curve depend on the scan rate, on the reversal potential, on the pre-treatment of the electrode and on the solution composition. The application of repetitive cyclic voltammetry is in itself a method of electrode cleaning and the appearance of this kind of voltammogram is an indication of a clean surface. This fact may constitute the basis of a method to detect biofilm formation in a flow system since the smallest deposit on the electrode surface will certainly change the pattern observed when the platinum electrode surface is clean.

Two types of electrochemical cells were used, a batch cell for cyclic voltammetry experiments and a flow cell (Figure 2). The microorganism used as a biofilm producer was *Pseudomonas fluorescens* isolated from river water. The optimum growth temperature is 27 °C and glucose was used as the limiting substrate. Batch cultures were performed in 0.5 L glass fermenters with stirring. The fermenter with the medium that contained 5 g/dm³ glucose, 2.5 g/dm³ peptone and 1.25 g/dm³ yeast extract, in phosphate buffer at pH=7 (0.3 M Na₂ HPO₄) was autoclaved at 120°C during 20 minutes. The batch fermenter was used as the immersion vessel for the electrodes where the biofilm was allowed to grow. The flow in the flow cell was achieved with a peristaltic pump.

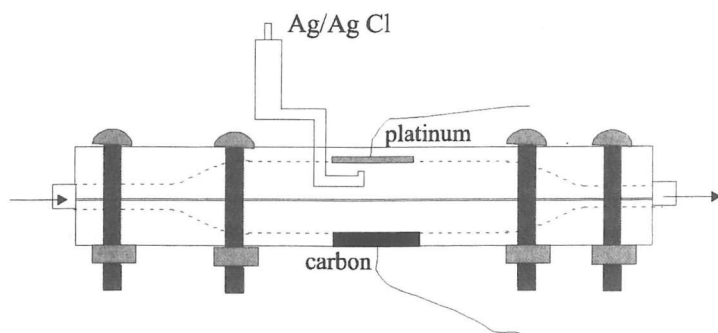


Figure 2 – Schematic view of the flow cell.

Figure 3 shows a series of cyclic voltammograms recorded on a batch cell and the different cycles show the effect of the different components used in the medium on the standard voltammograms shown in figure 1. Figure 4 shows the difference in the voltammetry on the batch cell at a clean electrode in the culture medium and immediately after the electrode with biofilm is immersed in the same solution. A clear difference is observed specially in the H₂ desorption region. Figure 5 shows the effect of recycling the potential on an electrode with biofilm.

The above preliminary experiments demonstrate that the platinum electrode and cyclic voltammetry may constitute the basis of an electrochemical detector of biofilms. These experiments will now have to be carried out in the flow cell that was constructed for this purpose.

Figure 6 shows the effect of the flow rate on the cyclic voltammograms recorded in such cell containing a buffer solution. Experiments where the biofilm is made to grow on the flow system are now under progress.

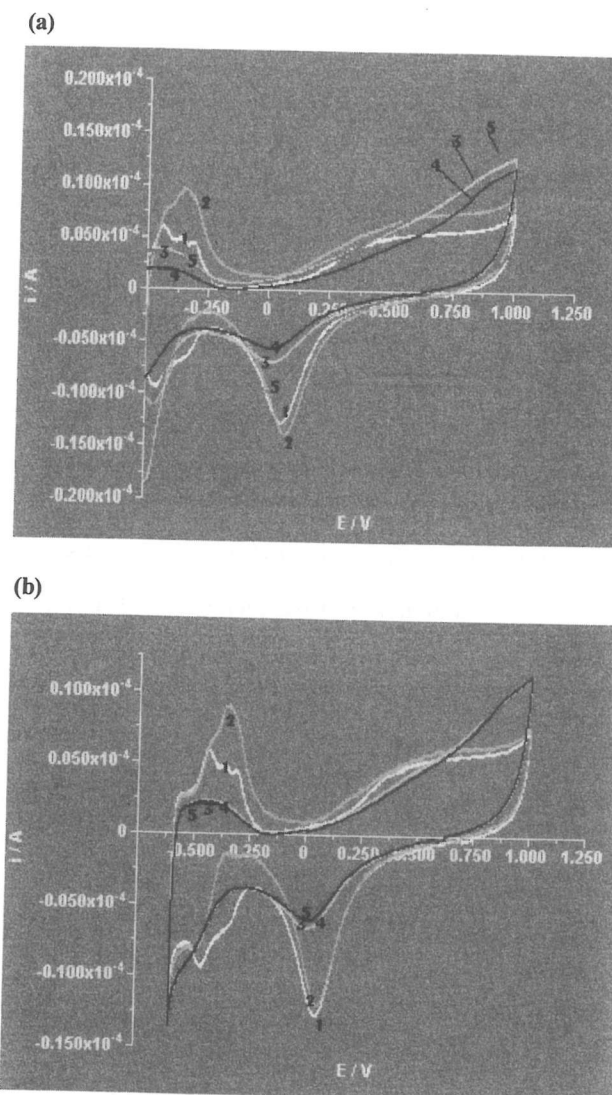


Figure 3 – Cyclic voltammograms recorded on a batch cell at a scan rate of 250 mV/s. (a) Voltammograms were obtained: (1) in a buffered solution; (2) in a buffered solution with 5 g/dm³ of glucose; (3) in a buffered solution with 2.5 g/dm³ of peptone; (4) in a buffered solution with 1.25 g/dm³ yeast extract; (5) in a buffered solution with 5 g/dm³ of glucose, 2.5 g/dm³ of peptone and 1.25 g/dm³ of yeast extract. (b) Voltammograms were obtained: (1) in a buffered solution; (2) in a buffered solution with 5 g/dm³ of glucose and 2.5 g/dm³ of peptone; (3) in a buffered solution with 5 g/dm³ of glucose and 1.25 g/dm³ of yeast extract; (4) in a buffered solution with 5 g/dm³ of glucose, 2.5 g/dm³ of peptone and 1.25 g/dm³ of yeast extract.

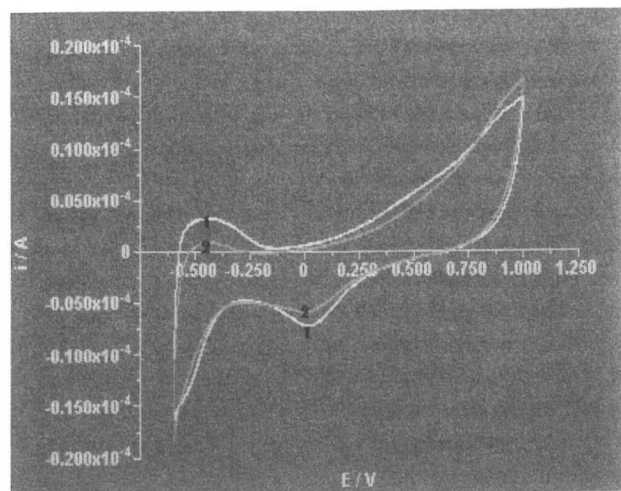


Figure 4 – Cyclic voltammograms recorded on a batch cell containing the culture medium at a scan rate of 250 mV/s. Voltammograms were obtained: (1) with a clean electrode in the culture medium; (2) immediately after an electrode with biofilm is immersed in the culture medium.

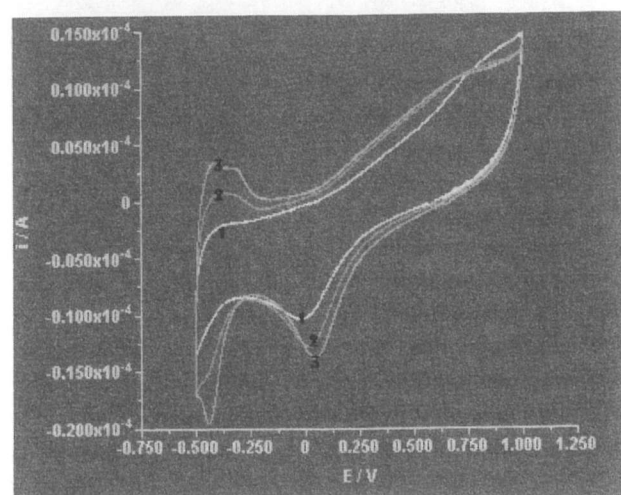


Figure 5 – Cyclic voltammograms recorded on a batch cell at a scan rate of 250 mV/s. Voltammograms were obtained: (1) immediately after the electrode with biofilm is immersed in the buffered solution; (2) after 5 cycles; (3) after 100 cycles.

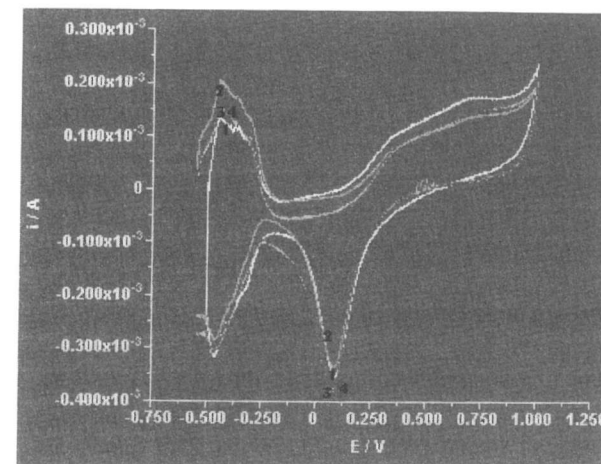


Figure 6 – Cyclic voltammograms recorded on a flow cell at a scan rate of 100 mV/s. Voltammograms were obtained in a buffered solution: (1) without flow; (2) with a flow rate of 4 mL/min; (3) with a flow rate of 15.8 mL/min; (4) with a flow rate of 33.3 mL/min.

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[2] J. Wang, "Analytical Electrochemistry", VCH Publishers, Inc., (New York, 1994).

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