AN ELECTROCHEMICAL DETECTOR FOR BIOFILM FORMATION

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ABSTRACT

On-line detectors of biofilm – platinum electrodes - were calibrated and validated in the laboratory under defined conditions, using a bacterium known as a good biofilm producer, *Pseudomonas fluorescens*. The electrochemical technique used was cyclic voltammetry.

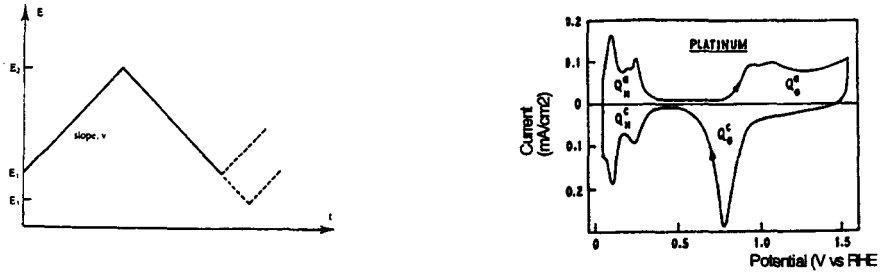
KEYWORDS

Cyclic voltammetry; biofilm detector; platinum; *Pseudomonas fluorescens*.

INTRODUCTION

It is widely recognized the need for on-line monitoring techniques that are reliable, easy to implement and cheap, to detect biofilm formation during continuous operation.
Electrochemical techniques are well known for their role in analytical chemistry allowing a large number of organic, inorganic and biological compounds to be determined and quantified. Therefore, they may also provide a convenient means of detecting the early stages of formation of biofilms in heat exchangers and water treatment systems (Illsley, R. A., 1997).

In the present work we set out to develop an electrochemical detector to monitor *in situ* the formation of biofilms in flow systems. The technique used is repetitive cyclic voltammetry applied to a platinum planar electrode of small area (7,85 x 10^{-3}cm^2) introduced in the system, which together with an auxiliary electrode and a reference electrode, constitute an electrochemical cell. The potential versus time function applied to the electrode is a succession of isosceles triangular waves (Wang, J., 1994) as illustrated in Figure 1(a).



(a) (b)

Figure 1 – (a) Potential–time profile used in repetitive cyclic voltammetry. (b) Voltammogram at a Platinum electrode in 1M sulfuric acid at 25°C; continuous triangular potencial sweeps at 40 mV sec^{-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)

When the solution where the electrode is immersed is air-free aqueous sulfuric acid, and the Pt electrode surface is clean, the current plotted *versus* electrode potential is a cyclic voltammogram depicted in Figure 1(b). This graph should be regarded only as an illustration of the type of cyclic voltammogram obtained at a clean Pt surface in sulfuric acid solutions. Details depend strongly on the scan rate, on the reversal potential, on the pre-treatment of the metal sample and on the solution composition. The application of repetitive cyclic voltammetry to Pt electrodes is in itself a method of electrode cleaning and the appearance of a voltammogram of the type shown in Figure 1(b) is an indication that the surface is free of impurities (Woods, R., 1976). This fact may constitute the basis of a method to detect formation of a biofilm in a flow system since the smallest deposit on the electrode surface will certainly change the pattern observed in figure 1(b).

MATERIAL AND METHODS

1. On-Line Biofilm Monitoring Devices based on electrochemical techniques

The biofilm on-line monitoring device is based on electrochemical techniques that have already been used successfully in basic and applied studies of corrosion (Mittleman, M. W., *et al*, 1994), but not fully developed in biofouling situations. In the present case, a platinum electrode was used, since this material is not expected to suffer a corrosion process. Therefore, if a biofilm is formed on the surface, the differences between cyclic voltammogramms obtained before and after biofilm formation on the surface can be used as a measure of the amount of pellicle formed.

The experiments were carried out in a batch cell, where the cyclic voltammetric behaviour of the platinum was recorded before and after a deposit has been previously grown on the surface.

"The batch cell" consists of a three electrode compartment all-glass cell, a platinum working electrode, a platinum auxiliary electrode and a silver/silver chloride reference electrode. Cyclic voltammograms were obtained using an AUTOLAB-PGSTAT 20 potentiostat to produce a repeating triangular function. Cyclic voltammograms were recorded on several platinum electrodes with biofim layers grown in a range of steps of development. This strategy allows to follow the electrochemical response as a function of different stages of biofilm formation.

The experimental procedure included the following steps:
- Recording of the cyclic voltammograms for each clean electrode, using buffered solutions or water.
- Immersion of the electrodes in the immersion vessel under conditions to allow biofilm formation on the surface of the electrodes. Then, new cyclic voltammograms were recorded at known time intervals. The differences between the voltammograms obtained with and without biofilm can be used as a measure of the biofilm on the surface. After the recording of each voltammogram the electrode was removed and the deposited film was characterised optically.

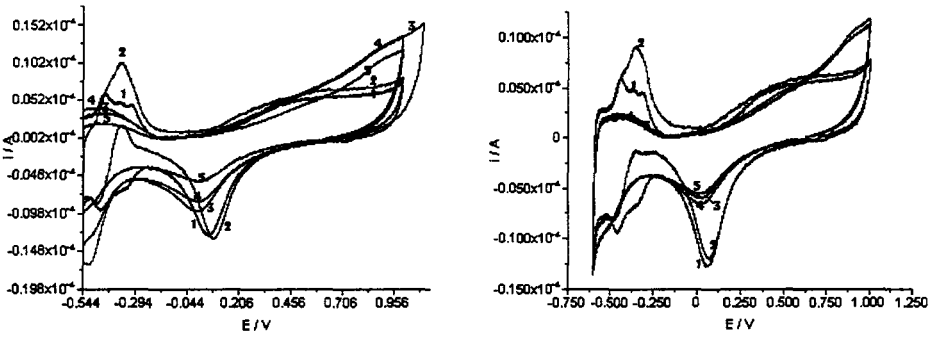
2. Cell Growth and Biofilm formation

The microorganisms used as a biofilm producers were the Gram-negative aerobic bacteria *Pseudomonas fluorescens*. Their optimum growth conditions are 27ºC, glucose as the limiting substrate and pH 7 (phosphate buffered).

A continuous culture of the bacteria was performed in a 0.5 L glass fermenter, aerated and agitated and fed with the sterile medium containing 5 g/dm^3 glucose, 2.5 g/dm^3 peptone and 1.25 g/dm^3 yeast extract, in phosphate buffer at pH 7 (0.0684 M Na_2HPO_4 and 0.1 M NaH_2PO_4). This culture was used to inoculate the immersion vessel used for biofilm formation on the electrode surface. The immersion vessel was operated in a continuous mode, under conditions to allow biofilm formation (residence time =1.5 h) and fed with the a medium containing 50 g/dm^3 of glucose, 25 g/dm^3 peptone and 12.5 g/dm^3 peptone.

RESULTS

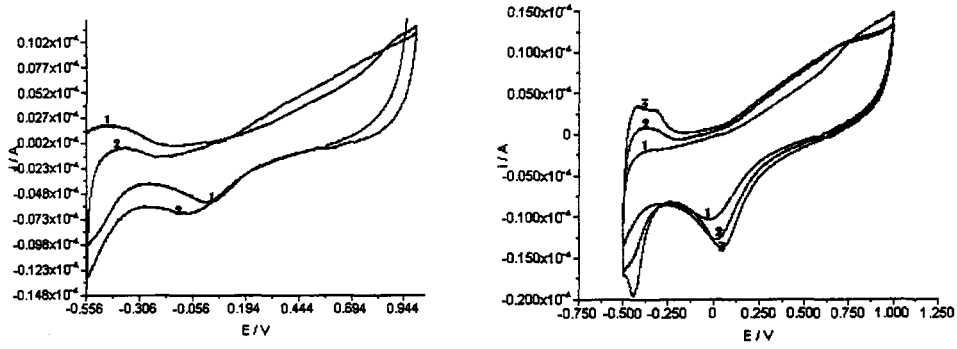
Figure 3 shows a series of cyclic voltammograms recorded on a batch cell and the different cycles show the effect of the different components used in the medium on the standard voltammograms shown in figure 1(b).



(a) (b)

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/dm^3 of glucose; (3) in a buffered solution with 2.5 g/dm^3 of peptone; (4) in a buffered solution with 1.25 g/dm^3 yeast extract; (5) in a buffered solution with 5 g/dm^3 of glucose, 2.5 g/dm^3 of peptone and 1.25 g/dm^3 of yeast extract. (b)Voltammograms were obtained: (1) in a buffered solution; (2) in a buffered solution with 5 g/dm^3 of glucose and 2.5 g/dm^3 of peptone; (3) in a buffered solution with 5 g/dm^3 of glucose and 1.25 g/dm^3 of yeast extract; (4) in a buffered solution with 5 g/dm^3 of glucose, 2.5 g/dm^3 of peptone and 1.25 g/dm^3 of yeast extract

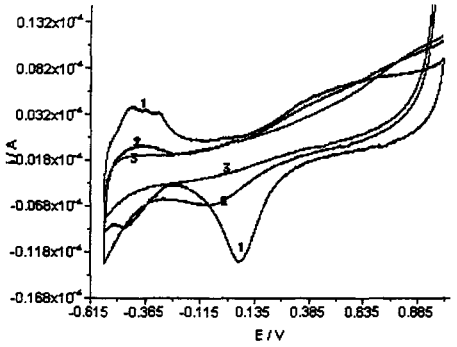
Figure 4(a) shows the difference in the voltammetry on the batch cell at a clean electrode in the culture medium and immediately after the electrode with biofilm is immersed in the same solution. A clear difference is observed specially in the H_2 desorption region. Figure 4(b) shows the effect of recycling the potential on an electrode with biofilm immersed in a bach cell containing buffered solution.



(a) (b)

Figure 4 – (a) 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. (b) Cyclic voltammograms recorded on a batch cell at a scan rate of 250mV/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 5 shows the difference in the response of the electrode when different amounts of biofilm are



attached.

Figure 5 – Cyclic voltammograms recorded on a batch cell at a scan rate of 250 mV/s. Voltammograms were obtained in (1) in a buffered solution; (2) immediately after the electrode with biofilm formed for 5 hours is immersed in the buffered solution; (3) immediately after the electrode with biofilm formed for 8 days is immersed in the buffered solution.

CONCLUSIONS

Experiments carried out in batch seem to indicate that an electrochemical cell containing a working platinum electrode may constitute the basis of a detector for a biofilm formation. Differences between the response of such electrode when it is clean and after a deposit has grown on its surface may be used as a measure of the amount of biofilm formed. It is likely that this detector may be useful in waste water treatment systems or heat exchangers to detect the early stages of biofilm formation. Experiments on a flow cell are now under progress.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support provided by Instituto de Biotecnologia e Química Fina. I. A. Pinho acknowledges a PRAXIS grant (PRAXIS XXI/ BD/ 11211/ 97).

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