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Monitoring biofilm formation by using cyclic voltametry – effect of the experimental conditions on biofilm removal and activity

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Abstract The effect of experimental conditions on cyclic voltammetry experiments on platinum electrodes covered with biofilms formed by *Pseudomonas fluorescens* for 2 hours was investigated. Results show that recycling the potencial stabilizes the shape of the cyclic voltammogram after 130 cycles, but the observation of this electrode by epifluorescence microscopy showed that cells are still adhered to the platinum surface. Some experimental conditions were changed during the electrochemical measurements – sweep rate, pH of the buffer and applied potential range. Some of these parameters had a strong impact on the bacteria that are adhered to the surface, increasing the death and removal in some circumstances.
Keywords Biofilm monitoring, *Pseudomonas fluorescens*, electrodes, cyclic voltammetry; viability; removal

Introduction

The unwanted accumulation of biofilms in industrial equipment is a natural phenomenon, due to the favourable conditions of nutrients, temperature and availability of microorganisms. The early detection of biofouling may allow the application of more effective strategies to eradicate biofilms. It is widely recognised the need for on-line monitoring techniques that are reliable, easy to implement and cheap, to detect biofilm formation during continuous operation (Kahlre and Flemming, 2000, Licina *et al*, 1994). Electrochemical techniques have been applied to a number of investigations in surface fouling by bacteria (Mittelmann *et al*, 1992, Illsley *et al*, 1997, Cachet et al, 2000). Cyclic voltammetry is a well-known method for its role in analytical chemistry allowing a large number of organic, inorganic and biological compounds to be determined and quantified. Therefore, it may also provide a convenient means of detecting the early stages of biofilm formation in heat exchangers and water treatment systems. The present work presents results obtained on the application of cyclic voltammetry to platinum electrodes with bacteria on the surface.

Material and Methods

Microorganism, cell growth and biofilm development

Pseudomonas fluorescens ATCC 13525, a Gram-negative aerobic bacterium, was used through this work. A reactor (0.5 L volume) was used to form biofilms on the surface of the electrode. It was inoculated with bacteria in exponential phase of growth. The system was continuously fed with a sterile medium containing 50 mg/l glucose, 25 mg/l peptone and 12.5 mg/l yeast extract in phosphate buffer (PB). The platinum electrodes (working electrodes) were immersed in the fermenter during 2 hours (eight electrodes were used for each condition tested). These electrodes with biofilm on their surface were used to carry out the electrochemical measurements and the observations under epifluorescence microscope. Prior to their utilisation, the electrodes were degreased, rinsed twice with water and sterilised using ethanol before being introduced inside the fermenter.

Electrochemical experiments

The working electrodes were platinum discs with 1 mm diameter. The electrodes were prepared by sealing a platinum wire into a glass tube and polishing the surface of the cross section with alumina powder, on a polishing cloth; the internal end of the platinum wire was sealed to a copper wire that provided the external contact. The reference electrode was a Metrohm silver/silver chloride electrode and all the data are reported versus this reference. The auxiliary electrode was a platinum spiral. The electrochemical experiments were carried out using a potentiostat Autolab type PGSTAT 10, Ecochemie that produced a repeating triangular function. The cyclic voltammetry experiments were carried out in a two-compartment, three-electrode cell at room temperature. Each electrode was electrochemically treated by immersion in the solution of interest and recycling the potential between the appropriate limits, and using different operating conditions, namely the pH of the buffer, the sweep rate and the potential limits. The experimental procedure included recording of the cyclic voltammograms for each clean electrode, the immersion of the electrodes in the fermenter to allow biofilm formation on the surface of the electrodes.

Then, the electrode was recycled between pre-selected limits for approximadely 30 minutes, and this procedure was followed by caracterization of the electrode surface.

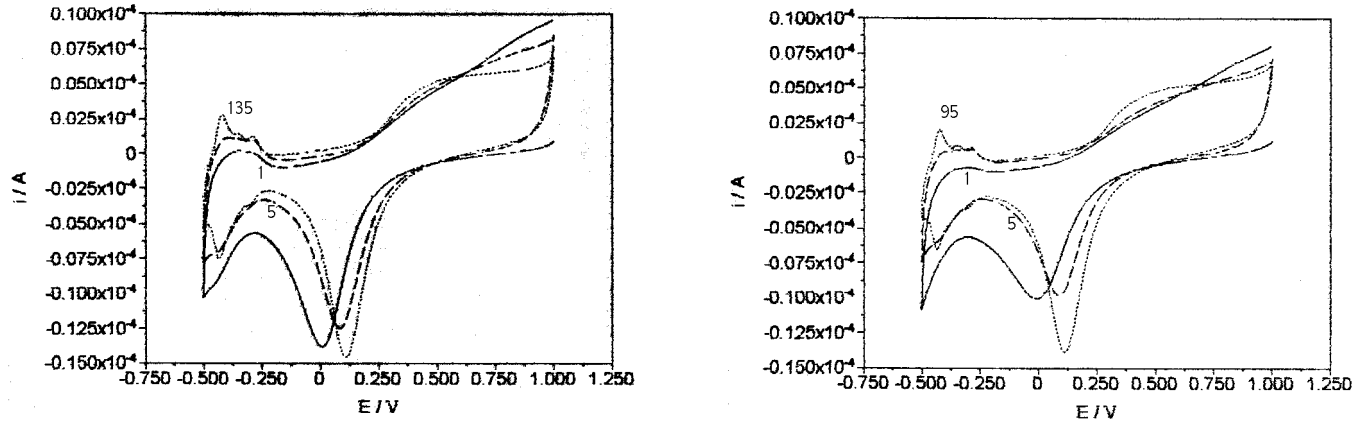
Epifluorescence observations

The platinum electrodes were visualized under epifluorescence microscopy after staining the surface with the *Bac*ligth viability kit, Molecular Probes. The two *Bac*Ligth stains, SYTO 9 and propidium iodide, dissolved in DMSO, were mixed together (130 µl + 130 µl) and dropped on the surface and incubated for 15 min. The electrodes were observed under epifluorescence (viable cells were fluorescent green, while dead cells were fluorescent red).

Results

Detection of biofilm by cyclic voltammetry

Figure 1 (a) shows that when cyclic voltammetry is applied to a platinum electrode with a biofilm with 2 hours on its surface, it is necessary to apply 130/135 scans to obtain a constant voltammogram (Fig 1a). However, when the same electrode was immersed in a 50ppm medium (without bacteria), 90/95 scans were sufficent to clean it (Fig. 1b).

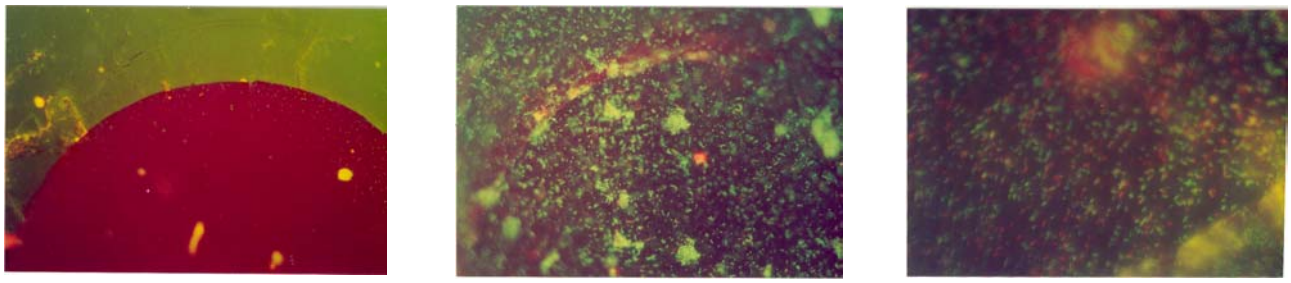


(a) (b)

Figure 1. Cyclic voltammetry when a) electrode with a 2 hours biofilm on the surface; b) electrode immersed in 50ppm medium.

Effect of the experimental conditions on the activity and removal of bacteria from the surface

The observation, by epifluorescence microscopy, of the platinum electrode surface after the cyclic voltammetry, experiment showed that, for the conditions used (phosphate buffer pH 7, sweep rate 250 mV s^{-1}, and potential applied from –0.5 to 1.0 V), the biofilm was not to removed from the surface and part of the remaining cells on the surface are active. Figure 2 shows the surface of the electrode prior to biofilm formation (a), electrode with a 2 hours biofilm on the surface (b) and a platinum electrode with a 2 hours biofilm on the surface after cycling the potencial (c).

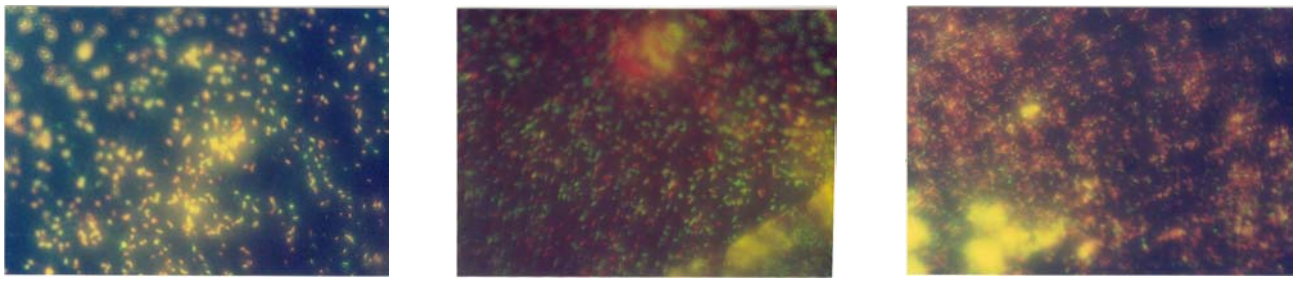


(a) (b) (c)

Figure 2. Epifluorescence microscopy photographs of a platinum electrode surface of the electrode prior to biofilm formation (a), electrode with a 2 hours biofilm on the surface (b) and a platinum electrode with a 2 hours biofilm on the surface after cycling for 30 min in PB pH 7, sweep rate 250 mV.s^{-1}, and potential applied from –0.5 to 1.0V (c).

Effect of the sweep rate

The observation by epifluorescence of the electrode surfaces presented in Figure 3 shows that the variation of the sweep rate from 150 m/s to 500 m/s increases the number of dead cells on the surface. However, lower sweep rates favour de removal of cells from the surface. (Figure 3)

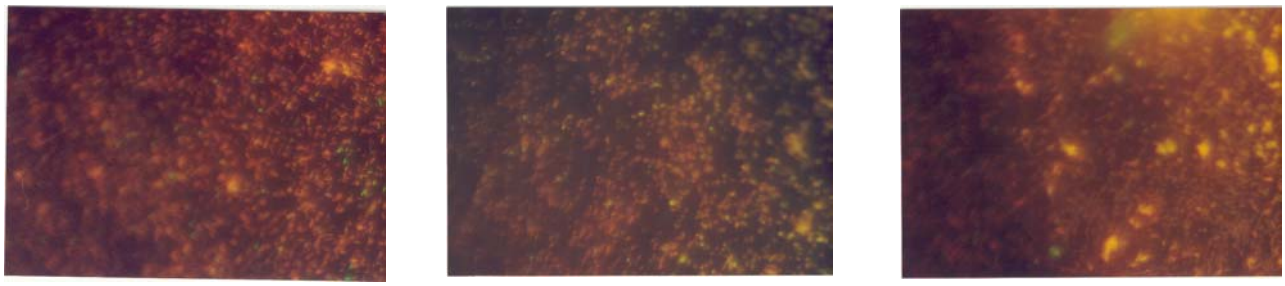


(a) (b) (c)

Figure 3. Epifluorescence microscopy photograph of a platinum electrode after 150 scans of cyclic voltammetry in PB pH 7, potential applied from –0.5 to 1.0 V, and sweep rate a) 150 $mV.s^{-1}$; b) 250 $mV.s^{-1}$; c) 500 $mV.s^{-1}$.

Effect of the pH

Runs carried out in phosphate buffer pH 4 showed that in these conditions, the cleaning is more effective. In fact, the bacterial cells on the surface are almost all dead only by immersion in pH 4 (Figure 4.a). Some of the bacteria can be removed by the application of cyclic voltammetry using the same pH (Figure 4 b). Using pH 7 during cyclic voltammetry, it seems that the cells are removed at a lesser extent (Figure 4c)

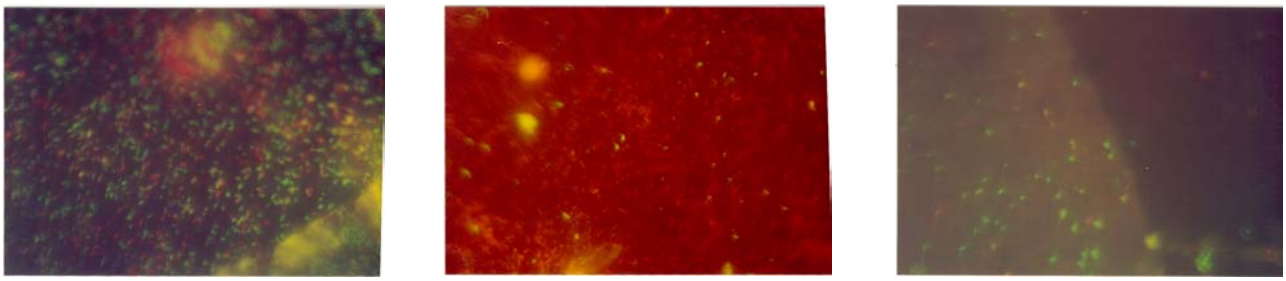


(a) (b) (c)

Figure 4. Epifluorescence microscopy photograph of a platinum electrode a) after 30 minutes immersed in pH 4; after cyclic voltammetry in b) pH 4; c) pH 7.

Effect of the applied potential range

Figure 5 b) shows that an increase on the positive potential to 1.5 V, does not clean furthers the electrode but all the bacterial cells are dead. Conversely, with a negative increase to –2.0 V the surface is almost clean, in spite of the fact that some viable cells are still on the platinum surface (Figure 5 c). When this potential was applied, hydrogen bubbles were formed at the surface of the electrode, which removed the bacteria.



(a) (b) (c)

Figure 5. Epifluorescence microscopy photograph of a) a platinum electrode after 30 minutes of cyclic voltammetry with a potential applied from –0.5 to 1.0 V; b) a platinum electrode after 30 minutes of cyclic voltammetry with a potential applied from –0.5 to 1.5 V.; c) a platinum electrode after 1.5 minutes of cyclic voltammetry with a potential applied from – 2.0 to 1.0 V.

Discussion and Conclusions

Previous results have shown that cyclic voltammetry can be very useful in detection of biofilm formation. Figure 1 (b) presents the effect of recycling the potential on the characteristics of the voltammogram: voltammograms obtained after 1, 5 and 135 potential cycles. It should be noticed that as the potential is cycled between the pre-set limits the pattern of the voltammograms approaches that corresponding to a clean surface. The evolution of the voltammograms shape as the potential is cycled may constitute a means of providing information on the coverage of the surface since the area under the peaks increases as the biofilm is removed. The number of cycles needed to obtain a voltammogram that shows no change increases with the amount of biofilm deposited. Results show that recycling the potencial stabilizes the shape of the cyclic voltammogram after 130 cycles, but the observation of this electrode by epifluorescence microscopy showed that cells are still adhered to the platinum surface (Figure 2 (a), (b) and (c)). However, some cells are dead after the process. The results presented in Figures 3, 4 and 5 show that some parameters that were changed during the electrochemical measurements – sweep rate, pH of the buffer and applied potential range - may have a strong impact on the bacteria that are adhered to the surface: in some cases – high sweep rates, low pH, higher potential limits- a higher number of dead cells are obtained, while in other cases – lower sweep rates, higher potential limits- less cells are present after the process. These results may be related to the formation in situ, at the platinum electrode, of products (such H2O2) that may act as biocides, application of potentials that may give rise to higher electrostatic repulsions (bacteria are negative at these conditions) and thus favour detachment from the surface and formation of bubbles that remove mechanically the bacteria from the surface.

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