MONITORING BIOFILM GROWTH AND ACTIVITY USING A SCALABLE MULTICHANNEL ELECTROCHEMICAL BIOSENSOR

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ABSTRACT

In this work we report on a multichannel biofilm monitoring platform that can monitor the growth and activity of a biofilm. This was possible by combining an electrochemical impedance spectroscopy (EIS) biosensor with a fuel cell activity sensor. We have previously shown an electrochemical sensor platform which was able to measure biofilm growth. This paper describes an improved system which can also monitor the biofilm activity. This additional information will enable the discrimination of dead from living biomass.

KEYWORDS

Impedimetric biosensor, Biofilm growth, Microbial fuel cell, Electrochemical impedance spectroscopy, Microfluidics

INTRODUCTION

The standard method for characterizing biofilms is optical microscopy, which is a cheap and simple but disruptive and offline technique. Alternative analytical methods include gravimetric sensor systems such as quartz crystal microbalances or surface acoustic waves, surface plasmon resonance sensors as well as electrochemical methods.[1] Among the latter, electrical impedance spectroscopy (EIS) has gained increasing interest recently [2].

EIS is a non-disruptive online technique which is easily scalable to mulit-channel sensor systems. It evaluates the resistance and reactance of a surface by means of applying a small-amplitude AC signal with variable frequency through a pair of electrodes while measuring the resulting current (Figure 1a). The results can be represented as a Nyquist diagram (Figure 1b) in which the real and imaginary parts (i.e., resistance and reactance) of the complex impedance are plotted as a function of frequency. The presence of a biofilm on top of the electrode will hinder the charge transfer between the electrodes, thus increasing the measured impedance. This change in the surface impedance will result in a curve shift in the Nyquist diagram which can be correlated to the growth of a biofilm on top of the electrodes.

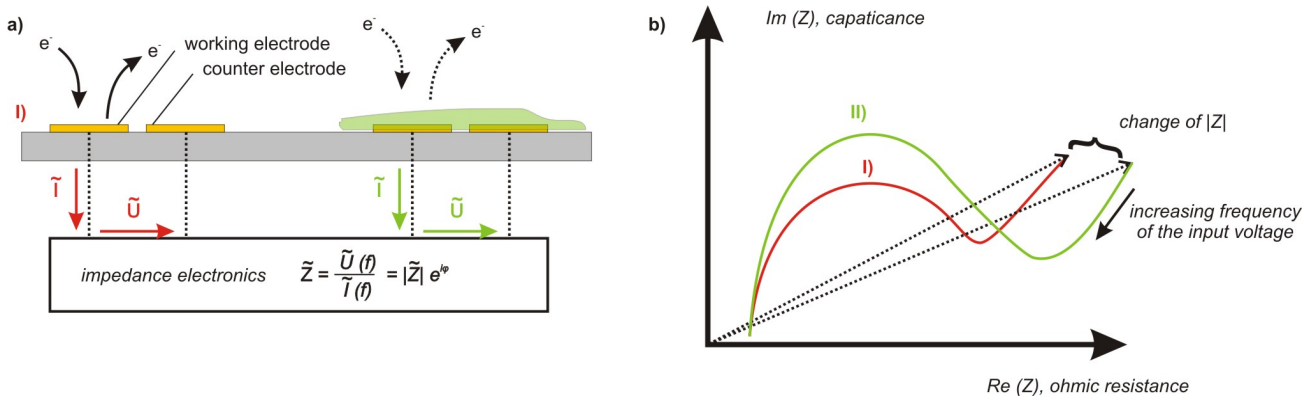

Figure 1 - a) Principal setup of EIS in biofilm detection – to a pair of electrodes a small AC voltage is applied and the current between the electrodes is measured. b) The impedance is usually displayed in the complex plane in form of a Nyquist diagram (displaying real and imaginary parts of the complex resistance).

EXPERIMENT

Our system consists of a polymer microfluidic flow cell with two channels (reference and measurement), planar gold electrodes sputtered on a cyclic olefin copolymer (COC) substrate, MFC activity sensor and custom made electronics. The MFC sensors are composed of a carbon rod in a closed reservoir which contains electrolyte solution and is fluidically separated from the channel through a proton exchange membrane (Figure 2).

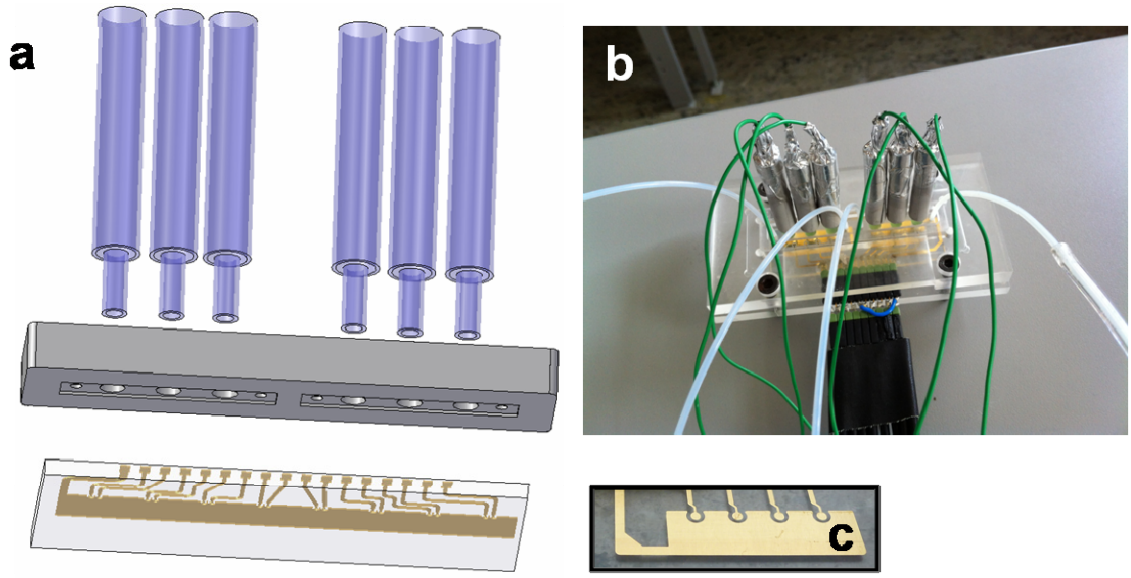

Figure 2 - View of the flow cell setup. a) Schematic view of flow cell, MFC activity sensors on the top, flow cell in the middle and planar electrodes at the bottom. b) Picture of the measurement cell. c) EIS planar electrode.

The MFC-based sensors were initially tested and characterized in a yeast microreactor. When the yeast catabolic cycle is activated by sugar water free electrons are generated and form a current which can be measured by the MFC activity electrode. As seen in Figure 3 the shaded areas represent the injection of yeast with sugar water whilst in the other intervals only sugar water was probed. The shaded intervals show a clear current increase.

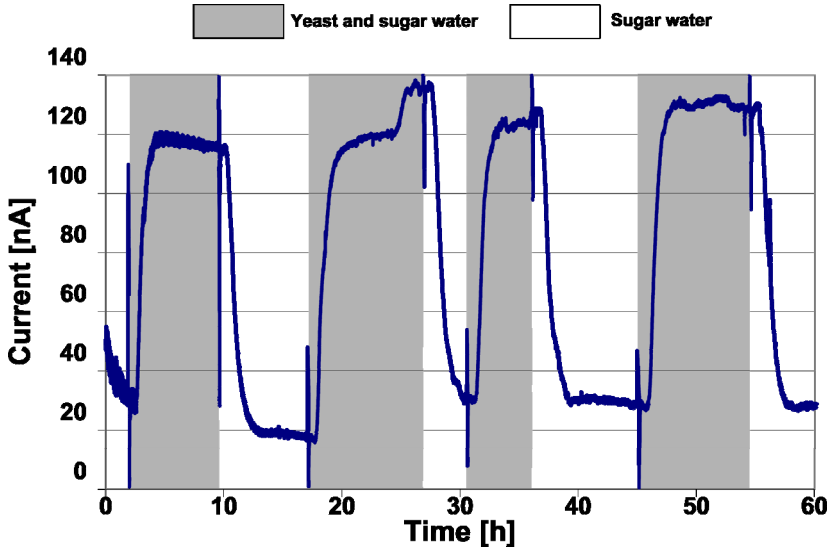

Figure 3 - Microbial fuel cell (MFC) activity sensor test in a yeast and sugar water microreactor. Gray shaded intervals mark the injection of yeast and sugar water. During other intervals only sugar water was probed across the sensor. The switching of the pump is responsible for the presence of peaks in the diagram.

RESULTS AND DISCUSSION

This setup was used to monitor the growth and metabolic activity of *Pseudomonas aeruginosa* and *Staphylococcus aureus* during 50 hour periods. This experiment was divided into two parts; the injection and the growth phase which took 3 and 47 hours, respectively. During the first part the bacterial suspension was probed across the measurement channel whilst the reference channel was probed with feeding medium (brain heart infusion broth). In the next stage both channels were probed with feeding medium. Drift effects (mainly due to ambient temperature changes) were compensated by subtracting the results of the measurement from the reference channel. The impedance increased over time because of biofilm formation on top of the measurement electrodes (Figure 4). Biofilm activity (Figure 5) also followed that trend as the number of bacteria increased. After the experiment the biofilm was stained and analyzed by means of fluorescence microscopy (Figure 4, inlay) confirming the presence and absence of the biofilm on the measurement and the reference electrodes, respectively.

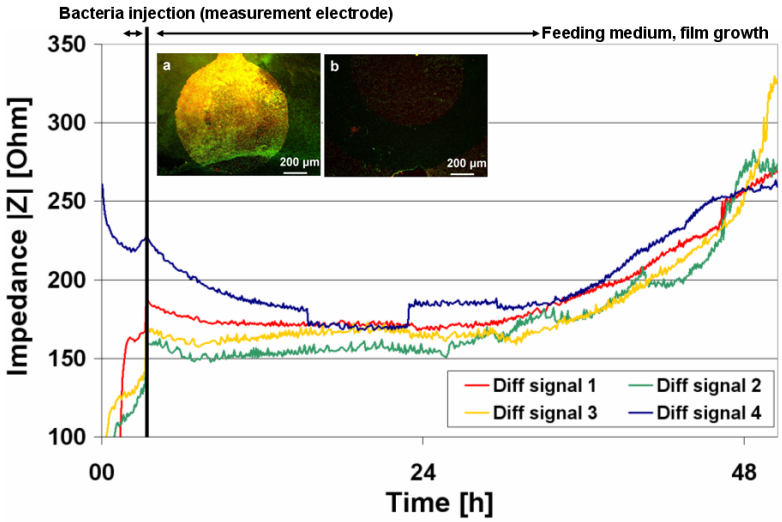

Figure 4 - Differential impedance measurement. During the first 3 hours of the experiment the measurement electrode was probed with *Staphylococcus aureus*, followed by feeding medium. Reference electrode only came in contact with feeding medium. After 48 hours the results tend to be affected by air bubbles. The inlay pictures show the live-dead staining of these electrodes. a) Measurement electrode b) Reference electrode.

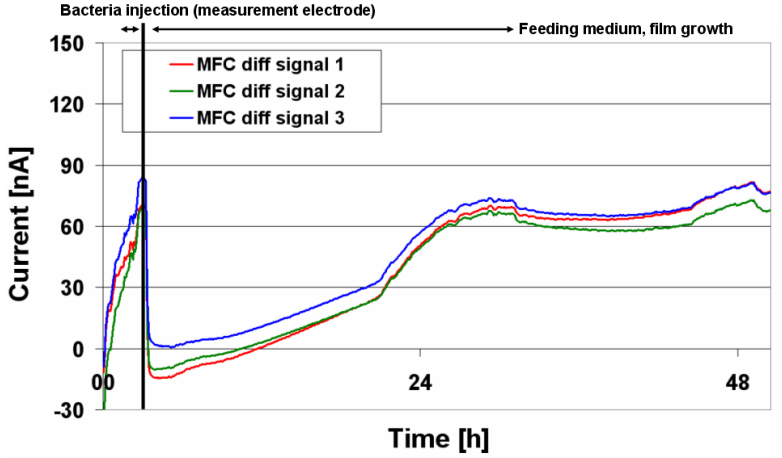

Figure 5 - Differential current measurement over 48 hours of the MFC electrodes. An increase in the signal can be observed which correlates to the increase in measured impedance.

CONCLUSION AND OUTLOOK

We presented a biosensor that combines EIS based sensors with a MFC activity measurement system, thus enabling the monitoring of the biofilm formation as well as its activity. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were successfully monitored for over 50 hours. Such a system can be used to provide decisive information on the effect of physical or chemical treatments of the desired biofilm, for instance enabling the discrimination of dead from living biomass.

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