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# Quantification of bio-anode capacitance in bioelectrochemical systems using Electrochemical Impedance Spectroscopy

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## HIGHLIGHTS

- Fluorinated Tin Oxide (FTO) anodes with electro-active biofilm produced 1.1 A $m^{-2}$.
- Impedance spectroscopy was used to quantify biofilm capacitance.
- Biofilm capacitance reached 450 μF $cm^{-2}$ while FTO capacitance was 25 μF $cm^{-2}$.
- Average biofilm yield of 0.55 g COD biomass/mol $e^-$ was determined.

## ARTICLE INFO

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## ABSTRACT

Understanding the electrochemical properties of bio-anodes is essential to improve performance of bioelectrochemical systems. Electrochemical Impedance Spectroscopy (EIS) is often used to study these properties in detail. Analysis of the EIS response, however, is challenging due to the interfering effect of the large capacitance of typically used graphite and carbon-based electrodes. In this study, we used flat electrodes made of conductive Fluorine-doped Tin Oxide (FTO) as anode, and monitored bio-anode performance. We show that with this configuration, it is possible to accurately separate the distinct contributions to the electrical response of the bio-anodes: charge transfer, biofilm and diffusion resistances, and biofilm capacitance. We observed that the capacitance of the biofilm increased from 2 μF $cm^{-2}$ to 450 μF $cm^{-2}$ during biofilm growth, showing a relationship with current and total produced charge. These results suggest that biofilm capacitance is a measure for the amount of active biomass in bioelectrochemical systems. At the end of the experiment, the biofilm was harvested from the FTO electrode and an average yield of 0.55 g COD biomass/mol $e^-$ was determined.

## 1. Introduction

Bio-anodes play an essential role in Bioelectrochemical systems (BESs) which can be used to recover electricity or produce chemicals from wastewater [1–3]. These bio-anodes are capable of extracellular electron transfer through direct and indirect mechanisms [4–6], and also of temporary charge (energy) storage in the form of electrons in multi-heme *c*-type cytochromes [7,8] or as organic polymeric molecules inside the bacterial cell [9]. All these processes together determine the rate and efficiency at which electrical current is produced. Quantification of these processes, like biofilm capacitance, charge transfer, biofilm and diffusion resistances [10–13], is essential for further development of BESs.

Electrochemical Impedance Spectroscopy (EIS) is often proposed as a powerful tool to separate these processes occurring in the bio-anode. EIS is based on the measurement of the response of a small alternating voltage (AC) perturbation at a given electrode potential. These EIS measurements are performed at different frequencies to reveal the characteristic response times of the different processes occurring in the bio-anode. Although the EIS measurement itself is relatively simple and can be performed in-situ, interpretation of the frequency dependent response is challenging. For accurate analysis, it is essential that the

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equivalent circuit that is used to interpret the data is an accurate physical representation of the system under study. Verification of the suitability of the equivalent circuit, e.g. by changing experimental settings and analyzing the response, is therefore crucial [10,12,14].

A special challenge in EIS analysis arises when the electrode under study has a high capacitance. This electrode capacitance can be of such magnitude that it is impossible to distinguish between biofilm capacitance and electrode capacitance. Even more, it may result in an overlapping response of charge transfer and diffusion processes, so that their separate contribution to the total impedance cannot be quantified [14]. So far, EIS studies on bio-anodes have been performed on porous electrodes that have high capacitance such as carbon-based plates, graphite felt, and carbon cloths [11,13,15]. Often, it remains unclear if the reported values for biofilm capacitance are related to electro-active biofilm, electrode, or the combination of both. In addition, the other resistances in the system may not be analyzed correctly when electrode capacitance is high, as high electrode capacitance will interfere with ionic diffusion in the EIS spectrum.

The aim of this study was, therefore, to quantify bio-anode properties, more specifically biofilm capacitance, charge transfer, biofilm and diffusion resistances, without interference of electrode capacitance. For this purpose, Fluorinated Tin Oxide (FTO) was used as electrode material to grow the electro-active biofilm on. Flat FTO is an attractive electrode material for electrochemical analysis of bio-anodes, because it is very stable and has a much lower capacitance, in the order of tens of $\mu F\ cm^{-2}$ [16], than typical carbon electrodes like graphite plates ($\sim 1\ mF\ cm^{-2}$) [11,17–19]. In this work, the development of an electro-active biofilm on FTO was monitored using EIS and polarization experiments. An equivalent circuit model is proposed to analyze the experimental data and to quantify electrochemical properties of the bio-anodes. Two independent experiments were performed to confirm the validity of the results.

## 2. Materials & methods

### 2.1. Bioelectrochemical system set-up with the FTO anode

Two independent experiments were performed, in which electro-active biofilms were grown on FTO electrodes. Bio-anode performance was studied for 25 days in the first experiment, and for 52 days in the second experiment. A two-chamber BES was used, each chamber having a volume of 35 mL and a projected (circular) surface area of $19\ cm^2$. The anode and cathode chamber were separated by a cation exchange membrane (FumaTech GmbH, Germany). 3.2 mm thick glass plates coated with fluorinated tin oxide (FTO) (provided by Xop Fisica, Spain) with 15 Ω/sq sheet resistance was used as anode electrode. Graphite felt of 2.8 mm thickness (CGT Carbon GmbH, Germany) was used as cathode electrode. The current collector was a 0.3 mm platinum wire for the anode, and a 0.8 mm titanium wire for the cathode. An Ag/AgCl/3 M KCl (+0.205 V vs. SHE) reference electrode was placed in the anode chamber, in between the electrode and the membrane. All potentials are reported vs Ag/AgCl.

### 2.2. Inoculation and media composition

Both experiments were started with clean electrodes and membranes and the BESs were inoculated with a mixed culture of anodic microorganisms from a BES fed with acetate. The anolyte contained $820\ mg\ l^{-1}\ CH_3COONa$ (10 mM acetate), $0.2\ g\ l^{-1}\ NH_4Cl$, $0.13\ g\ l^{-1}$ KCl, $1\ ml\ l^{-1}$ vitamin and $1\ ml\ l^{-1}$ mineral solution [20] in a phosphate buffer solution ($4.58\ g\ l^{-1}\ Na_2HPO_4$ and $2.77\ g\ l^{-1}\ NaH_2PO_4{\cdot}2H_2O$ (50 mM)). The anolyte was sparged with $N_2$ for 30 min before introducing it into the BES. The catholyte contained 100 mM potassium hexacyanoferrate[III] in a 50 mM phosphate buffer solution. Both anolyte and catholyte were recirculated via a bottle (500 mL) from the bottom to the top of the chamber with pump speeds of 70 mL $min^{-1}$ and 100 mL $min^{-1}$ 500 mL of the 530 mL total anolyte volume was regularly replaced with fresh medium to ensure sufficient nutrients and acetate, and stable pH (6.9–7.1). The reactor was operated inside a temperature controlled cabinet at 30 °C.

### 2.3. Electrochemical control and measurements

In both experiments, the anode potential was controlled at −0.35 V vs Ag/AgCl by a potentiostat (Ivium n-stat with IviumSoft v.2.462, Eindhoven, The Netherlands) in a three electrode setup, with the anode as working electrode, Ag/AgCl as the reference electrode, and the cathode as counter electrode. The anode potential of −0.35 V was chosen, as at this potential it is possible to observe the different contributions to charge transfer without excessive interference from diffusion in the EIS spectra. Bio-anode performance was characterized using EIS measurements (typically repeated 3 times; in some cases 2 or 4 times) and polarization curves, both of which were recorded at least once every 3 days. EIS measurements were performed at the same controlled anode potential of −0.35 V. An amplitude of 10 mV was used for the AC signal in a frequency range of 10 kHz–50 mHz. For some experiments, lower frequencies down to 5 mHz were used to visualize the full spectrum, including diffusion. After each EIS measurement, a polarization curve was measured. During the polarization curve, the anode potential was increased from −0.45 V to −0.30 V with each step of 0.05 V lasting for 5 min. The current was recorded each second, and the average current of the last 10 s at each anode potential was used for presentation in the polarization curves.

### 2.4. Electro-active biofilm quantification

At the end of the second experiment, the electro-active biofilm was removed from (scraped off) the FTO electrode to determine the total biomass weight (in g of COD). The biomass was homogenised in 10 mL of mili-Q water by ultrasound treatment prior to analysis of the chemical oxygen demand (COD) with a cuvette test (Hach Lange).

## 3. Results and discussion

### 3.1. Bio-anode performance on FTO

Two independent experiments were performed in order to confirm the validity of our analyses. In both cases an electro-active biofilm was grown on the FTO electrode, at constant anode potential of −0.35 V vs Ag/AgCl. First of all, the general performance in terms of current density and polarization behaviour is analyzed, to get a general insight in performance of the electro-active biofilm on FTO electrodes.

Fig. 1A shows the current density as a function of time for both experiments. In the first experiment, the onset of current production was on day 5, followed by a steep rise in current on day 13. In the second experiment, the onset of current production was on day 13, followed by a steep rise in current on day 21. This delay in the onset was attributed to the early replacement of medium and subsequent dilution of inoculum. The maximum current density was $0.74\ A\ m^{-2}$ for experiment 1, and $1.1\ A\ m^{-2}$ for experiment 2.

A selection of the polarization curves, obtained for experiment 2, is presented in Fig. 1B. The polarization curves showed a similar increase in bio-anode performance with time. In experiment 1, the maximum current density reached in the polarization experiment was $1.1\ A\ m^{-2}$ (day 26) at −0.30 V. In experiment 2, the maximum current density was $1.6\ A\ m^{-2}$ (day 51) at −0.30 V. The current achieved on this flat surface with low capacitance is comparable to the current achieved on other, more capacitive electrode materials, like 2D graphite-based electrodes [21]. FTO is thus a suitable electrode material to grow highly active electro-active biofilms, despite its low specific surface area and smooth surface.