Simultaneous Detection of Electroactive Bacteria with EIS and CLSM

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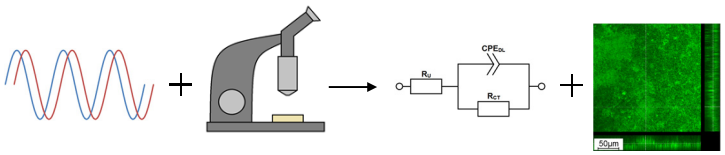
The Idea

Understanding the attachment of **electroactive bacteria (EAB)** to electrode surfaces and their subsequent biofilm formation is one of the major challenges for the establishment and improvement of **bioelectrochemical systems (BES).**



By a **simultaneous** combination of the two powerful monitoring methods **Electrochemical Impedance Spectroscopy (EIS)** and **Confocal Laser Scanning Microscopy (CLSM):**

- Reaction kinetics at the electrode,
- Diffusion processes,
- Cell attachment to the electrode surface, and
- Biofilm structure of established electroactive colonies.

Can be studied under the same conditions and with minimal influence to the system.
To fulfil this idea a flow cell was constructed.

Flow Cell development

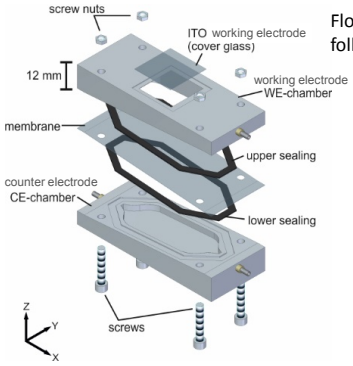


Fig. 1: Exploded view of developed flow cell.

Flow cell development (**fig. 1**) underlay the following requirements:

- Applicability as a **BES**
- continuous and non-destructive *in-situ* microscopy of working electrode (**ITO working electrode**)
- EIS measurements and Cyclic Voltammetry
- Two chamber system for separated working and counter electrode
- Homogeneous flow conditions (**fig. 2**)

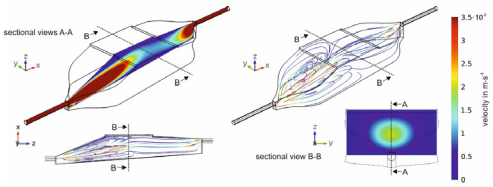
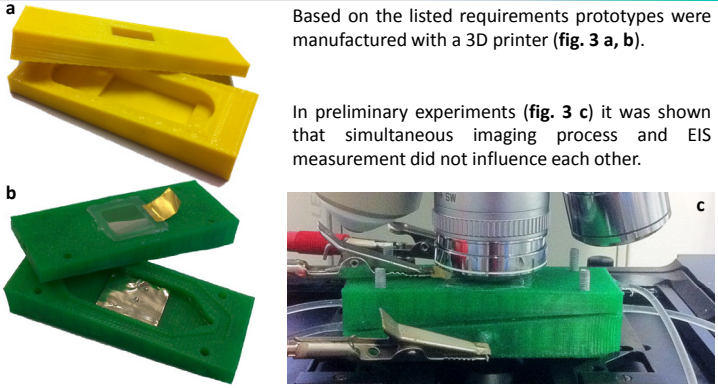


Fig. 2: CFD analysis to simulate laminar fluid conditions at the working electrode (flowrate of 5 mL · min-1)

3D printed prototype



Based on the listed requirements prototypes were manufactured with a 3D printer (**fig. 3 a, b**).

In preliminary experiments (**fig. 3 c**) it was shown that simultaneous imaging process and EIS measurement did not influence each other.

Fig. 3: 3D printed prototypes of the flow cell (a)(b). Electrically connected cell under CLSM ocular.

Final PEEK flow cell with electrodes

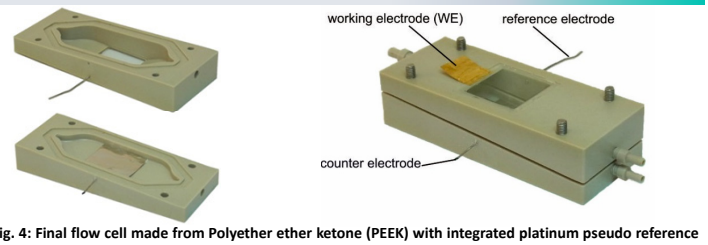


Fig. 4: Final flow cell made from Polyether ether ketone (PEEK) with integrated platinum pseudo reference electrode.

EIS and CV measurements with Fe(II) / Fe(III) redox probe

Prior to the application of the flow cell as a BES, EIS and cyclic voltammetry (CV) measurements with the redox probe Fe(+II) / Fe(+III) were performed. 1 mM $K_4[Fe(CN)_6]$ in anaerobic 0.5 M Na_2SO_4 was applied.

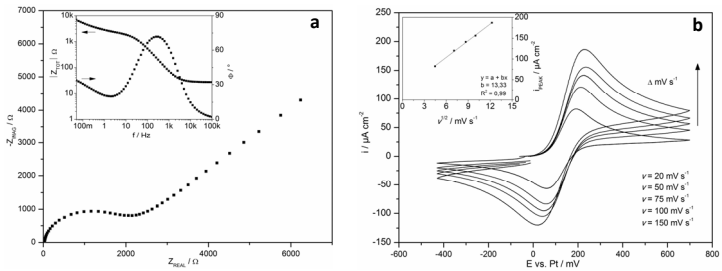


Fig. 5: Impedance (a) and CV (b) measurements of Fe(+II) / Fe(+III) in the developed flow cell.

EIS measurement produced a standard pattern (**fig. 5a**) of the well known iron redox system, indicating that the flow cell is suitable for EIS measurements. The same applied to the CV measurements (**fig. 5b**). With increasing scan rate peak currents over the square root of the scan rate gave a linear correlation, typical for a diffusion control.

Application in MFC mode with *Shewanella oneidensis*

Attachment of the electroactive model organism *Shewanella oneidensis* to the ITO working electrode was observed in MFC mode (**ITO = anode**).
EIS measurements showed that the R_{ct} was lowered over time (decreasing size of the semicircle in the Nyquist plot (**fig. 6**)).
Simultaneously, the number of attached *S. oneidensis* cells to the ITO electrode was increased (**fig. 7**).

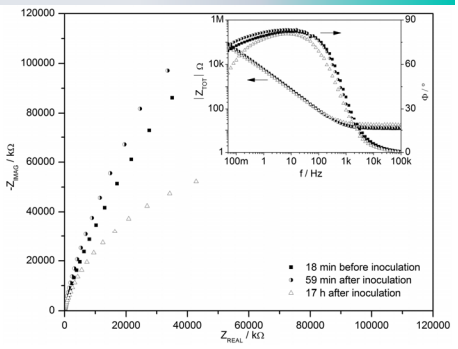


Fig. 6: Impedance measurements during attachment of *S. oneidensis* cells, taken through the course of the experiment.

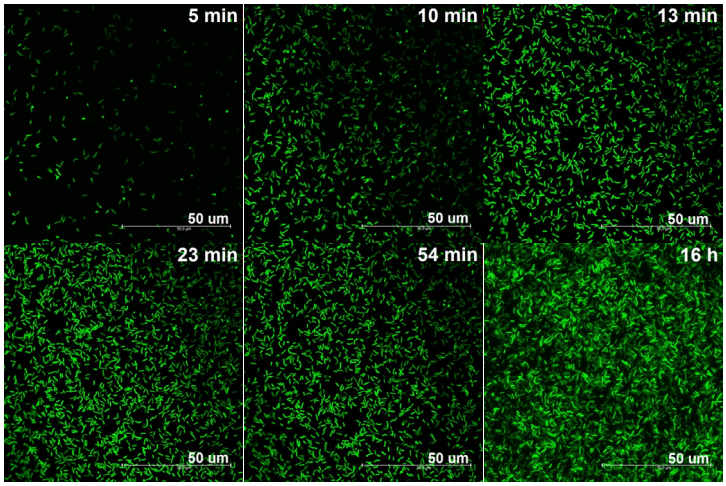


Fig. 7: CLSM images of attached *S. oneidensis* cells, taken through the course of the experiment.