Experimental report

05/01/2022

Proposal: 8-02-955 **Council:** 4/2021

Title: Structural changes in photosynthetic bacteria on electrode surfaces during different stages of the electron transfer mechanism.

Research area: Chemistry

This proposal is a new proposal

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Samples: Carbon films/silicon substrates
Synechocystis sp. PCC 6803
Nostoc sp. ATCC 27893

Instrument	Requested days	Allocated days	From	To
FIGARO	3	2	06/09/2021	08/09/2021

Abstract:

This proposal builds on previous successful neutron reflectometry (NR) experiments studying the structural changes of smaller redox species and proteins at electrode surfaces under electrochemical control. Here, we wish to extend this project to study the structural changes of redox-active cyanobacterial cells adhering to carbon electrode surfaces as they form biofilms under applied potential to better understand this key stage in the creation of bioelectronic devices and the underlying reasons why two bacterial strains behave so differently at this interface.

Experimental report: 8-02-955: Electrochemistry of photosynthetic bacteria

Background

This proposal aimed to investigate the structural changes occurring at the interface between redox-active cyanobacteria and carbon electrode surfaces. Such bacteria are used in bio-photovoltaic (BPV) devices—an emerging renewable energy technology in which biofilms of photosynthetic bacteria are grown onto electrodes and used to harvest solar energy. Such devices remain surprisingly inefficient, however, with inexplicably slow electron-transfer at the bacteria/electrode interface. Our previous work has demonstrated that the biofilms only become electroactive after several hours of 'conditioning' (being allowed to settle at an applied potential of +0.30 V vs Ag/AgCl). Additionally, the strain *Synechocystis* sp. PCC 6803 shows a significantly lower electron-transfer efficiency than another cyanobacterial strain, *Nostoc* sp. ATCC 27893. In this work, we aimed to use a custom-made electrochemical cell to investigate the biofilm/electrode interface and monitor any structural changes over time and as a function of applied potential.

Results

A key challenge in this experiment was to ensure good coating of the electrode surfaces with the bacteria—this was rendered difficult as the bacteria usually prefer rough or patterned surfaces rather than the ultra-flat substrates used in NR. To aid this, a pre-layer of polylysine

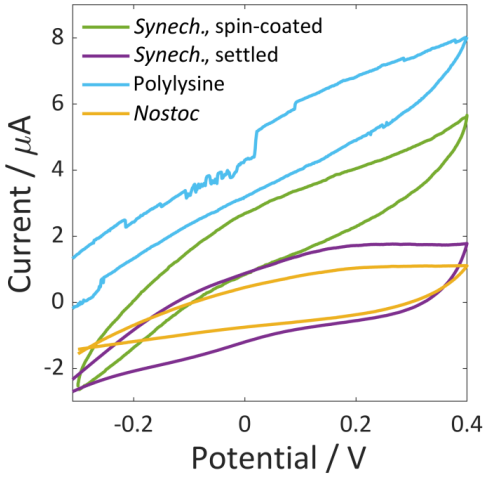

Figure 1: Comparison of CVs (2 mV s^{-1}) for the carbon surface with polylysine (blue), spin-coated (green) and settled (purple) *Synechocystis* and settled *Nostoc*.

was added, as this is known to improve adhesion for biological species.

We also attempted to spin-coat some samples with biofilms but this proved an unreliable approach and gave very patchy layers (e.g. Figure 1 shows the CV for the spin-coated sample of *Synechocystis* had a much higher resistivity compared with the other samples). The horizontal sample environment available at FIGARO was therefore crucial as it allowed us to instead use the settling approach, whereby we introduced bacterial solutions into the experimental cell and then allowed the bacteria to slowly settle onto the surface at an applied potential of +0.30 V whilst continually measuring NR profiles to monitor any changes. This approach proved reliable; when no further changes were seen in the NR data the cells were rinsed and for both strains the rinsing solution ran clear (the bacteria are a strong green colour). When the experiment was finished and the cells disassembled, the biofilms were clearly visible on the carbon surfaces. In addition, the CVs run pre- and post-rinsing appeared largely identical.

As D_2O was not conducive to the bacterial health, we used an H_2O contrast for most of the experiments, with the result that only small shifts were observed. (The polylysine layer was initially characterised in both H_2O and D_2O in order to discount its contribution.) Small changes were observed over the span of 4 hours as the biofilm was allowed to form at

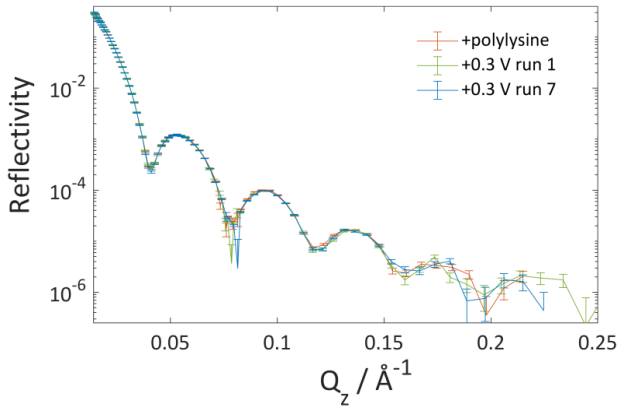

Figure 2: Raw H-PBS data for the polylysine-coated carbon substrate with settled *Synechocystis* showing subtle changes over time as the biofilm forms.

an applied potential of +0.30 V (Figure 2) in good agreement with our previously observed electrochemical measurements, suggesting the bacteria are slowly adhering to the surface. Even considering the poor contrast, however, the changes seen were much smaller than expected, particularly given the biofilms were visible by eye at the end of the experiment. We believe that there may be a relatively thick layer of buffer between the carbon surface and bacterial membranes that gives rise to these small shifts, although we have yet to complete fitting to confirm this.

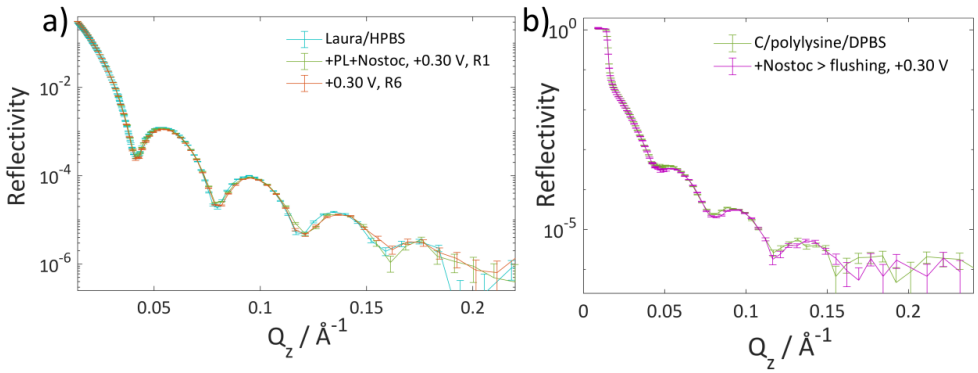

Figure 3: Raw data for the carbon substrate in a) H-PBS and b) D-PBS with *Nostoc*.

Similar results were seen for *Nostoc* (Figure 3). For this sample, we flushed with D-PBS at the end of the experiment in an attempt to improve the contrast to conduct a final characterisation; as shown in Figure 3b, the shift was still relatively small, in possible agreement with the large water interlayer hypothesis.