UNIVERSITY OF Southampton
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ELECTROCHEMICAL SENSING FOR MARINE BACTERIAL BIOFILMS

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INTRODUCTION

The ability to detect biofouling on marine platforms, heat exchangers and pipelines in-service is often limited by a lack of suitable techniques capable of sensing the presence of these biofilm species. Biofilms are a problem since they can cause the reduction in heat transfer performance, fluid frictional resistance increases, fluid flow restriction and biocorrosion [**1**]. Understanding how marine biofouling organisms such as bacteria, algae and animal larvae, attach to metallic surfaces to ultimately develop a generic means to control all biofoulers is a great challenge [**2**]. This has led to the present approach of selecting one particular type of marine organism, *i.e.* bacteria species, in order to focus on bacterial / interfacial properties and sense *in situ* its presence on metallic surfaces using an electrochemical technique.

OBJECTIVE

To detect the formation and growth of marine aerobic bacterial biofilms using gold (Au) microelectrodes and electrochemical impedance spectroscopy (EIS).

BIOFILM ELECTROCHEMICAL SENSING

The presence of a bacterial biofilm can modify the electrochemical properties and the mass transport near the interface, which ultimately alters the interfacial impedance response, see **Figure 1** [**3**].

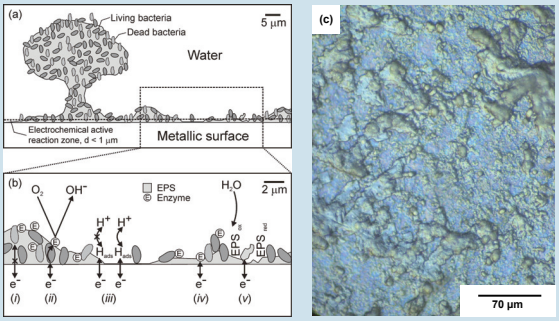

Figure 1. A schematic of a bacterial biofilm colonisation: **(a)** an overview of a typical biofilm morphology, **(b)** processes detected by electrochemical measurements [**3**], **(c)** the initial biofilm formation on titanium (Ti) immersed for 168 hours in seawater at the National Oceanography Centre, Southampton (NOCS).

METHODOLOGY

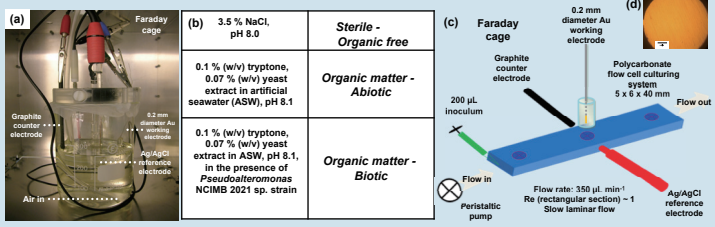

Figure 2. Experimental set-up in laboratory temperature controlled conditions at 18 ± 1 °C: **(a)** Electrochemical cell under controlled static conditions, **(b)** bio-chemical characteristics of the media, **(c)** electrochemical sensor embedded in a flow cell device under controlled hydrodynamic conditions, and **(d)** optical microscopy image x500 showing 0.2 mm diameter Au microelectrode after final surface polishing using 0.3 mm alumina (Al_2O_3) powder.

EIS measurements in continuously aerated media at 18 ± 1 °C laboratory controlled temperature over 72 h were made using a Gamry Instruments potentiostat (PC4-750) and EIS300 software at the open circuit potential (OCP), with an applied sinusoidal potential of 10 mV_{rms} and a frequency range of 0.1 to 100,000 Hz. ASW was used containing dissolved salts and metal-ions, vitamins and nutrients [**4**]. In addition, tryptone and yeast extract were added to enhance the organic carbon levels relevant to open seawater at pH 8.1 resulting in a similar test condition to [**5**].

RESULTS AND DISCUSSION

The EIS data are presented in the form of Bode |*Z*| (|impedance| *vs.* frequency in semi-log coordinates) plots to assess the extent of the oxygen reduction reaction (ORR) kinetics enhanced by enzymatic processes, see **Figure 1(b)*(ii)***. In addition, the physico-chemical properties of the media were measured before (data not shown) and after each test to report any potential changes with regard to typical values in-service conditions. Optical microscopy images x200 and x500, respectively of the Au microelectrode after testing *vs.* the control surface in **Figure 2(d)** were used to corroborate the EIS response, see **Figure 3**.

- **Figures 3(a)** and **(b)** show that the interfacial impedance was uniform with exposure time, with a capacitive response between 10 to 100,000 Hz and diffusion / resistive response between 0.1 to 10 Hz. The physico -chemical properties of the media were similar to summer surface seawater from the North Atlantic Ocean equilibrated with atmospheric pressure [**6**].

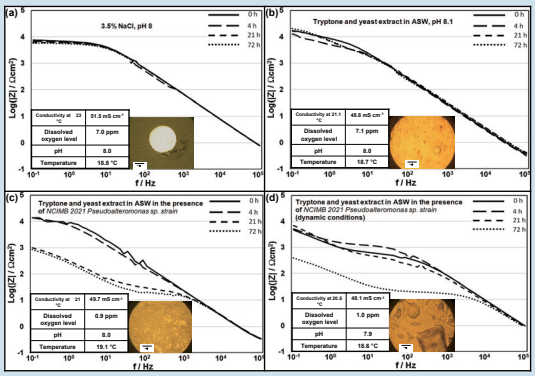

Figure 3. Physico-chemical properties of the media, optical microscopy image x200 and x500, respectively after test and Bode (|Z| *vs. f*) plots at OCP over a 72 h immersion of a 0.2 mm diameter Au microelectrode in continuously aerated **(a)** 3.5 % NaCl solution, pH 8.0, **(b)** 0.1 % (w/v) tryptone, 0.07 % (w/v) yeast extract in ASW, pH 8.1, **(c)** in the presence of *Pseudoalteromonas* NCIMB 2021 sp. strain , under controlled static conditions and **(d)** under controlled hydrodynamic conditions.

- **Figure 3(b)** shows that the presence of a conditioning film (*i.e.* the adsorbed organic material on the Au surface) did not affect the overall impedance response with exposure time.
- **Figure 3(c)** shows a more complex interfacial impedance response with time. It was initially similar to **Figure 3(b)** during the first 4 hours, associated with the conditioning film formation and the adhesion of the first pioneering bacteria [**7**]. Whereas, **Figure 3(d)** reveals enhanced diffusion at the low frequency part of the spectra, 0.1 to 10 Hz, related to enhanced mass transport in the hydrodynamic conditions which affected the ORR kinetics over the first 21 h.
- **Figures 3(c)** and **(d)** show a significant (10-fold) change in impedance (the charge transfer resistance) after prolonged exposure associated with an enhancement of the ORR kinetics due to enzymatic processes. The presence of patchy surface features on the Au microelectrodes was consistent with bacterial biofilms. The dissolved oxygen (DO) levels decreased with time from ~7 to 1 ppm and the turbidity of the media increased (not shown) over 72 h. The increase of turbidity can be explained by the formation of bacterial metabolism by -products present in the test media [**6**]. The decrease of DO levels can be associated with the inability of the DO probe to function properly (*i.e.* membrane hindered by adsorbed organic material, thus the DO depleted was not constantly replenished).

CONCLUSIONS

- The EIS response in continuously aerated NaCl (sterile), ASW (abiotic), ASW in the presence of *Pseudoalteromonas* NCIMB 2021 sp. strain (biotic) has been studied over a duration of 72 hours under both static and controlled hydrodynamic conditions.
- NaCl solution and ASW gave similar EIS behaviours – predominately a capacitive response between 10 to 100,000 Hz and diffusion / resistive response between 0.1 to 10 Hz. The EIS response was relatively uniform with time.
- For ASW in the presence of *Pseudoalteromonas* NCIMB 2021 sp. strain, the EIS response was more complex, with a significant change by 10-fold in impedance with time, associated with an enhancement of the ORR kinetics by enzymatic processes within the bacterial biofilms.
- The charge transfer resistance has the potential to be used to gauge the extent of bacterial biofilm formation.
- Overall, using ASW in the presence of *Pseudoalteromonas* NCIMB 2021 sp. strain, an electrochemical sensor is capable of monitoring for the initial biofilm development over a duration of 72 hours under both static and controlled hydrodynamic (laminar flow) conditions.

FURTHER WORK

- Enhance experimental controllability by using a defined carbon source in ASW, *e.g.*, glucose / acetate.
- Study the effect of bacterial growth phases (exponential, stationary and death) on the EIS response in aerated ASW in the presence of *Pseudoalteromonas* NCIMB 2021 sp. strain.
- Study the effect of solution turbidity on the EIS response.
- Monitor the initial bacterial biofilm development under turbulent flow conditions.

ACKNOWLEDGEMENTS

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