

Electrochemical Investigation of Aerobic Biocathodes at Different Poised Potentials: Evidence for Mediated Extracellular Electron Transfer

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Microbial Fuel Cells (MFCs) are a promising new technology for the conversion of organic wastes to electrical energy. They work by oxidising organics at the anode using bacteria, and combining this with the Oxygen Reduction Reaction (ORR) at the cathode. Pt and other chemical catalysts have been used at the cathode in MFCs. However, their high cost and issues with long term stability could limit their application in MFCs. To have a more sustainable MFC system, one idea is to use aerobic bacteria to catalyse the ORR at the cathode, which would make the MFC technology cheaper and more robust. Carbon electrodes modified with these bacteria lower the overpotential required for ORR, but little is understood about the organisms that constitute these biofilms and their mechanisms of electron transfer. In the current work, mixed communities of biofilms catalysing the ORR were grown in electrochemical half-cells poised at potentials of +200mV and -100mV vs Ag/AgCl and the electrochemical behaviour of these biofilms was studied using Cyclic Voltammetry (CV). These investigations suggest a shift in the mechanism of Extracellular Electron Transfer (EET) as the potential changes.

1. Introduction

Microbial Fuel Cells (MFCs) are devices that use bacteria for the conversion of organic waste to electrical energy. In an MFC, bacteria donate electrons to an insoluble anode, which travel through an external circuit to a cathode electrode where they combine with oxygen and protons to form water. The circuit is completed with the passage of protons from the anode to the cathode, and electrical work is possible by electrons passing through the external circuit. MFCs are being developed for energy recovery from organic wastes, and could potentially be used for domestic wastewater treatment. Around 1.5% of the total energy used in the US is used for wastewater treatment (Logan, 2009). Given that as much as 9.3 times the energy that exists in the wastewater is used to treat it, technologies for energy recovery, such as MFCs, are vital for future water sustainability (Logan, 2009)

The Oxygen Reduction Reaction (ORR) has slow kinetics on the surface of graphite/carbon cathode electrodes, and so expensive, unsustainable precious metal catalysts such as Pt are required to catalyse the reaction (Yu et al., 2007). Aerobic biocathodes are a cheap, self-regenerating catalyst, so much research in recent years has focused on their development for MFCs (He and Angenent, 2006). An aerobic biocathode is a biofilm of a mixed community containing autotrophic, electro-trophic bacteria which use the electrode as part of their energy metabolism. They directly accept electrons from poised potential electrodes and use these to reduce O₂ as part of an electron transport chain in order to generate ATP (Ter Heijne et al., 2010). However, very little is known about the dominant electro-trophic organisms in these biofilms and their mechanisms of electron transfer, although extracellular electron transfer is thought to occur by two different mechanisms; Direct Electron Transfer (DET) via membrane-bound structures such as cytochromes, or by Mediated Electron Transfer (MET) via electron mediators (Rosenbaum et al., 2011).

The mechanisms established for anode biofilms vary between species. For example, Fe-reducing bacteria such as *Shewanella oneidensis* MR-1 are known to produce electron shuttles (Marsili et al., 2008), whereas Fe-reducing *Geobacteraceae* do not appear to produce electron shuttles and are thought to require direct contact with the electrode surface (Bond and Lovley, 2003). Recently, it has been suggested that *Shewanella oneidensis* MR-1 switches from DET to MET depending on the potential of the electrode (Roy et al., 2014).

Trying to determine which mechanisms of electron transfer aerobic electrorophic bacteria use at the surface of electrodes is key to understanding aerobic biocathodes in general and to the development of the MFC technology as a whole. In the current study therefore, aerobic biocathodes were grown on potentiostatically-poised carbon electrodes at two different potentials; -100mV and +200mV vs Ag/AgCl. This was done in order to understand the electrochemical behaviour of the biocathodes and establish information regarding the mechanism of electron transfer at the electrode surface. The resulting aerobic biocathode biofilms were investigated by Cyclic Voltammetry (CV) which is a powerful method for gaining mechanistic information about electrochemical processes occurring at electrodes.

2. Methodology

2.1 Cell setup, Inoculation and Operation

3-electrode half-cells for enrichment and study of aerobic biocathode biofilms were assembled. They each contained a Working Electrode (WE), Counter Electrode (CE) and Reference Electrode (RE). The WE was constructed by attaching a piece of acetone washed carbon felt (2.5 x 5 x 0.5 cm) to a graphite plate current collector using a PTFE holder with PTFE screw bolts. Only one side of the CF was exposed to electrolyte, and the other pressed up against the graphite plate current collector. The CE was piece of coarse platinized mesh of 20 cm² geometric area and used a Ti wire current collector. The RE assembly was made by slotting a glass Ag/AgCl RE purchased from Pine Research Instrumentation through a rubber bung and sealing this inside a conical polypropylene tube filled with a 3M NaCl agar gel. Unless otherwise stated, all potentials stated in this paper were recorded against the Ag/AgCl reference electrode. The half-cell chamber body was made from polypropylene with various ports for the WE, CE and RE. The chamber also had a sampling port and gas inlet and outlet ports. The reactor volume was 1L and the WE and CE electrode spacing was approximately 2cm.

At the beginning of the experiment, the half-cell chamber was filled with 1L of a trace nutrient medium adapted from Ter Heijne et al. (Ter Heijne et al., 2010) for the growth of aerobic biocathode biofilms and containing the following; 50mM pH 5.8 phosphate buffer, 10ml/L of a macro nutrients solution, 1ml/L of a micro nutrients solution and 1ml/L of a vitamin solution. This trace medium was inoculated using 10% by V of activated sludge inoculum obtained from Tudhoe wastewater treatment plant which is located in the North East of the UK. The macronutrients solution contained 28g/L NH₄Cl, 10g/L MgSO₄·7H₂O, and 0.57g/L CaCl₂·2H₂O. The micro nutrients solution contained 2g/L FeCl₂·4H₂O, 1g/L CoCl₂·6H₂O, 0.5g/L MnCl₂·4H₂O, 0.05g/L ZnCl₂, 0.05g/L H₃BO₃, 0.04g/L CuCl₂·2H₂O, 0.07g/L (NH₄)₆Mo₇O₂₄·4H₂O, 1g/L NiCl₂·6H₂O, 0.16 g/L Na₂SeO₃·5H₂O and 2ml/L 37% HCl. The vitamin solution contained 1g/L pyridoxine.HCl, 0.5g/L nicotinic acid, 0.25g/L riboflavin, 0.25g/L thiamine.HCl, 0.2g/L biotin, 0.2g/L folic acid and 0.01g/L vitamin B12. The cell was then hooked up to a Sycopel potentiostat and the WE polarised at -100mV vs. Ag/AgCl. The current was then measured every day. Through the gas inlet, a gas sparger was inserted to provide continuous aeration from an aquarium pump. The medium was changed for fresh biocathode growth medium every 2-3 weeks. When the medium was changed in the cell, the RE drift, pH and OCP were also measured.

2.2 Cyclic Voltammetry (CV)

The half-cell WEs were characterised by Cyclic Voltammetry (CV) at the beginning of the experiment in the non-inoculated medium. CV was performed on an AUTOLAB PGSTAT302 potentiostat by scanning the electrode from +500mV to -200mV at a rate of 5mV/s in quiescent non-stirred solution and 4 scans were taken for each CV experiment, with the 4th scan taken as the stabilized CV response. At 83 days, the carbon felt electrodes were again assessed by CV in exactly the same way. After the biocathode biofilm was enriched in the electrochemical half-cell, a third identical half-cell was setup and inoculated using effluent from the first. When this cell developed an aerobic biocathode, it was assessed by CV in the same way as before. This cell was then sparging with N₂ for 1hr whilst the WE was polarised at -100mV in order to remove all dissolved O₂ from the electrolyte. After removing dissolved O₂, an N₂ blanket was maintained on the surface of the electrolyte, and a series of CV scans were carried out at increasing scan rates from

1mV/s to 100mV/s over a range of +500mV to -200mV. At each scan rate, two scans were taken and the second scan was taken as the stabilised response. For the CV at different scan rates in the absence of O_2 , a reduction peak and oxidation peak were observed. For the reduction peak, the scan rate and square root of the scan rate were plotted separately against the peak height. The peak heights were corrected for capacitance by subtracting a linear baseline extrapolated from a capacitive region of the curve.

3. Results and Discussion

The half-cell started exhibiting increased reduction current above the background ORR current from carbon after 50 days of operation, as observed in the CA graph (Figure 1). This increase in performance was attributed to the formation of a biocathode biofilm containing electrothrophic aerobic bacteria which are able to use the electrode to gain metabolic energy and act as catalysts for the ORR. The electrochemical performance of the cell was assessed by CV at the beginning and at 83 days of operation (Figure 1). In comparison, the electrode performance increased considerably above the non-inoculated cell containing just the carbon electrode in the process electrolyte. The Open Circuit Potential (OCP) of the electrode at 83 days after inoculation was +460mV and the onset potential for the ORR occurred at values which were slightly less than this. In comparison, the non-inoculated cell at the beginning of the experiment had an OCP of +100mV and an onset potential for ORR of -100mV. Another key feature of the CV was the presence of a double wave in the reduction current as the potential was swept from +500mV to -200mV, which was accompanied by a peak on the reverse scan.

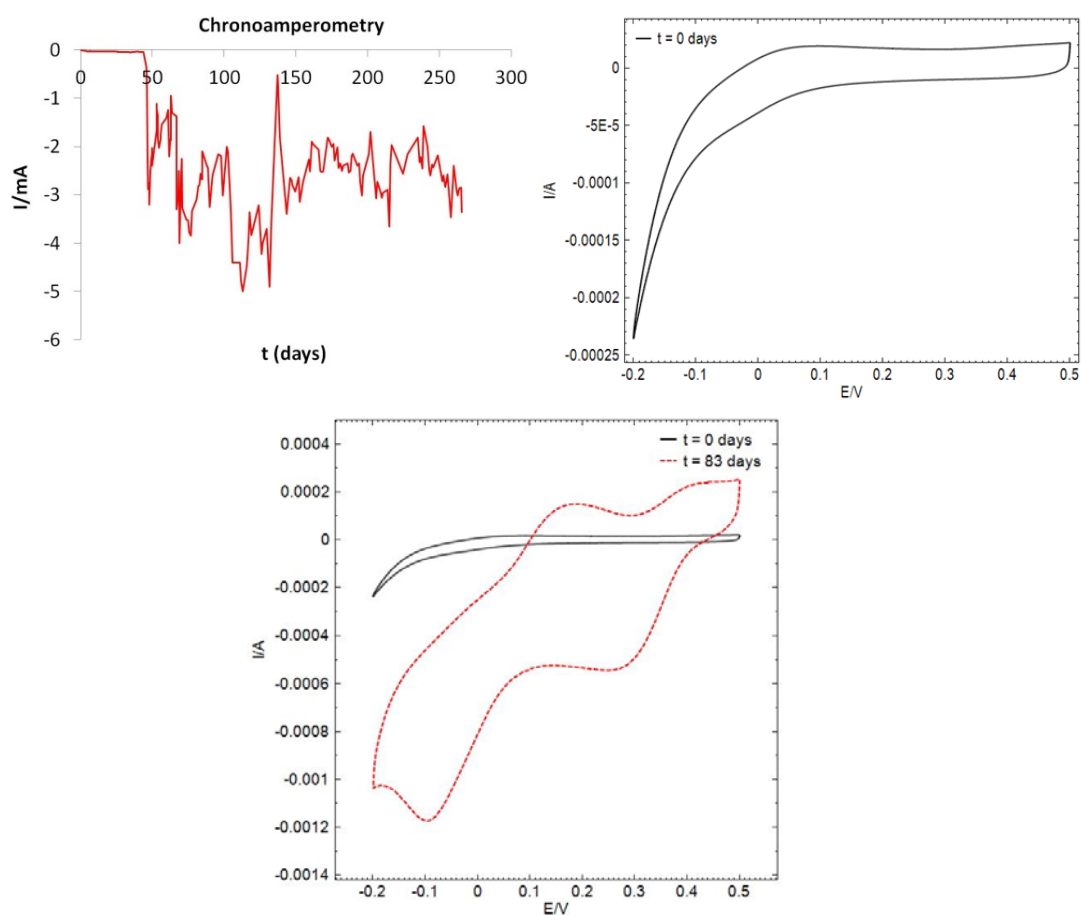


Figure 1: CA graph showing growth of an electrothrophic aerobic biocathode biofilm on a carbon felt electrode polarised at -0.1V vs Ag/AgCl (Top Left). CV of the blank carbon felt electrode at $t = 0$ days (Top Right). CV at $t = 0$ days and $t = 83$ days for this carbon felt electrode, showing biocathode formation (Bottom).

In order to confirm this result, a second cell using effluent from the first was enriched for a biocathode biofilm. This cell took 5 days to enrich and was assessed by CV during operation at 41, 50, 103 and 104 days after inoculation. This cell exhibited the same CA profile, CV and OCP as the biofilms initially inoculated from activated sludge, and the CV for this biofilm at 103 days after inoculation is presented in Figure 2. O_2 was removed from this cell by sparging with N_2 and polarising the electrode at -100mV for 1 hour, and the CV was recorded again. When this was done, the first wave corresponding to ORR by the biocathode was removed and the second reduction peak and the oxidation peak remained. This reduction and oxidation peak had the same area in the CV, thus showing that they were part of the same redox couple. Further to this, the mid-point potential for this redox couple was estimated at $+100\text{mV}$ by taking the average of the two peak positions.

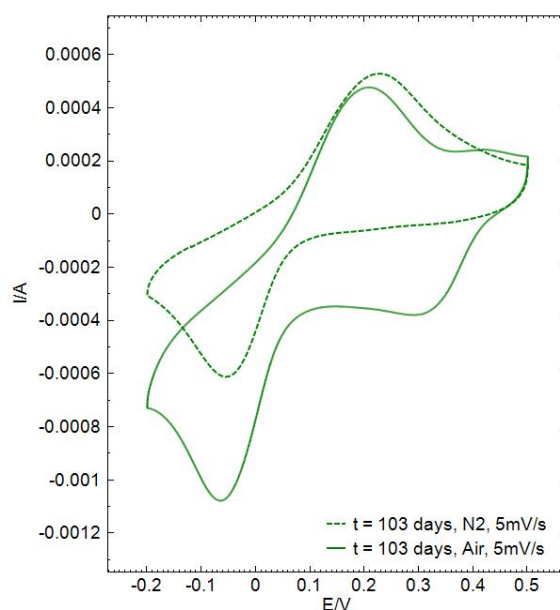


Figure 2: CV of a biocathode biofilm taken at 103 days after inoculation in aerated solution (solid line) and in non-aerated solution (dashed line)

In the absence of O_2 , the WE was cycled at increasing scan rates from 1mV/s to 100mV/s , and the peak height plotted against the scan rate and the square root of the scan rate (Figure 3). Linearity in the graph when the peak height is plotted against the square root of the scan rate indicates semi-infinite diffusion, whereas linearity when the peak height is plotted against the scan rate indicates that the redox species are adsorbed on the surface of the electrode (Léger and Bertrand, 2008). In the case of this cell, the same result was demonstrated on 4 separate occasions; the peak height was linear with the square root of the scan rate, indicating semi-infinite diffusion. This suggests that in the case of the electrode polarised at -100mV , there is an electroactive redox species present in the biofilm which is both diffusible and reversible, suggesting the presence of an electron mediator which can enable the biofilm to accept electrons indirectly from the electrode. This redox active diffusible species could not be detected separately in the CV of abiotic carbon electrodes in the electrolyte from the half-cell containing the biocathode electrode, suggesting that the diffusible redox active species was confined within the biofilm matrix.

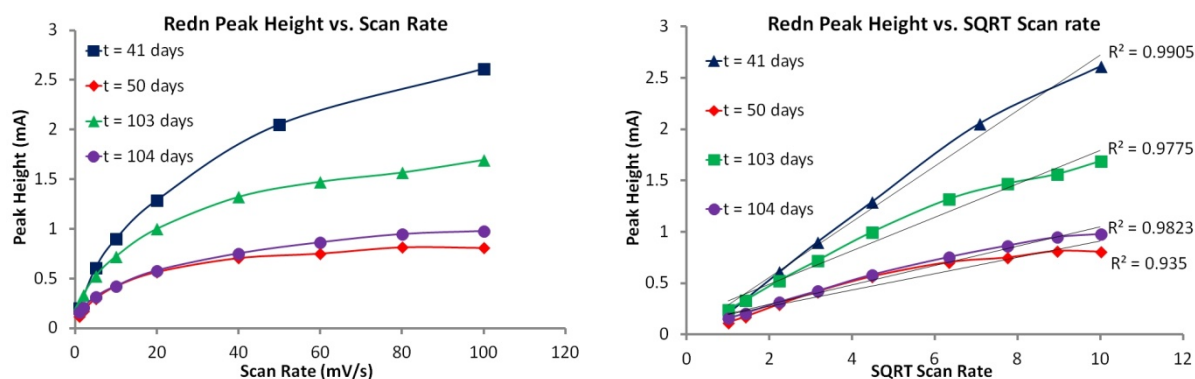


Figure 3: Peak height for the reduction peak observed in the CV for the aerobic biocathode grown at -100mV in the absence of air vs scan rate (left) and vs the square root of the scan rate (right) at scan rates increasing from 1 to 100mV/s . The CV were recorded and analysed at $t = 41, 50, 103$ and 104 days after inoculation

In order to provide further evidence for this hypothesis, an identical cell was inoculated from the original half-cell, but this cell was polarised at $+200\text{mV}$, a potential where the biofilm would not be able to use a mediator with a mid-point potential of $+100\text{mV}$. This third cell developed an aerobic biocathode in exactly the same way as the previous two cells, but analysis of the electrode by CV at 26 days after inoculation in air and in the absence of air after N_2 sparging shows that the resulting aerobic biocathode exhibits none of the redox features previously observed (Figure 4). In common with the electrodes polarised at -100mV , the electrode polarised at $+200\text{mV}$ also exhibited a large positive OCP of $+460\text{mV}$ and an onset potential for ORR occurring at values slightly less than this.

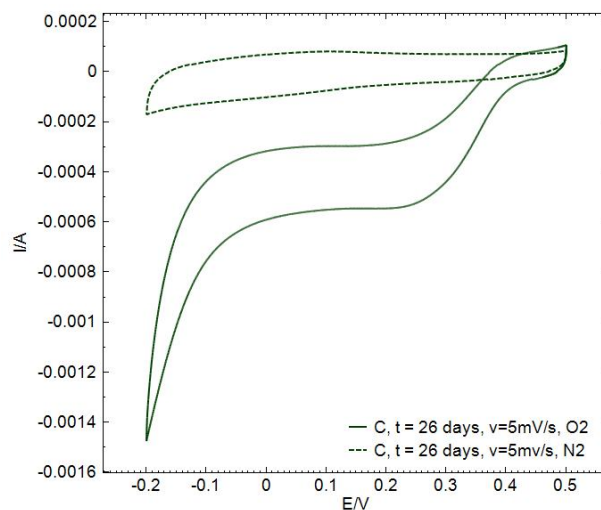


Figure 4: CV in air (solid line) and in the absence of air (dashed line) for a biocathode grown at a potential of $+200\text{mV}$.

4. Conclusions

In terms of the energy available for the electrotrophic bacteria within the biocathode biofilm, this is greater at the lower potential of -100mV , as compared to an electrode polarised at $+200\text{mV}$, as the potential of O_2 reduction as the terminal electron acceptor for the bacteria at pH 5.8 is 670mV . At -100mV , there is evidence for a soluble redox species present in the vicinity of the electrode surface, whereas at $+200\text{mV}$, there are no additional redox features observed in the CV. All the biofilms in this study set an OCP of $+460\text{mV}$ and the onset potential for ORR occurred at values slightly less than this. The presence of a

diffusible species at lower electrode potentials suggests a shift in mechanism to mediated electron transfer by the biofilm. The reasons for this could be that there is a competitive advantage for the bacteria in switching from DET to MET when the electrode potential is far away from the potential of the redox enzyme used by the bacteria. For example, the kinetics of mediated enzyme electrocatalysts are influenced by the electron transfer driving force according to;

$$\Delta E_T = E_{enz}^0 - E_m^0 \quad (1)$$

Where E_{enz}^0 is the formal redox potential of the enzyme and E_m^0 is the formal redox potential of the mediator (Chakraborty and Barton, 2011). As the redox potential of the mediator is shifted further from the redox potential of the active site, the rate of enzyme-mediator electron transfer rate increases exponentially due to the increased driving force (Kavanagh and Leech, 2013). This might give the aerobic biocathode microbes an effective means of utilising the additional energy available from the electrode by utilising a mediator with a mid-point potential which is close to the electrode poised potential. More effective use of the energy available from the electrode confers a survival advantage in these bacteria over other species present in the biofilm.

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