Milner EM, Scott K, Head IM, Curtis T, Yu EH.
Evaluation of porous carbon felt as an aerobic biocathode support in terms of hydrogen peroxide.
Journal of Power Sources (2017)
DOI: https://doi.org/10.1016/j.jpowsour.2017.03.079

Copyright:
© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)

DOI link to article:
https://doi.org/10.1016/j.jpowsour.2017.03.079

Date deposited:
10/05/2017

This work is licensed under a Creative Commons Attribution 4.0 International License
Evaluation of porous carbon felt as an aerobic biocathode support in terms of hydrogen peroxide

Edward M. Milner, Keith Scott, Ian M. Head, Tom Curtis, Eileen Hao Yu

School of Chemical Engineering and Advanced Materials, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom

School of Civil Engineering and Geosciences, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom

**highlights**

- Novel 4-electrode cell developed for assessment of H$_2$O$_2$ from carbon electrodes.
- H$_2$O$_2$ production from carbon electrode inhibits aerobic biocathode biofilm formation.
- To minimize H$_2$O$_2$ formation, poised-potentials $> 0.2$ vs. Ag/AgCl are desired.

**abstract**

Aerobic biocathodes provide a low-cost and sustainable substitute for expensive precious metal catalysts at the cathode of Microbial Fuel Cells (MFCs). However, the abiotic formation of peroxide, which is catalyzed by the porous carbon support at certain cathode potentials, may be detrimental to their activity. Two different carbon felt supports, one treated with nitric acid, the other untreated, were characterized electrochemically through a series of chronoamperometry (CA) experiments using a novel 4-electrode electrochemical setup, in order to determine the potential at which peroxide is initially formed. Peroxide was detected at a potential of $0.2$ V (all potentials are against Ag/AgCl) for the untreated carbon felt electrode and at a potential of $0.05$ V for the nitric acid treated carbon felt. Given these results, two half-cells poised at $0.2$ and $0.1$ V were set up in order to study biocathode formation. The half-cell poised at $0.2$ V did not develop an aerobic biocathode, whereas the half-cell poised at $0.1$ V developed an aerobic biocathode. This study shows that to develop aerobic biocathodes on carbon felt, cathode electrode potentials more positive than $0.2$ V must be applied.

© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

**1. Introduction**

Microbial Fuel Cells (MFCs) are a promising technology that could be used for the production of electricity from wastewater [1–8]. In MFCs, organic substrates present in wastewater, are oxidized by a biofilm of bacteria which deliver electrons to an anode [1]. These electrons then move through an external circuit and go onto reduce oxygen at a cathode. The net result is the production of electrical power from the cell. Typically, the oxygen reduction reaction (ORR) at the cathode is catalyzed by precious metal catalysts such as Pt. Cheap and sustainable materials are required to make wastewater microbial fuel cells economically viable. Alternatives to precious metal catalysts used for the ORR at the cathode are therefore needed to lower the cost of MFCs. The chemical catalysts currently used for the ORR in MFCs, such as Pt and metal macrocycles [9–11], are expensive and unsustainable. Aerobic biocathode biofilms which catalyze the ORR are one alternative, being both cheap and sustainable [12], and have studied extensively in the literature [13–23].

However, the effect of reactive oxygen species produced from the carbon support from the reduction of abiotic reduction of oxygen at carbon on the development of aerobic biocathodes is something which is often neglected. Cultivation of aerobic biocathodes on carbon materials can potentially be complicated by the abiotic formation of H$_2$O$_2$ on carbon, H$_2$O$_2$ kills bacteria through the generation of reactive oxygen species, such as superoxide, which damage the bacterial cells [24]. Therefore, a means of determining the potential at which H$_2$O$_2$ is first produced on porous carbon electrodes would be advantageous in understanding how H$_2$O$_2$ forms.

* Corresponding author.
E-mail address: eileen.yu@ncl.ac.uk (E.H. Yu).

http://dx.doi.org/10.1016/j.jpowsour.2017.03.079
0378-7753/© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
production affects the growth of aerobic biocathode biofilms, and for determining a potential window over which an aerobic biocathode can be safely cultivated in poised-potential half-cells on carbon, without the possibility of oxidative stress. This information is summarized in Fig. 1. Fig. 1 shows that any cathode potential more negative than the reduction potential for the terminal electron acceptor (O₂) for the bacteria (blue line in Fig. 1), but more positive than the onset potential for peroxide formation from the abiotic ORR on carbon felt (red line in Fig. 1), is suitable for the development of aerobic biocathodes (green line in Fig. 1).

Electrochemical oxygen reduction can occur to either water or peroxide on different carbon materials [25], as described by the following equations [25]:

\[
\text{O}_2 + 4e^- + 4H^+ = H_2O \quad E^0 = 0.60V \text{ vs. } \text{Ag/AgCl (pO}_2 = 0.2, \text{ pH} = 7)
\]  

(1)

\[
\text{O}_2 + 2e^- + 2H^+ = H_2O_2 \quad E^0 = 0.13V \text{ vs. } \text{Ag/AgCl (pO}_2 = 0.2, \text{ pH} = 7, [H}_2O_2] = 5mM)
\]  

(2)

Further reduction of H₂O₂ to OH⁻ or H₂O is also possible, dependent on several factors, such as the applied potential and the pH [25]. The degree to which the ORR occurs by 4 e⁻ or 2 e⁻ reduction is given by the electron number, \(n_e\), which is measured using a rotating ring disk electrode (RRDE) [25]. Given the data from an RRDE experiment, \(n_e\) can be calculated [25]:

\[
\frac{4I_d}{I_d + \frac{N}{2}} = n_e
\]

where \(I_d\) is the disk current, \(I_k\) is the ring current, and \(N\) is the collection efficiency. The ORR occurs via 4 e⁻ and 2 e⁻ pathways on carbon, leading to \(n_e\) values that lie between 2 and 4 [25]. \(n_e\) values closer to 4 imply that water is the primary product, whilst \(n_e\) values closer to 2 imply that peroxide is the primary product. The 4 e⁻ and 2 e⁻ ORR pathways are electrode potential-dependent, and therefore \(n_e\) is also electrode potential-dependent for any given carbon electrode. Additionally, a higher ratio of nitrogen- to oxygen-containing surface functional groups on the carbon electrode surface increases \(n_e\) [26], which is believed to be due to a difference between the orientation of O₂ adsorption at nitrogen and oxygen catalytic sites on the carbon surface [27]. Nitrogen surface functional groups can be introduced through ammonia heat treatment under N₂ gas [26], whilst both oxygen and nitrogen functional groups can be introduced by heating with concentrated HNO₃ acid [28]. Nitric acid treatment has been shown to increase the ORR catalytic activity of carbon materials [29,30], which could be due to an increase in active surface area [30] and/or and in increase in surface oxygen/nitrogen functional groups [30]. \(n_e\) is measured using a rotating ring disk electrode, but this type of experiment is only possible for carbon powders or carbon supported catalysts which can be immobilized as inks onto the supporting glassy carbon rotating disk electrode of the RRDE [25]. This presents a problem for calculating \(n_e\) for porous carbon materials, such as carbon felt [31], carbon brush [32], or a three dimensional carbon material [33–36], which cannot be easily immobilized. Porous carbon materials are cheap, readily-available, conductive, and support a large biofilm [1], and therefore they increase power densities and lower the costs of MFCs. The use of porous carbon materials for MFCs is desirable, but they require assessment for peroxide production which is detrimental to biofilm growth. One way to carry out this assessment is to determine the maximum potential at which peroxide can be detected using a system designed for porous carbon materials. This information gives a minimum potential at which it is possible to cultivate aerobic biocathodes on porous carbon materials in half-cells without also producing peroxide.

In the current study, a novel 4-electrode system was developed to detect H₂O₂ produced from porous carbon felt. In this system, the primary working electrode (PWE), made from carbon felt, was polarized in the presence of a Pt secondary sensing electrode (SSE), which was itself polarized at a potential at which H₂O₂ is oxidized. This allowed for the determination of the potential at which peroxide is first produced in quantities sufficient so to detect an oxidation current at the SSE. A detectable current at the SSE indicates the presence of peroxide in solution, which is inhibitory to microbial growth and development [24]. In order to test the 4-electrode experimental method, two different carbon felts were compared. Untreated carbon felt was compared with the same carbon felt treated with nitric acid. Nitric acid treatment of the carbon is predicted to increase the potential at which peroxide is detected by increasing either the surface area and/or the number of surface oxygen functional groups of the carbon material which catalyze 2e⁻ ORR. To test whether the formation of peroxide had an effect on biofilm growth, cultivation of aerobic biocathode biofilms on untreated carbon felt was conducted at two different poised potentials. One potential at which peroxide is not produced from the carbon support, and the second at a potential at which peroxide is produced.

2. Experimental

2.1. Experimental setup

A novel 4-electrode system was designed (Fig. 2A) and was used to determine the minimum applied potential, \(E_{\text{min}}\) for formation of
peroxide on carbon felt and HNO3 treated carbon felt. This system is analogous to an RRDE (See supplementary material for details on RRDE), but is designed for porous carbon materials. The primary working electrode (PWE) was porous carbon felt and a secondary sensing electrode (SSE) was made from Pt. The PWE (porous carbon felt) was polarized at a potential at which oxygen reduction occurs, whilst the SSE was simultaneously polarized in the same chamber at a potential at which H2O2 is oxidized, therefore detecting H2O2 produced by the PWE. All of the carbon felt electrodes used in the abiotic studies had an area of 60 × 20 mm. The carbon felt was purchased from VWR (Cat. No. 43200.RR, Alfa Aesar, UK), and had a strand density of 3.65 g/cm³ (20 °C) and a thickness of 0.5 cm. All carbon felt electrodes were washed in acetone prior to use to remove oil residues left over from manufacture, and left to air dry for 12 h. The SSE used to detect H2O2 in all of the studies was platinitized titanium mesh with a geometric area of 2 cm², and the counter electrode was a larger piece of platinitized titanium mesh (35 cm² geometric area).

HNO3 treatment of the carbon felt was carried out using a method based on that used by Wang et al. and Scott et al. for the modification of carbon black supports [37,38]. HNO3 treatment of the carbon felt was carried out by placing the carbon felt electrode in a round-bottomed flask attached to a reflux condenser with 400 ml of concentrated HNO3 (69% by weight, AnalR NORMAPUR® analytical reagent). The solution was refluxed for 2 h at 122 °C until the colour of the gas in the reflux condenser went from dark brown to light brown. The contents of the flask were cooled to room temperature before removing the HNO3 and the carbon felt. This carbon felt electrode was washed repeatedly with deionized water until the washings reached pH 6. The carbon felt electrode was then air dried for approximately 12 h before use.

The cell used to test the materials was constructed from two polypropylene centrifuge tubes, one forming the chamber for the two working electrodes, and the other centrifuge tube used for the counter electrode chamber (Fig. 2A). Each centrifuge tube was modified with a polypropylene flange, and glued into place using hot melt glue from a glue gun. The flanges allowed fixture of an anion exchange membrane (Fumasep FAD, Fumatech, Germany) of 1 cm² geometric area between the two tubes (Fig. 2A), allowing the passage of anions between the two chambers, but not peroxide. One of the tubes contained the PWE and SSE, as well as an Ag/AgCl reference electrode (RRPEAGCL2 miniature Ag/AgCl reference electrode, Pine Research Instrumentation, US). All potentials in the study were recorded against the Ag/AgCl reference electrode, which has a potential of 0.208 V vs. the standard hydrogen electrode. Both working electrodes were maintained in a fixed position relative to each other, and both were connected to titanium wire. Likewise, the platinum mesh counter electrode was fixed into position in the other chamber in the same way. The experiments were performed in 50 mM phosphate buffer (pH 7.0), and the buffer was stirred using a magnetic stirrer in order to improve the detection of H2O2 at the SSE.

2.2. Electrochemical characterization

All electrochemical experiments were carried out using a potentiostat (Autolab PGSTAT302, Metrohm, UK) fitted with a bipotentiostat module. In chronoamperometry (CA) experiments, the SSE was polarized for 15 min at +0.6 V to get a stable background current, before simultaneous polarization of both the PWE and SSE for 1 h. When both electrodes were polarized simultaneously, the SSE was maintained at +0.6 V. The potential of the PWE was also kept constant but the applied potential used was different for different experiments. The SSE potential was fixed at +0.6 V as at this potential the rate of peroxide oxidation on platinum electrodes is limited by mass transfer to the electrode rather than the kinetics of oxidation [39]. Experiments were performed at different PWE potentials; −1, −0.5, −0.1, −0.05, 0, +0.05 V for HNO3-treated carbon felt and −1, −0.5, −0.2, −0.1 V for untreated carbon felt. All CA experiments were conducted twice, and raw CA data were smoothed using a Savitky-Golay filter, and the average data were plotted.

2.3. Experimental setup, operation and electrochemical characterization of poised-potential aerobic biocathode half-cells

Two poised-potential aerobic biocathode half-cells (Fig. 2B) were used for the cultivation of aerobic biocathodes biofilms in which the working electrode potential was fixed. In one of the half-cells, the potential was poised at −0.1 V, whilst in the other, the potential was poised at −0.2 V. This is a 3-electrode setup with a working electrode, counter electrode and reference electrode...
(Fig. 2B). The reactor chamber body was cylindrical with a 1 L volume and was made from polypropylene with ports for the working, counter, and reference electrodes, a sampling port and gas inlet/outlet (Fig. 2B). A larger reactor volume of 1 L for the aerobic biocathode cultivation half-cell (Fig. 2B), in comparison to the smaller 50 mL volume used for the peroxide detection half-cell (Fig. 2A), was chosen in order to ensure a larger volume of inoculum, and therefore a better chance of biocathode enrichment. The working electrode and counter electrode faced each other with a 3 cm electrode spacing. The working electrode was a rectangular piece of carbon felt with an area of 12.16 cm² (1.9 x 6.4 cm) and a thickness of 0.5 cm (VWR Cat. No. 43200.RR, Alfa Aesar, UK). The carbon felt was acetone washed beforehand and held in a polytetrafluoroethylene (PTFE) holder, comprising a front and backing plate held together with nylon bolts, exposing only one side of the carbon felt to solution. A graphite plate was used to make contact between the carbon felt and the external circuit. The counter electrode was a two-sided, rectangular piece of Pt mesh with an area of 35 cm² attached to a titanium wire, and the reference electrode was Ag/AgCl (RE-5B, BASi, UK) housed within a polypropylene luggin capillary containing a 3 M NaCl agar salt bridge.

The inoculum used for both half-cells was 1 L of effluent from a single pre-enriched aerobic biocathode half-cell from our previous study [40]. 50% by volume of this pre-enriched effluent (0.5 L) went into each half-cell, and the remaining 50% by volume for each half-cell (0.5 L) was made up using fresh medium, giving a final volume of 1 L (final pH 6.85). The fresh medium was a minimal growth medium containing 50 mM phosphate buffer (pH 7.0) and trace nutrients for the bacteria. These trace nutrients have been described previously [40].

The two cells were connected to a quad potentiostat (Winstonbrook Technologies) with one poised at -0.1 V and the other poised at -0.2 V and the current was measured continuously. The cells were operated under conditions of continuous aeration in the dark at 30 °C. The cells were characterized electrochemically using a potentiostat (AUTOLAB PGSTAT302) potentiostat at t = 0 days and at t = 7 days. The biocathodes were electrochemically characterized by cyclic voltammetry (CV). Four CV scans were recorded at 5 mV/s from -0.2 to +0.5 V, with the last scan taken as the stable CV response. The solution pH and reference electrode drift were measured for both cells at the beginning and end of the experiment to ensure that the change was less than 0.5 pH units and 5 mV respectively.

3. Results and discussion

3.1. Potentials for the formation of peroxide on HNO₃-treated and untreated carbon felts

In the CA experiments, the SSE was polarized at +0.6 V for 15 min, before simultaneous polarization of both the PWE and the SSE, with the PWE polarized at different potentials depending on the experiment. The CA curves for the SSE and PWE for HNO₃-treated and untreated carbon felt are given in Fig. 3. Initial polarization of the SSE produced a rapidly decaying capacitive current, tending towards a low background oxidation current less than 1 μA/cm². This was the initial SSE oxidation current at t = 0 s (Fig. 3B and D). An increase in this oxidation current was then observed for the SSE when the PWE was also polarized, due to the oxidation of peroxide (Fig. 3B and D). The oxidation current either

![Fig. 3](image-url)
increased then plateaued over the hour-long simultaneous working electrode polarizations, or increased then peaked, before falling away, depending on the potential of the PWE (Fig. 3A and C). Therefore, the SSE acted as a sensor for the detection of peroxide in solution, given off by the PWE.

In the CA profile of the PWE for both materials, the reduction current initially decreases rapidly due to capacitive discharge, then plateaus to a limiting reduction current (Fig. 3A and C). At more negative potential, the observed CA reduction current continues to decrease after the initial capacitive discharge for the entire experimental period. The H₂O₂ product is produced in solution, but its decomposition is catalyzed by the carbon electrode [41], and spontaneously in solution [42,43]. The kinetics of peroxide decomposition are described by a 1st order rate equation [41–43]:

\[ 2H_2O_2 \rightarrow 2H_2O + O_2 \]  

\[ -\frac{d[H_2O_2]}{dt} = k[H_2O_2] \]  

where \([H_2O_2]\) is the concentration of hydrogen peroxide, \(i\) is the time in seconds, and \(k\) is the 1st order rate constant for the decomposition of hydrogen peroxide. The implications of the 1st order rate equation are that the rate of removal of peroxide increases as the concentration of peroxide increases in solution. In the experiments, the amount of carbon (catalyst) for the decomposition of peroxide is constant, which means that the 1st order rate is also constant for the system. As peroxide is both simultaneously produced and removed in the system, the concentration of peroxide in solution plateaus and reaches a maximum value with time. The SSE is polarized at +0.6 V where the oxidation current due to peroxide oxidation on platinum is limited by mass transport and not electrode kinetics [39]. Therefore, at this potential for the SSE, the current observed at the SSE is proportional to the concentration of peroxide present in solution. The maximum oxidation current at which the SSE eventually plateaued was dependent on the potential applied at the PWE; the more negative the potential, the higher the maximum oxidation current reached on the SSE. This behaviour was observed in all of the SSE CA experiments for both the HNO₃-treated and untreated carbon felts, apart from where the PWE was polarized at −0.5 and −1 V in the case of HNO₃-treated carbon felt, and −1 V for the untreated carbon felt. In these cases, the peak is caused by the falling rate of production of peroxide from the PWE as the experiment proceeds, combined with the decomposition of peroxide in solution.

The potential of the PWE at which the oxidation current on the SSE increases above background levels is the potential at which peroxide is first detected at −0.05 V, whereas for the untreated carbon felt this occurs at −0.2 V. On further examination of the PWE CA data for the two materials (Fig. 3A and C inset), the most negative potential on the PWE which yields no increase in SSE oxidation current is 0 V for the HNO₃-treated carbon felt, and −0.1 V for the untreated carbon felt. This is consistent with the improved catalysis and ORR onset potential on treatment of the carbon felt with HNO₃ treatment. The acid treatment has this effect due to an increase in electrochemically active surface area [30], and/or through an increase in surface oxygen groups [30], which catalyze 2e⁻ ORR. Therefore, HNO₃ surface treatment enhances the abiotic catalysis of the carbon felt, but increases the potential at which peroxide is first formed on the electrode.

Considering that the bacterial terminal electron acceptor is 4 e⁻ ORR with an E° equal to 0.60 V (pH 7.0, pO₂ = 0.2), the useable potential window over which growth of aerobic biocathodes using poised-potentials can be attempted is reduced on treatment of the carbon felt with HNO₃. For HNO₃-treated carbon felt, potentials between 0 and 0.60 V (600 mV potential window) can be applied to the carbon felt, whilst for untreated carbon felt, this potential window is from −0.1 to 0.60 V (700 mV potential window).

3.2. Mathematical model describing the solution peroxide concentration with time

A simple descriptive model of the current observed at the SSE was developed. Key to this model was the assumption that the current observed at the PWE was due entirely to 2e⁻ ORR, although this is not usually the case, as for most carbon materials, the average electron numbers, \(n_e\), are higher than 2.0, and are potential-dependent [26]. For example, Watson et al. obtained \(n_e\)-values from RRDE experiments of approximately 2.3 for glassy carbon, and 2.5 for carbon black, which both varied to some degree with potential [26]. With the assumption that \(n_e = 2\), the current at the PWE is approximated as equal to the rate of peroxide production according to [44]:

\[ \text{Rate} \left( \text{mol s}^{-1} \right) = \frac{dN}{dt} = \frac{i}{nF} \]  

where \(i\) is the current, \(F\) is the Faraday constant, and \(n = 2\) for 2e⁻ ORR. Therefore, the rate of production of peroxide in the system from the PWE at any point in time is known and is given the value \(r\). This value varies with time, and is calculated directly from the PWE current and fed into the model. Additionally, the non-electrochemical decomposition of peroxide to O₂ and water catalyzed at the PWE and occurring in the bulk solution is well-known to follow 1st order kinetics [41–43]. By considering the rate of production at the PWE, \(r\), which is known, and the rate of decomposition of peroxide, it is possible to write an equation for the concentration of peroxide in solution at time \(t\):

\[ \text{Rate of production of peroxide at PWE} = r \]  

\[ \text{Rate of decomposition of peroxide} = k[H_2O_2] \]  

| Carbon felt     | PWE potentials (V vs. Ag/AgCl) | E_{min} for an oxidation current at the SSE (V vs. Ag/AgCl) |
|-----------------|--------------------------------|----------------------------------------------------------|
| Untreated       | −1, −0.5, −0.2, −0.1           | −0.2                                                     |
| HNO₃-treated    | −1, −0.5, −0.1, −0.05, 0, +0.05 | −0.05                                                   |

Table 1: The potential at which peroxide is first detected for HNO₃ and non-HNO₃ treated carbon felts.
Overall rate \( \frac{d[H_2O_2]}{dt} = r - k[H_2O_2] \) \hspace{1cm} (9)

\[ [H_2O_2] = \frac{r}{k} \left( 1 - e^{-kt} \right) \] \hspace{1cm} (10)

where \( r \) is the rate of production of peroxide from the PWE which is known, \( k \) is the 1st order rate constant for the decomposition of peroxide, \( t \) is the time in seconds, and \([H_2O_2]\) is the concentration of peroxide in solution. The derivation of Equation (9) from Equation (8) is included in the supporting material for this article. Across all experiments, \( k \) is constant, but \( r \) is approximated as being proportional to the observed current at the PWE (Equation (6)). Therefore, both \( r \) and \( t \) are variables which are input into Equation (9). In order to predict the peroxide concentration in solution with time, values of \( k \) for HNO3-treated and untreated activated carbons were taken from the literature and were used for the HNO3-treated and untreated carbon felt. These values were 0.007 and 0.019 min\(^{-1}\) respectively [41].

For the two carbon felt materials, modelling of \([H_2O_2]\) in solution with time yields the curves seen in Fig. 4. It is observed that when the PWE is polarized at potentials more negative than \(-0.1\) V, the current takes time to plateau, leading to a falling value of \( r \), and therefore to a peak in the current observed at the SSE for both carbon felt materials. For the simulation, \([H_2O_2]_{\text{max}}\) is given by \( r/k \), which is the point at which the SSE current plateaus. For untreated carbon felt, the modelled solution \([H_2O_2]\) and the SSE current agree reasonably well (Fig. 4A). However, a comparison of the current observed at the SSE for HNO3-treated carbon felt and the modelled \([H_2O_2]\) is constant, but \([H_2O_2]\) is affected by how fast \( H_2O_2 \) is transported from the PWE, and the timescale of this process in relation to \( H_2O_2 \) decomposition. The decomposition of \( H_2O_2 \) molecules before they reach the SSE explains why the signal response at the SSE is lower than at the PWE. Detection for this experiment could be improved by improving \( H_2O_2 \) mass transport through better mixing of the electrolyte.

3.3. Biocathode development with applied poised potential

In order to examine the effect of peroxide production from the carbon felt electrode on biocathode biofilm formation, two half-cells were set-up and both were inoculated with a consortium of aerobic biocathode bacteria from an existing aerobic biocathode half-cell. One of the half-cells was poised at \(-0.1\) V, whilst the other was poised at \(-0.2\) V. Both used medium buffered to pH 7.0, and were kept under conditions of continuous aeration. During this period, the reduction current increased considerably for the electrode poised at \(-0.1\) V in comparison to the electrode poised at \(-0.2\) V (Fig. 5A). This indicates that a biocathode biofilm formed in the cell poised at \(-0.1\) V, whereas no biofilm formed in the cell poised at \(-0.2\) V.

CV showed that cells poised at \(-0.1\) and \(-0.2\) V both exhibited the features typical of abiotic oxygen reduction on an uncatalyzed carbon electrode, with reduction current increasing from right to left as the potential is swept to more negative values (Fig. 5B). However, after 7 days of operation for both half cells, in the cell poised at \(-0.1\) V the CV showed a considerable improvement in catalysis as evidenced by the large increase in reduction current, whereas the cell polarized at \(-0.2\) V showed no change from abiotic oxygen reduction at an uncatalyzed carbon electrode (Fig. 5B). This indicated growth of an aerobic biocathode biofilm on the electrode poised at \(-0.1\) V, but not when the working electrode was poised at \(-0.2\) V. Therefore, the electrochemical data show whether an electroactive biocathode biofilm was able to form or not, because an electroactive biocathode biofilm gives an electrochemical response in CA and CV (Fig. 5).

The CV for the cell poised at \(-0.1\) V possesses a characteristic high onset potential \( E_{\text{onset}} \) for ORR of approximately \(+0.4\) V, which has been observed previously in our recent aerobic biocathode study [40], and in a study by Rothballer et al. [23]. In both of these studies, the bacterial community of the aerobic biocathode was found to be dominated by uncultured Gammaproteobacteria [23,40]. Additionally, the aerobic biocathode community is autotrophic, fixing CO2 from the air into biomass (no organic donor is present in the medium) [40], and the bacteria likely accept electrons directly from the cathode for energy, coupling this with the...
reduction of oxygen \[23,40]. Both half-cells were inoculated using pre-enriched aerobic biocathode inoculum (half-cell effluent) from our previous study \[40\], and this explains why the CV for the half-cell poised at \(-0.1\) V (Fig. 3B) developed similar electrochemical features (\(E_{\text{onset}}\) for ORR of \(+0.4\) V) to those observed for biocathodes from our previous study \[40\]. Beyond electrochemical characterization, microscopy characterization of the biofilm has not been carried out, but would be a useful tool for future studies, and for determining whether non-electroactive biofilms are present or not.

It has been shown previously through microscale measurements of peroxide, pH and \(\text{O}_2\) at the surface of continuously polarized glassy carbon electrodes, that a sustained high pH and surface concentration of peroxide (80 \(\mu\)M) limit biofouling and slow the rate of cathode degradation \[46\]. Low surface concentrations of peroxide have also been reported to inhibit the formation of biofilms on a polarized carbon fabric scaffold cathode \[47\] (\(-25\) \(\mu\)M), and on a polarized conductive carbon nanotube-poly(vinyl alcohol) composite ultrafiltration membrane \[48\] (<10 \(\mu\)M). The peroxide surface concentration reported in these studies lie in the range of the maximum peroxide concentration of \(-20\) \(\mu\)M reported here for untreated carbon felt at a poised-potential of \(-0.2\) V (Fig. 4B), and the potential at which an inhibitory effect on aerobic biocathode formation has been observed (Fig. 5).

In the case of MFCs and microbial electrolysis cells (MECs), the cathode potential is not controlled using a potentiostat, and the cathode potential is influenced by many factors, including the bioanode \[49,50\]. For an MFC with a carbon cathode, the maximum rate of peroxide production occurs when the system is operated over a low external resistance, which can then be increased further by supplying an additional voltage input from a power source (MEC). MFCs and MECs designed for peroxide synthesis using a carbon cathode exploit this to increase bulk catholyte peroxide concentration. For peroxide synthesis MFCs, values of 2.32 mM \[51\], 0.02 mM \[52\] and 5.78 mM \[53\] have been reported in the literature, whilst for peroxide synthesis MECs, significantly higher values of 38 mM \[54\] and 91 mM \[55\] have been achieved. Increasing cathode carbon current density and peroxide production by circumventing bioanode extracellular electron transfer and utilizing the oxidation of microbially-produced \(\text{H}_2\) at a platinized anode has also been demonstrated, giving a catholyte peroxide concentration of 61 mM \[56\].

The catholyte peroxide concentrations reported in MFCs/MECs for peroxide synthesis are in excess of the maximum peroxide concentration determined to be inhibitory in the present study for untreated carbon felt (\(-20\) \(\mu\)M at \(-0.2\) V poised-potential). Peroxide formation may therefore be a problem for aerobic biocathode formation in MFCs, and MECs in particular. In an MFC with a bioanode and biocathode, where the biofilms develop gradually and simultaneously from the point of inoculation, there may be little peroxide production if electrons are taken up directly by the developing biocathode instead of the underlying carbon support. However, this scenario will depend on the viability of the biocathode inoculum. With a biocathode inoculum which is not pre-enriched, bioanode formation may occur before biocathode formation, causing formation of peroxide prior to biocathode formation. Choosing aerobic biocathode material supports which favor oxidation of microbially-produced \(\text{H}_2\) at a platinized anode or which have a high over-potential for ORR, may be important strategies to circumvent this problem. Conversely, the production of peroxide in MFCs/MECs may be exploited to inhibit the formation of unwanted aerobic biofilms, such as in the case of the non-electroactive biofilms on the surface of MFC activated carbon cathodes, which cause long-term cathode performance degradation \[57\].

4. Conclusions

The results demonstrate how the poised potential can affect the development of aerobic biocathodes which is linked to abiotic peroxide production from the carbon support.

In this work, a minimum potential at which peroxide is produced by two different porous carbon felts in sufficient quantity to be detected electrochemically using a novel 4-electrode technique has been determined. This potential was found to be dependent on properties of the carbon felt. This potential is hypothesized to be the minimum safe potential at which the aerobic biocathode community can be grown without the possibility of suffering from the adverse effects of peroxide formation on the carbon electrode support. Given that the potential of the terminal electron acceptor for the aerobic biocathode is \(+0.6\) V (pH 7.0, \(\text{pO}_2\) = 0.2), a potential window over which the bacteria can be grown using a potentiostatically-poised carbon electrode is inferred. For the two carbon felt electrodes, these potential windows are: 0 to +0.60 V for \(\text{HNO}_3\)-treated carbon felt, and \(-0.10\) to +0.60 V for untreated carbon felt. This demonstrates that the potential window available for biocathode growth is dependent on the material properties of the carbon support.

For the untreated carbon felt, it has been shown that it is not possible to enrich for an aerobic biocathode biofilm in a half-cell using an enriched source of aerobic biocathode bacteria as inoculum when the working electrode potential is \(-0.2\) V, but it is possible when the working electrode potential is \(-0.1\) V. Therefore, the cultivation of aerobic biocathodes in half-cells can be carried out at \(-0.1\) V on untreated carbon felt, so as to maximize the energy potentially available to the bacteria. This illustrates the importance...
of understanding abiotic electrochemical reactions which may compromise the ability to develop oxygen reducing biocathodes. Further to this, the 4-electrode method is predicted to be useful for poised-potential studies using other porous carbon materials, and also for bioanode poised-potential half-cell studies.

Acknowledgement

The authors thank EPSRC Supergen Biological Fuel Cells (EP/H019480/1) for funding this project. Data supporting this publication is openly available under an 'Open Data Commons Open Database License'. Additional metadata are available at: http://dx.doi.org/10.17634/091409-3. Please contact Newcastle Research Data Service at rdm@ncl.ac.uk for access instructions.

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpowsour.2017.03.079.

References

[1] B.E. Logan, Microbial Fuel Cells, John Wiley & Sons, Inc., 2008.
[2] B.E. Logan, J.M. Regan, Environ. Sci. Technol. 40 (2006) 5172–5180.
[3] B.E. Logan, Nat. Rev. Microbiol. 7 (2009) 375–381.
[4] D.R. Lovley, Nat. Rev. Microbiol. 4 (2006) 497–508.
[5] A. Janiak, Y. Fan, H. Liu, Biofuels 5 (2014) 79–92.
[6] H. Wang, J.-D. Park, Z.J. Ren, Environ. Sci. Technol. 49 (2015) 3267–3277.
[7] G. Mohanakrishna, S. Srikanth, D. Pant, Bioelectrochemical systems (BES) for microbial electroreduction: an advanced wastewater treatment technology, in: Applied Environmental Biotechnology: Present Scenario and Future Trends, Springer, 2015, pp. 145–167.
[8] D. Pant, A. Singh, G. Van Bogaert, S. Irving Olsen, P. Singh Nigam, L. Diels, N. Rodriguez, R. Amils, V.M. Fernandez, A.L. De Lacey, Biosens. Bioelectron. 26 (2011) 1263–1268.
[9] E.M. Milner et al. / Journal of Power Sources xxx (2017) 1–8

Please cite this article in press as: E.M. Milner, et al., Journal of Power Sources (2017), http://dx.doi.org/10.1016/j.jpowsour.2017.03.079