**EVALUATION OF THE PERFORMANCE OF A MEDIATORLESS
MICROBIAL FUEL CELL BY ELECTROCHEMICAL IMPEDANCE
SPECTROSCOPY**

Eliseo Herrero-Hernandez^{a,1,*}, David Greenfield^a, Thomas J. Smith^b, Robert Akid^c

^a Materials and Engineering Research Institute (MERI), Sheffield Hallam University, City Campus, Howard Street, Sheffield S1 1WB, UK

^b Biomedical Research Centre, Sheffield Hallam University, City Campus, Howard Street, Sheffield S1 1WB, UK

^c School of Materials, University of Manchester, Manchester M13 9PL, UK

¹ Present address: Institute of Natural Resources and Agrobiolgy (IRNASA-CSIC), Department of Procesos de Degradación del Medio Ambiente y su Recuperación, Salamanca 37008, Spain.

* Corresponding author: E-mail address: elihh@usal.es

Fax: +34 923219609.

This is the peer reviewed version of the following article: *Electroanalysis* - Weinheim 31: 1189-1194 (2019), which has been published in final form at <https://doi.org/10.1002/elan.201900120>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

Abstract

A mediatorless microbial fuel cell was developed using *Escherichia coli* bacteria and platinised titanium mesh as electrodes, producing a maximum power density of 627 mW·m⁻². The performance characteristics of the fuel cell were evaluated using both electrochemical and optical techniques. Cyclic Voltammetry showed that an anaerobically grown cell suspension of *E. coli* was electrochemically active, and are consistent with a role for *E. coli*-secreted mediators in the functioning of the cell, after the formation of a biofilm on the surface of the electrode. Electrochemical impedance spectroscopy (EIS) data show a variation in the internal resistance during bacterial growth. EIS analysis based on an equivalent circuit revealed that the initial internal resistance of the cell (5.6 MΩ) initially reduces by around 50 % over an 8 hour period; more or less the same time where the fuel cell reaches its maximum potential of 860 mV, whereupon the resistance begins to increase resulting in the corresponding fall in potential; this trend was reversible upon the introduction of further nutrients into the cell.

Keywords: electrochemical impedance spectroscopy; *Escherichia coli*; mediatorless; microbial fuel cells.

1. Introduction

Microbial fuel cells (MFCs) are bio-electrochemical transducers that use microorganisms to convert chemical energy to electrical energy generated by the metabolism of organic substrates [1,2]. The current generated by MFCs arises from the transfer of electrons liberated at the anode when bacteria oxidize organic material to carbon dioxide, protons, and electrons. In most MFCs the electrons that reach the cathode combine with protons that diffuse from the anode through a cation exchange membrane and oxygen provided from air; the resulting product is water, although most experimental MFCs use chemical oxidizers in the cathode chamber.

Microbial fuel cells are classified into two types depending on how electrons are transferred from the bacteria to the anode. Mediator-based MFCs are systems in which electroactive metabolites, or 'mediators', are added to the system to transfer the electrons between microorganisms and electrodes [3]. The second type of cell is that of a mediatorless fuel cell, which does not require the addition of any electroactive metabolite to transfer the electrons [4]. Many successful mediatorless MFCs rely mainly on the presence of metal-reducing bacteria, such as *Shewanella* [5], *Rhodospirillum rubrum* [6] and *Geobacteraceae* [3,7], which transfer electrons directly to the anode via molecular nanowires and electrochemically active redox enzymes in their outer membrane.

Escherichia coli has frequently been investigated as a catalyst for the electrochemical oxidation of glucose to construct biosensors and microbial fuel cells with suitable mediators. In this way, several types of MFCs employing the redox reaction of glucose using *E. coli* as a catalyst have been documented in the literature [8,9]. However, Wang et al. [10] pointed that the over-loading of exogenous mediators to *E. coli* may

cause damage to the electron flow in microbial metabolic pathways and decrease the substrate oxidation activity of the microbes. Recent studies have indicated some favourable results with mediatorless *E.coli*-based MFCs [11-12] and so it is now possible to perform detailed studies of such systems that might inform improvements in performance.

The development of MFC technology is advancing in different fields like design, material selection and the exploitation of different sources [13-16], allowing MFCs to reach their full potential in the field of waste treatment; however its potential as an energy producer has not been developed in the same way due to the variety of factors that can affect the overall performance of an MFC [17]. While the Internal Resistance, R_{int} , has been recognized as an important parameter [18,19], very little information can be found concerning R_{int} of MFCs. Advanced electrochemical techniques such as electrochemical impedance spectroscopy (EIS), which has become a useful tool in areas such as corrosion research and the characterisation of electrochemical systems [20-21], are rarely used to study the electrochemical properties of MFCs. In EIS, an ac signal is applied over a wide frequency range (usually 100 kHz–1 mHz) at a fixed operating potential amplitude to characterize the properties of the system. Since only a small perturbation is applied to the open circuit potential of the specimen for the recording of an impedance spectrum, EIS is essentially a non-destructive technique, able to separate different contributors of total internal resistance [22] (Charge transfer, mass transport, electrolyte resistance). The experimental spectra can be analysed and fitted to equivalent circuits [23] generating data from the real part (Z_{re}) and imaginary part (Z_{im}) that are presented in the form of a Nyquist plot. A Nyquist plot typically consists of a semicircle and a linear portion, which represents electrolyte and charge-transfer resistance and diffusion resistance, respectively.

A slow electron-transfer process, which is anticipated in MFCs, will result in a relatively large semicircle and a relatively small or non-existent linear portion. On the Nyquist plot, the portion in the high-frequency (HF) range (the intercept of the semicircle with the Z_{re} axis) is the electrolyte resistance; while in the low-frequency (LF) range the total internal resistance (summation of all contributions) can be found. The difference between the HF and LF represents the charge-transfer and diffusion resistances.

To our knowledge, there has been limited application of EIS to assess the electrochemical characteristics of MFCs [24-27]. Even the electrolyte resistance is crucial to the performance of a MFC and, since this parameter is affected by reactor configuration and fuel composition, EIS has a future role to play in understanding MFC performance. The aim of this work is to employ EIS to evaluate and understand the catalytic activity of *E. coli* cells in a mediatorless microbial fuel cell in terms of electron transport and depletion of the carbon source as it is oxidised by the bacteria.

2. Experimental

2.1. Bacterial Culture Conditions

Bacterial strains of *Escherichia coli* (INV α F'; Invitrogen) were grown on Luria-Bertani, (LB), agar plates aerobically incubated at 37 °C for 24 h, and then stored at 4 °C. Cell suspensions for experiments were produced by growing cultures of *E. coli* in 5 mL aliquots of buffered LB broth (tryptone (10 g L⁻¹); yeast extract (5 g L⁻¹); NaCl (5 g L⁻¹); Na-phosphate buffer 0.05 M, pH 7.2).

2.2. Cyclic voltammetry

Direct electrode reactions of bacterial cells were examined by cyclic voltammetry using a conventional three-electrode electrochemical cell. Cyclic voltammograms (CV) of the LB medium and bacterial cell suspension were obtained using a potentiostat / galvanostat (Radiometer Copenhagen PGP 201, Crawley, UK). A platinised titanium mesh working electrode (Ti-shop, UK), a platinum counter electrode (Thermo Electron Corporation, Fife, Scotland) and a saturated calomel reference electrode (Thermo Electron Corporation, Fife, Scotland) were used in the electrochemical cell. Measurements were carried out at 37 °C under anaerobic conditions at a scanning rate of 5 mV·s⁻¹ in the potential range - 0.8 to + 1.0 V. All experiments were performed in duplicate as a minimum.

2.3. Microbial fuel cell construction

The microbial fuel cell was constructed according to a novel design previously described by the authors [28]. Each chamber contained a platinised titanium mesh electrode. The total accessible geometrical surface of the electrodes was 8 cm². The electrodes were attached to the external system using copper wire with all exposed surfaces sealed with non conductive silicone.

The anode chamber was loaded with 500 mL, or where indicated, a different volume of freshly autoclaved buffered LB growth medium and bacterial suspension. When necessary, additional carbon source was added during the experiment in the form of 15 mL of glucose (40 % w/v). The cathode chamber was loaded with 0.050 M potassium hexacyanoferrate (III) solution. Nitrogen was continuously purged through the anode compartment to maintain anaerobic conditions. The fuel cell was immersed in a water bath to maintain the temperature at 37 °C.

2.4. Data Acquisition and Calculations

The fuel cell was continuously monitored using a digital multimeter Mastech MAS-343, connected to a computer with a RS232C cable (Mastech Enterprise Co., China). The circuit was operated under a fixed external load resistance (R_{load}) of 10 k Ω . The current (I) in amperes (A) was calculated using Ohm's law, $I = V / R_{load}$, where V is the potential drop in volts (V) across the external load resistor (R_{load}) in Ohms (Ω). To calculate the power output P in watts (W) $P = I \times V$ or the power density PD (W m⁻²) $PD = I \times V / A$, where A (m²) is the area of the anode, various resistances were set using a variable resistor box (RS Components, UK).

2.5. Impedance measurements

The effect of bacterial growth on the R_{int} of the anode chamber (R_{int}) was evaluated using EIS. Experiments were performed using a system comprising an EG&G 263A potentiostat and a 5210 EC lock in amplifier at a frequency range of 0.1-120,000 Hz. A platinised Ti mesh served as the working electrode, the counter electrode was made from a solid Pt band of area 2 cm² and the reference was a saturated calomel electrode (SCE) in saturated KCl. Measurements were taken in the LB medium both with and without the addition of a 5 mL bacterial suspension. The cell was maintained at 37 °C in a water bath for the full duration of the experiments. The impedance response of the system was monitored as a function of time, starting immediately after the addition of the bacterial suspension to the LB medium. The value of the impedance was observed to fall for the first 10 hours whereupon an increase in the value was noted. When the impedance approached its initial value, 50 % of the LB medium was replaced with a fresh preparation to assess the response on R_{int} . The evolution of the cell potential (vs. SCE) was also measured during the EIS evaluation of the cell containing the bacteria. Further EIS measurements, using the same experimental parameters as given above, were carried out

to evaluate the value of R_{int} of the LB medium without bacteria and to ascertain how quickly the addition of bacteria altered the value of R_{int} .

3. Results and discussion

3.1. Cyclic voltammograms

Cyclic voltammetry (CV) tests were conducted to determine whether *E. coli* cultures were electrochemically active. Cyclic voltammograms were recorded at various times after the addition of the bacterial inoculum. CVs of the medium were also recorded as a negative control. Fig. 1 shows the cyclic voltammograms the MFC containing buffered LB medium and also the same medium containing *E. coli* cells at various stages of bacterial growth.

No changes were observed in the CVs for medium without *E. coli*. The anaerobically grown cell suspension of *E. coli* was electrochemically active; the CV shows that the bacterial cell suspension had an oxidation peak at - 0.45 V (versus SCE) in the anodic scan, which was seen to increase with time, corresponding to bacterial growth. The fact that the oxidation peak increased with increasing density of bacteria in suspension in the medium suggests that diffusible mediators derived from the bacteria play a role in electron conduction from the bacteria to the cathode. Some authors have pointed in previous works the possibility of operate a mediatorless MFC with *E. coli* suggesting that when *E. coli* forms biofilms some compounds excreted by cells can act as mediators in the transport of electrons from the cells to electrode surface [11, 25, 29]. Fluorescence microscopy photographs included by the authors in a previous work [28] showed that bacterial cells were attached to electrode surface. The involvement of such

diffusible mediators is also consistent with the fact that conductive nanowires and outer membrane cytochromes that are known in electrogenic *Geobacter* spp. and *Shewanella* spp. [30-31] have not been reported in *E. coli*.

3.2. Performance of the microbial fuel cell under open circuit and external resistance conditions

Once it had been established that *E. coli* could generate electricity in a mediatorless MFC, the performance of the fuel cell was monitored. The anode compartment was loaded with 500 mL of freshly prepared buffered LB growth medium and inoculated with a suspension of bacteria (5 mL) from a fresh overnight culture. Titanium platinised mesh electrodes were used in both cell compartments. The cell was operated in open circuit mode and over an external load resistance of 10 k Ω . When the cell was operated under these conditions for an extended period (Fig. 2.a), the potential difference across the external resistance reached a maximum of over 800 mV at approximately 6 hours after the start of the experiment, falling to levels below the initial value after a further 5 hours. When the cell was operated in open circuit mode, the measured potential is equal to the difference in potential between the anode and cathode chambers. The potential remained effectively constant around 200 mV for up to 5 hours, at which point it began to increase and reached the maximum potential at 950 mV over a further 3 hour period. The potential then remained constant at this maximum value until the end of the experiment.

In order to better understand what was occurring in the two electrode chambers, the potential relative to the SCE was monitored separately at the anode and cathode during bacterial growth (Fig. 2.b). The greatest changes were observed at the anode with a decrease in the potential of approximately 1 V, five hours after the start of the experiment. At the same time the cathode potential decreased very slowly, falling by less than 100 mV

in 25 hours. This result indicates that the change in measured open circuit potential is due to changes in the anode chamber, where the bacteria reside. The increase in negative potential at the cathode during the first 10 hours of the experiment can reasonably be explained by the increase in cell density and the production of chemical mediators by the growing culture.

Polarization curves (Figure 3) were obtained by varying the external resistance from 0.1 to 100 k Ω . The maximum power density achieved was 627 mWm⁻², at a current of 1.0 mA. The power density is an order of magnitude higher when compared to that obtained in recent studies using a two-chamber MFC of a similar configuration [32,33]; and is comparable to that seen in some studies using single-chamber MFCs, which have lower internal resistance.

3.3. Influence of sample volume

The volume of buffered LB culture medium added to the anode compartment was varied between 250 mL and 800 mL (maximum capacity of anode chamber). In each case the experiment was started by adding a bacterial inoculum consisting of a fresh 5 mL overnight culture of *E. coli* in the same medium). The maximum potential reached by measuring the voltage drop across an external resistance of 2 k Ω (Fig. 4.a) increased with increasing the sample volume. The experiment was repeated with the cell in open circuit mode, with anode-chamber culture volumes of 400 and 800 mL (Fig. 4.b). The experiment with smaller volume of culture showed a longer delay before a steeper increase in the open circuit potential, compared with that seen with the larger volume of culture. However, the maximum open circuit potential achieved was the same for both volumes of analyte (Fig. 4.b).

3.4. Assessment of internal resistance using an EIS equivalent circuit

The Nyquist plots produced by the EIS study are shown in figures 5.a and 5.b. In this figure is also represented the equivalent circuit where R_s is the solution resistance, Q is the constant phase element and R_{int} is the internal resistance. The constant phase element was used in the Randles Circuit as the depressed nature of the semicircle on the Nyquist plot did not allow the data to be modelled using a capacitor. The general trend shown in Fig. 5.a is for the R_{int} of the cell to increase as time progresses, indicated by the magnitude of the low frequency semicircle portion of the spectra. This trend is in line with the results of the separate experiment where cell potential was monitored as a function of time (Figure 2.a), where it can be seen that after an initial rise in potential to 860 mV, the output gradually drops off until it levels out at approximately 65 mV between 15 to 20 hours from the start of the experiment.

The low frequency semicircle portions of the Nyquist plots were analysed using equivalent circuit models, based on a standard Randle's circuit, (which is shown in Fig. 5). The equivalent circuit consisted of parameters characterizing the solution resistance (R_s), the constant phase element and the charge transfer resistance (R_{ct}) which is associated with the internal resistance to extract values for R_{int} . This analysis involved extrapolation of these portions of the curve to determine the point of intersection on the Z_{re} axis of the plot. The resulting resistance values (Figure 6.a) illustrate the effect of the composition of the medium upon the efficiency and effectiveness of the cell. In agreement with the timescale of the decay in the electrical potential (Figure 2.a), R_{int} drops from an initial value of around 5.6 M Ω to a minimum of just over 3.0 M Ω after around 5 hours. After this point, R_{int} gradually increases until, approximately 17.5 hours after the start of the experiment, it has returned to the initial level. Upon replenishment of 50 % of the

medium, R_{int} falls again to around the minimum level observed in the first cycle of the experiment.

It is interesting to note that this second fall in resistance occurs ~~is~~ more rapid than the fall in resistance noted at the commencement of the test. However, the rate at which the internal resistance recovers is similar in both cases. This result suggests that once the bacteria in the cell have been initially activated, it is easier to re-energise them with subsequent re-fuelling.

The data presented in figure 6.b shows a comparison of the values of R_{int} for the cells both with and without the addition of bacteria. The value of R_{int} for the abiotic cell is several orders of magnitude less than that displayed by the biotic cell. Furthermore, a high value of R_{int} is attained quickly upon the addition of bacteria to an abiotic environment; the increased magnitude of R_{int} is achieved within the time delay between the two data points as a result of bacterial addition.

4. Conclusions

Cyclic Voltammetry showed that *E. coli* can self-mediate the extra-cellular electron transfer to electrode surface through the excretion of different molecules that can act as mediators, those self-produced mediators play a role in the degradation of organic compounds by bacteria.

Electrochemical impedance spectroscopy (EIS) has been successfully used to evaluate the internal resistance R_{int} of the anode chamber of a mediatorless microbial fuel cell. Specifically EIS data shows that there is a variation in the internal resistance of the cell which changes with bacterial growth; with an initial reduction of around 50 % and a

posterior increase of the resistance resulting in a corresponding fall in output, the introduction of further nutrient into the cell produces a change in this trend with a new reduction of the internal resistance and the consequence increase in the output. This finding can significantly extend the possibility of using this microbial fuel cell in a semi-continuous flow mode, introducing new nutrients once the output starts to fall.

Acknowledgements

This work was supported by a Strategic Investment Fund grant from Sheffield Hallam University.

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Figure captions

Figure 1. Cyclic voltammograms of the MFC with a titanium platinised mesh electrode placed in: buffered LB medium alone, or in LB medium plus *E. coli* at various stages of bacterial growth, after 3 hours and after 5 hours. The experiment was carried out at 37 °C; scan rate 5 mVs⁻¹.

Figure 2. Evolution over an extended time of: (a) the open circuit potential and the difference in potential across an external resistance of 10 k Ω ; (b) the potential with the bacterial growth in the cathode chamber and in the anode chamber (vs SCE). The MFC was charged with 500 mL of buffered LB medium inoculated with *E. coli*.

Figure 3. Polarisation curve analysis of MFC with titanium platinised mesh electrodes, containing *E. coli* and buffered LB medium in the anode chamber at 37 °C (represents cell voltage and power density).

Figure 4. Influence of the volume of LB medium added to the anode chamber on the difference of potential (a) across an external resistance of 2 k Ω (a1) 250 mL, (a2) 400 mL, (a3) 600 mL, (a4) 800 mL; and (b) in the open circuit mode (b1) 400 mL, (b2) 800 mL. In all the cases the anode chamber was inoculated with *E. coli*.

Figure 5. Nyquist plots produced by the Electrochemical Impedance Spectroscopy (EIS) characterisation of the MFC inoculated with *E. coli*. Panel (a) shows the results from the cell with the original batch of buffered LB medium, (a1) scan 1 after 0.08 h, (a2) scan 3 after 4.25 h, (a3) scan 5 after 9.58 h, (a4) scan 7 after 14.82 h, (a5) scan 8 after 17.58 h; 18 hours after inoculation, half of the culture from the anode chamber was removed and replaced with an equal volume of fresh buffered LB medium. Panel (b) shows the EIS spectra recorded subsequently, (b1) scan 9 after 20.25 h, (b2) scan 11 after 25.58 h, (b3) scan 12 after 28.25 h, together with (a5) recorded shortly before the change of growth medium, which is duplicated from panel (a) in order to highlight the effect of refuelling the cell with fresh medium. For clarity, some of the intermediate scans have been omitted.

Figure 6. (a) Evolution of the resistance of the MFC, calculated by extrapolation from analysis of the low frequency semicircle portions of the Nyquist plots in Fig 5. (b) Comparison of the internal resistance of biotic and abiotic LB medium. Arrow indicate the addition of bacteria to LB medium; a 5mL bacterial suspension was added to the medium

between the acquisition of scans 3 and 4 whereupon R_{nt} attained a value equitable to that seen at the beginning of the experiment illustrated in part (a).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6