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The use of electrochemical impedance spectroscopy (EIS) in the evaluation of the electrochemical properties of a microbial fuel cell

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Abstract

Electrochemical impedance spectroscopy (EIS) has been used to determine several electrochemical properties of the anode and cathode of a mediator-less microbial fuel cell (MFC) under different operational conditions. These operational conditions included a system with and without the bacterial catalyst and EIS measurements at the open-circuit potential of the anode and the cathode or at an applied cell voltage. In all cases the impedance spectra followed a simple one-time-constant model (OTCM) in which the solution resistance is in series with a parallel combination of the polarization resistance and the electrode capacitance. Analysis of the impedance spectra showed that addition of *Shewanella oneidensis* MR-1 to a solution of buffer and lactate greatly increased the rate of the lactate oxidation at the anode under open-circuit conditions. The large decrease of open-circuit potential of the anode increased the cell voltage of the MFC and its power output. Measurements of impedance spectra for the MFC at different cell voltages resulted in determining the internal resistance (R_{int}) of the MFC and it was found that R_{int} is a function of cell voltage. Additionally, R_{int} was equal to R_{ext} at the cell voltage corresponding to maximum power, where R_{ext} is the external resistance that must be applied across the circuit to obtain the maximum power output.
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Keywords: Microbial fuel cell; Electrochemical impedance spectroscopy; Internal resistance of a microbial fuel cell

1. Introduction

EIS is frequently employed in corrosion studies, where it is used to evaluate corrosion protection by inhibitors, polymer coatings and anodic layers, pitting of alloys, and microbiologically influenced corrosion (MIC) [1,2]. EIS has also been used in microbiologically influenced corrosion inhibition (MICI) studies. Nagiub and Mansfeld [3,4] used EIS and electrochemical noise analysis (ENA) to show that *Shewanella ana* and *Shewanella algae* prevented pitting of Al, tarnishing of brass and rusting of steel in artificial seawater containing a growth medium.

Despite the successful application of EIS in corrosion science, very few studies have been published in which EIS has been used to evaluate the properties of MFCs and the parameters that determine its power output. This might be due to the perception that it is difficult to use EIS and to analyze the impedance spectra [5]. In the present studies EIS is being used to evaluate the electrochemical behavior of the anode and the cathode of a mediator-less MFC at their open-circuit potentials under different experimental conditions [6–8]. This was made possible by the introduction of Ag/AgCl reference electrodes in the anode and cathode compartments. EIS data are also being obtained for the MFC at different applied cell voltages. As previously discussed, [6] a better understanding of how an MFC works and how its power output can be improved, can only be obtained if a number of different electrochemical techniques such as EIS, potential sweeps, potentiodynamic polarization and cyclic voltammetry are applied in combination with other techniques such as surface analysis and microbiological investigations.

2. Experimental approach

A dual compartment MFC was used for all experiments. Bare graphite felt (GF-S6-06, Electrolytica) was used as the anode and the cathode. The cathode was electroplated with platinum at a loading of 0.15 mg/cm^2 [9]. A proton-exchange membrane (Nafion® 424, DuPont) was used to separate the

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anode and cathode compartments. Each electrode had an apparent surface area of 20 cm^2 and was connected to Pt-wire leads by a conductive carbon epoxy (EPOX-4, Electrolytica).

The assembled MFC was autoclaved at 121 °C for 15 min prior to the addition of any liquid media. Sterile Ag/AgCl reference electrodes were inserted into both the anode and cathode compartments after autoclaving. Anaerobic conditions were maintained in the anode compartment of the MFC by continuously passing filtered nitrogen gas through the compartment at a rate of 20 mL/min. Aerobic conditions were maintained in the cathode compartment by continuously passing air at a rate of 40 mL/min.

Electrochemical measurements were performed using two different anolytes: 1) buffer and lactate (20 mM) and 2) buffer, lactate (20 mM) and *Shewanella oneidensis* MR-1 (MR-1).

The buffer solution contained 50 mM PIPES ($C_8H_{18}N_2O_6S_2$) and 7.5 mM NaOH (pH 7.0). The same buffer solution was used in the anode and the cathode compartments. The same cell was used for the two sets of experiments.

The MFC was allowed to remain at the open-circuit cell voltage V_o for several hours such that a stable open-circuit cell potential (OCP) could be observed for both electrodes before measurements were taken.

2.1. Bacterial growth conditions

2.1.1. Shewanella oneidensis

MR-1 was grown in a PIPES-buffered minimal media (pH 7.0) containing 18 mM lactate as the sole electron donor, 50 mM PIPES, 7.5 mM NaOH, 28 mM NH_4Cl, 1.3 mM KCl, 4.3 mM $NaH_2PO_4 \cdot H_2O$ and 10 mL/L each of vitamin, amino acid and trace mineral stock solutions. The cells were harvested and injected into the MFC such that an OD_{600} of 0.4 was achieved in the anode compartment (the buffer served as the diluting medium) [9].

2.2. Electrochemical techniques

A Gamry PCI4/300 potentiostat was used for all electrochemical measurements. Gamry EIS300 software was used for recording of impedance spectra, while DC105 software was used for recording of potential sweeps, monitoring of the potential difference between the two reference electrodes and for measuring the *I–t* curve at an applied cell voltage.

2.2.1. Electrochemical impedance spectroscopy (EIS)

EIS measurements were carried for the anode, the cathode and the MFC in a frequency range of 100 kHz to 1 mHz with an ac signal of 10 mV amplitude. Anode impedance spectra were recorded using the anode as the working electrode and the cathode as the counter electrode. Cathode impedance spectra were recorded using the cathode as the working and the anode as the counter electrode. During these measurements, the Ag/AgCl reference electrode in the compartment of the working electrode was used as the reference electrode. When EIS measurements were performed for the MFC at several applied cell voltages, the anode was used as the working electrode and the cathode was used as the reference as well as the counter electrode.

2.2.2. Potential sweeps

Potential sweep experiments were carried out at a scan rate of 0.1 mV/s from the open-circuit cell voltage (V_o), where zero current is passed across the circuit ($I=0$), to the short-circuit cell voltage (V_{sc}), where current is at a maximum ($I=I_{max}$). From the *V–I* curves power (*P*)–*V* curves were calculated. The *P–V* curves were used to determine the cell voltages at which the maximum power (V_{max}) and half the maximum power (V_1) occur.

2.2.3. Voltage–time (V–t) and current–time (I–t) curves

The potential difference between the two Ag/AgCl reference electrodes was monitored for about 30 min to estimate a baseline value for determination of the membrane resistance (R_m). Current–time curves were then measured at three different applied cell voltages, i.e. V_{max}, V_1, and V_{sc}. The cell current flowing between the anode and the cathode, and the potential difference between the two reference electrodes, were monitored at each applied cell voltage for 3 h. From these measurements an estimated value of R_m under MFC operational conditions was made using Ohm's law.

2.3. Test procedure

The MFC was assembled with the anode compartment containing buffer solution and lactate. The cycle of electrochemical experiments was started by collecting an impedance spectrum for the anode and the cathode at their OCP followed by a potential sweep experiment.

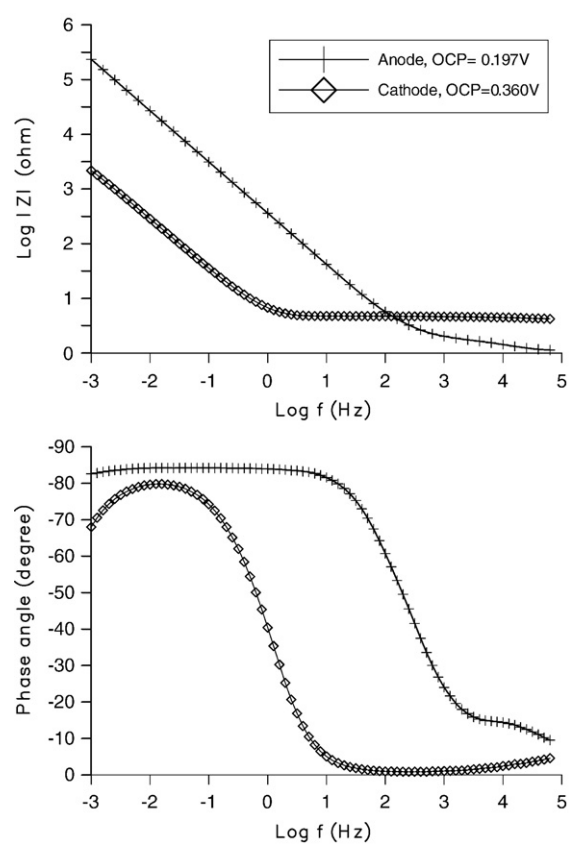

Fig. 1. Impedance spectra for the anode and cathode at their OCP for test with buffer and lactate as the anolyte.

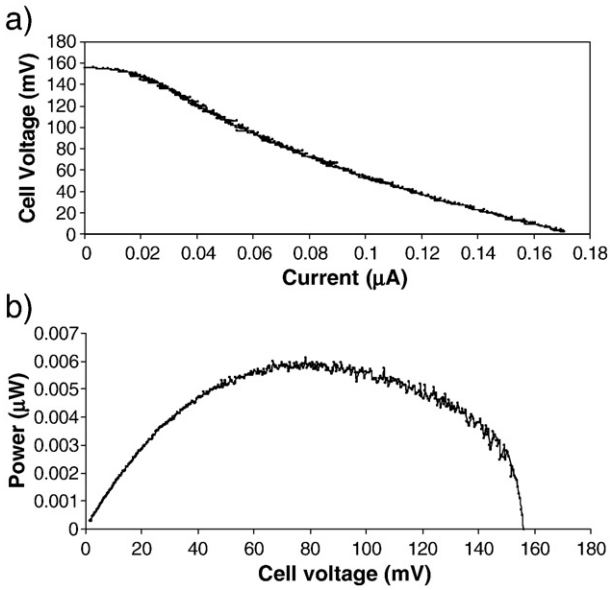

Fig. 2. *V–I* (a) and *P–V* (b) curves for test with buffer and lactate as the anolyte.

Monitoring the potential difference between the two reference electrodes was started at the open-circuit condition. After about 30 min of monitoring this potential difference, the required cell voltage was applied across the cell without interrupting the monitoring of the potential difference between the two reference electrodes. *I–t* curves were recorded for 3 h and then an EIS measurement was carried out for the MFC at the same applied cell voltage. All three processes were carried out for each cell voltage V_{max}, V_1 and V_{sc}, respectively.

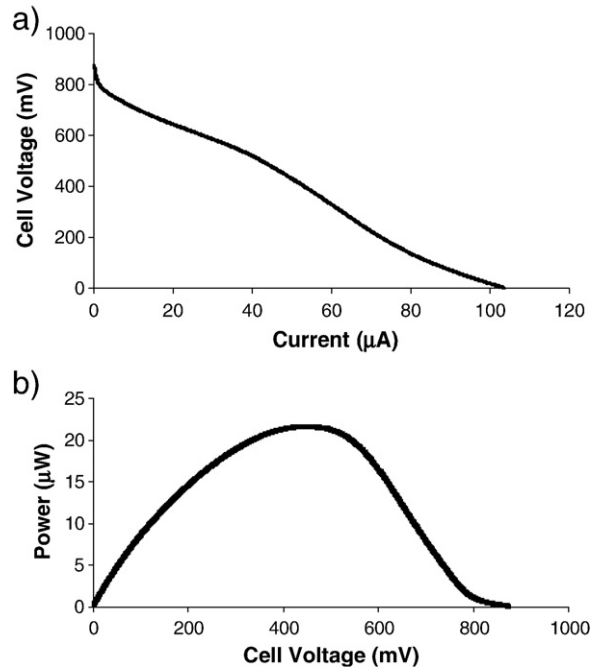

Fig. 4. *V–I* (a) and *P–V* (b) curves for MFC with buffer, lactate and MR-1 as the anolyte.

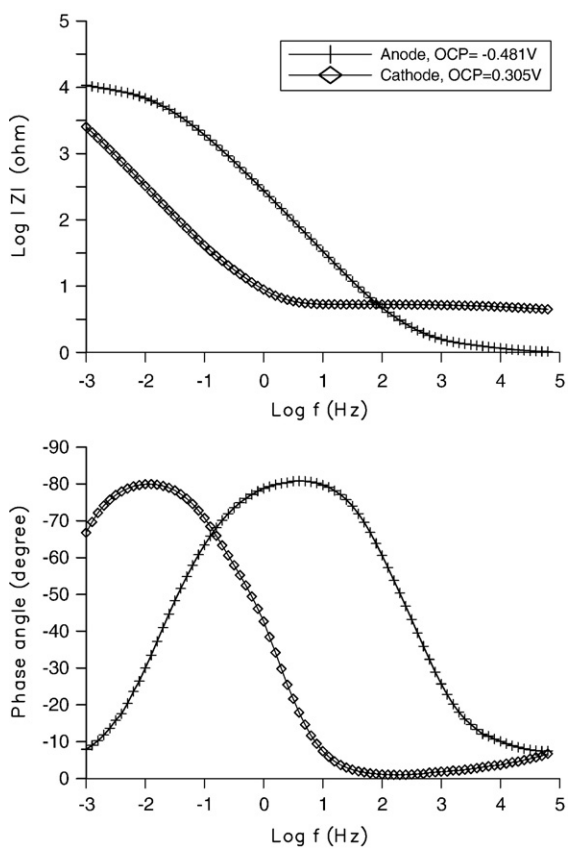

Fig. 3. Impedance spectra for the anode and cathode at their OCP for test with buffer, lactate and MR-1 as the anolyte.

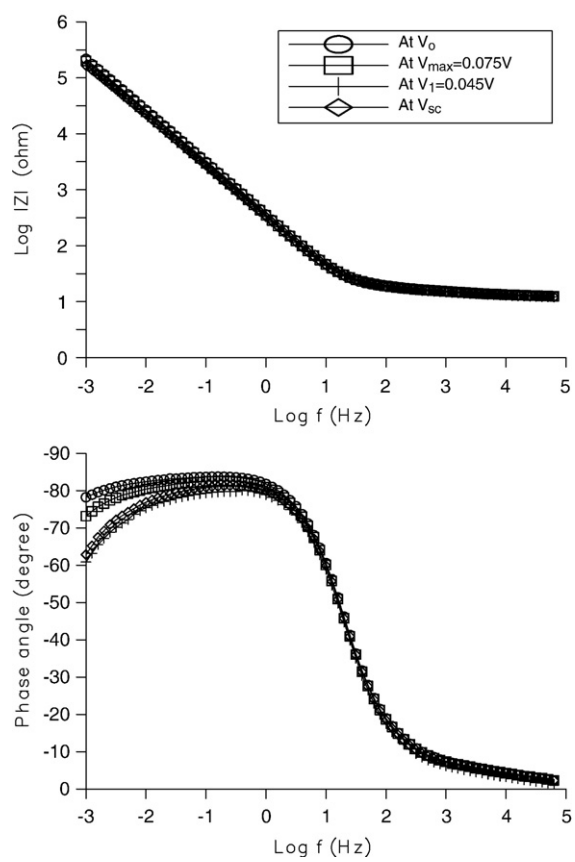

Fig. 5. Impedance spectra for the MFC at different applied cell voltages with buffer and lactate as the anolyte.

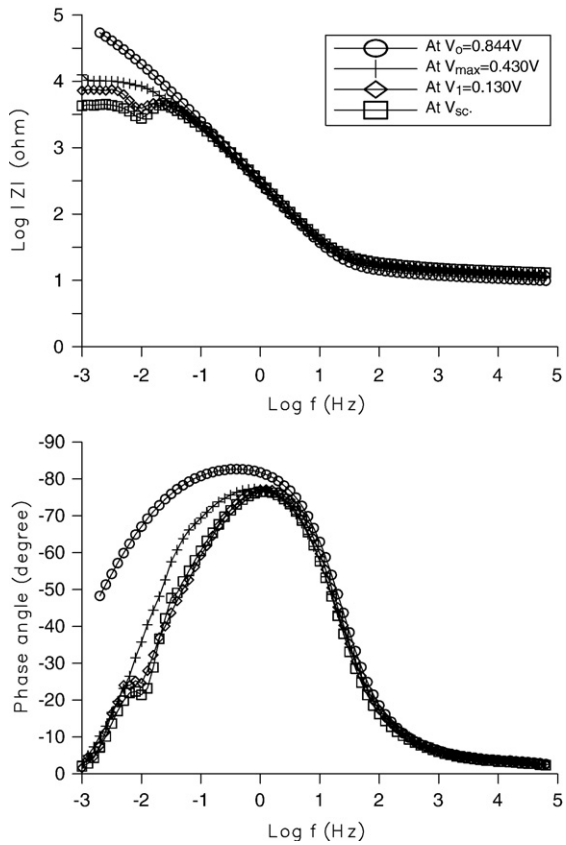

Fig. 6. Impedance spectra for the MFC at different applied cell voltages with buffer, lactate and MR-1 as the anolyte.

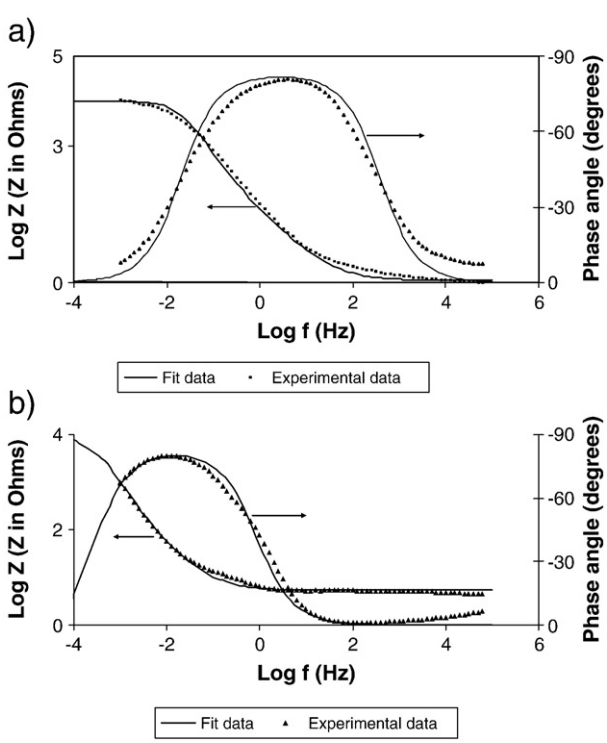

Fig. 7. Comparison of experimental and fitted impedance spectra (data of Fig. 3) of anode (a) and cathode (b).

Table 1
Fit parameters for the spectra of the anode and cathode (anolyte: buffer and lactate)

Parameter	Anode	Cathode
R_p (Ω)	$7.79 * 10^6$	$8.32 * 10^3$
C (F)	$9.22 * 10^{-4}$	$6.22 * 10^{-2}$
R_s (Ω)	1.5	5.5

This same sequence of experiments was repeated after the anode solution was completely replaced with a suspension containing buffer, lactate and MR-1. The same MFC assembly, i.e. the same electrodes and membrane, was used for all tests.

3. Results and discussion

The EIS data are presented in the form of Bode plots in which the logarithm of the impedance modulus $|Z|$ and the phase angle Φ are plotted vs. the logarithm of the frequency f of the applied ac signal. The impedance spectra recorded for the anode and cathode at their OCP with buffer and lactate as the anolyte are shown in the Fig. 1. The impedance spectra of both the anode and the cathode follow the one-time constant model (OTCM), in which the solution resistance (R_s) is in series with a parallel combination of the capacitance of the electrode (C) and its polarization resistance (R_p) [1,2]. R_p is inversely proportional to the exchange current density (I_o) of the reaction that takes place at each electrode [7]. It can be seen from Fig. 1 that the frequency dependence of Φ in the low-frequency region of the spectra indicates that the polarization resistance of the cathode (R_p^c) is much lower than the polarization resistance of the anode (R_p^a), i.e. the rate of oxygen reduction at the cathode is much faster than the rate of lactate oxidation at the anode. Additionally, these spectra show a much higher capacitance value for the cathode (C^c), which is due to the higher active surface area of the Pt-plated graphite cathode [6–8].

Fig. 2a shows the V–I plot for buffer and lactate as the anolyte. The open-circuit cell voltage was found to be 156 mV and a maximum current of 0.17 μA was measured at short-circuit. The P–V curve in the Fig. 2b shows that the V_{max} was about 80 mV and the maximum power that was obtained from this cell was 6 nW.

Fig. 3 shows the impedance spectra for the anode and cathode when the anode solution contained buffer, lactate and MR-1. The spectrum for the anode was very different from the condition when no bacteria were present in the anode compartment (Fig. 1). The frequency dependence of $|Z|$ and Φ at low frequencies demonstrated that R_p^a decreased considerably in the presence of MR-1. This decrease in R_p^a was accompanied by a sharp decrease of OCP^a from 0.197 V to −0.481 V. The decrease of R_p^a and OCP^a when MR-1 is present in the anode

Table 2
Fit parameters for spectra of the anode and cathode (anolyte: buffer, lactate and MR-1)

Parameter	Anode	Cathode
R_p (Ω)	$1.02 * 10^4$	$8.51 * 10^3$
C (F)	$9.70 * 10^{-4}$	$6.62 * 10^{-2}$
R_s (Ω)	1.1	5.3

Table 3
Fit parameters for spectra of the MFC at different applied cell voltages (Anolyte: buffer and lactate)

Parameter	V_o	V_{max}	V_1	V_{sc}
R_p^{cell} (Ω)	$9.79*10^6$	$2.94*10^6$	$1.50*10^6$	$5.23*10^5$
C^{cell} (F)	$7.11*10^{-4}$	$7.28*10^{-4}$	$8.33*10^{-4}$	$1.03*10^{-3}$
R_s^{cell} (Ω)	14.6	14.6	14.7	14.2

compartment suggests that the rate of the anodic redox reaction is considerably increased [6–8]. The cathodic impedance spectrum for the case when MR-1 is present at the anode is very similar to that without MR-1 (Fig. 1).

The MFC *V–I* curve for the case when MR-1 was present in the anolyte is shown in the Fig. 4a. V_o increased to 0.844 V and the maximum current that passed through the cell was 104 μA, which was much higher than the maximum current that was obtained without MR-1 (Fig. 2). The *P–V* curve (Fig. 4b) shows that the maximum power (P_{max}) had increased to 23 μW at V_{max}=0.430 V.

Fig. 5 shows the MFC impedance spectra that were obtained at four different applied cell voltages V_o, V_{max}, V_1, and V_{sc} with buffer and lactate as the anolyte using a two-electrode technique [1,2]. When comparing these results with those shown in Fig. 1, several interesting points are observed. The solution resistance (R_s^{cell}) of the MFC is higher than R_s for the anode and cathode since it includes the resistance of the anolyte and catholyte between the anode and the cathode (R_Ω) and the resistance of the membrane (R_m.). EIS data obtained in the presence of MR-1 at four different applied cell voltages are shown in the Fig. 6. The low-frequency dependence of the impedance spectra indicates that R_p^{cell} of the MFC decreases significantly as the applied cell voltage decreases, i.e. the cell current increases.

The EIS results shown in Figs. 1, 3, 5 and 6 were analyzed using the OTCM in the ANALEIS software developed by Shih and Mansfeld [2,10,11]. Fig. 7 shows a comparison of the experimental and the fitted spectra for the data in Fig. 3. Very good agreement between the two data sets was obtained for the EIS data obtained with and without MR-1. The results of the data analysis are tabulated in Tables 1 and 2. The only significant difference of the fit parameters is the drastic decrease of R_p^a in the presence of MR-1. According to mixed potential theory [12] this decrease of R_p^a, accompanied by the marked negative shift of OCP^a is due to the large increase of the rate of lactate oxidation that occurs at OCP^a in the presence of MR-1 [6–8].

The results of the analysis of the impedance spectra obtained for the cell at different cell voltages and under different anode conditions, i.e. without and with MR-1, are given in Tables 3 and 4. As will be discussed in more detail elsewhere [8] the internal resistance R_{int} of an MFC is defined as $R_{int}=R_p^a+R_p^c+R_\Omega+R_m$. For the MFC under investigation $R_p^a+R_p^c>>R_\Omega+R_m$. Since R_p^{cell} is

Table 4
Fit parameters of the MFC at different applied cell voltages (anolyte: buffer, lactate and MR-1)

Parameter	V_o	V_{max}	V_1	V_{sc}
R_p^{cell} (Ω)	$7.20*10^4$	$9.81*10^3$	$7.24*10^3$	$4.58*10^3$
C^{cell} (F)	$7.84*10^{-4}$	$9.24*10^{-4}$	$9.13*10^{-4}$	$7.11*10^{-4}$
R_s^{cell} (Ω)	14.2	13.4	12.8	11.7

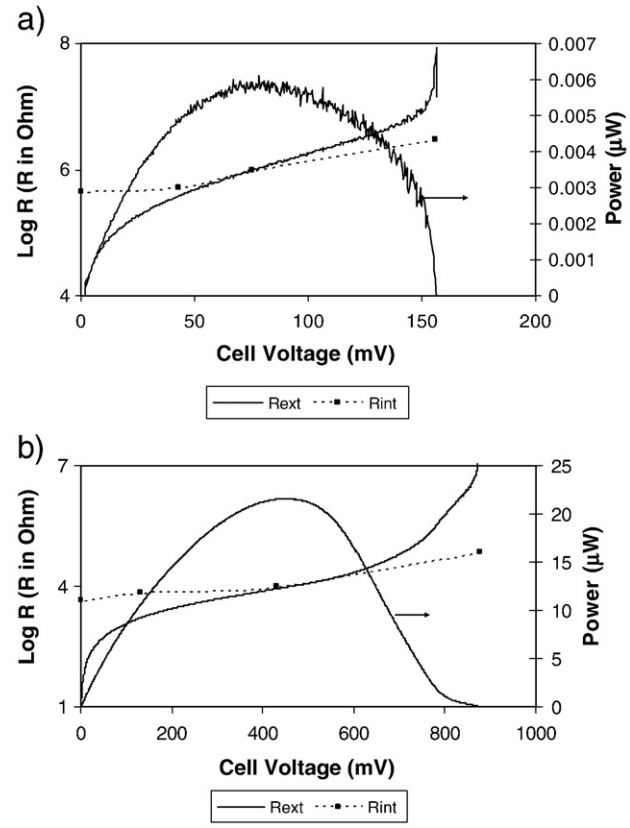

Fig. 8. The dependence of R_{int} and R_{ext} on *V* and the *P–V* curves for tests with buffer and lactate (a) buffer, lactate and MR-1 (b) as the anolyte.

equal to R_{int}, the data in Tables 3 and 4 show that R_{int} is much larger in the absence of MR-1 and decreases as the cell voltage decreases and the cell current increases (regardless of the presence

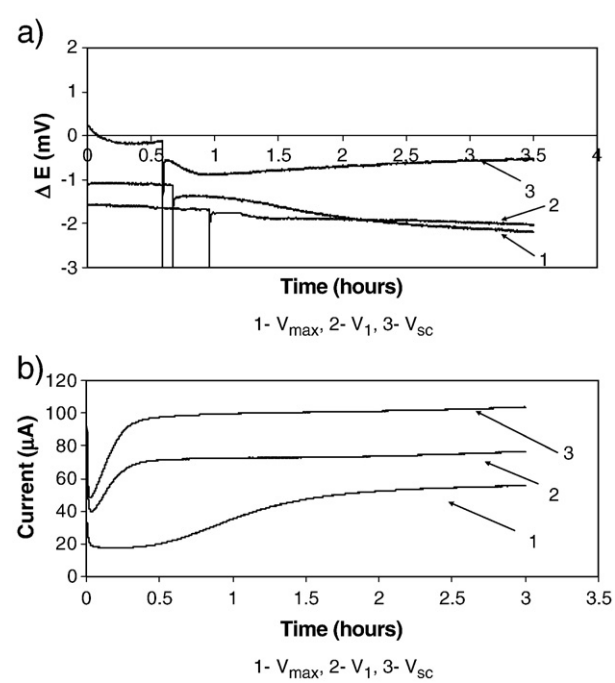

Fig. 9. Potential difference—*t* and *I–t* curves at three different cell voltages with buffer, lactate and MR-1 as the anolyte.

Table 5
Variation of $R_m+R'_\Omega$ with applied cell voltage

Applied cell voltage	$R_m+R'_\Omega$ (Ω)
V_{max}	5.85
V_1	5.40
V_{sc}	3.52

of bacteria at the anode). The cell capacitance (C^{cell}), which contains contributions from the capacitance of the anode and the cathode and $R_s^{cell}=R_\Omega+R_m$, were independent of the applied cell voltage.

Fig. 8 shows the $P-V$ curves for both anodic conditions and the dependence on cell voltage of R_{int} and the external resistance R_{ext} that has to be applied to obtain the desired power production form the cell ($R_{ext}=P/I^2$). These figures demonstrate that $R_{ext}=\infty$ at V_o, and $R_{ext}=0$ at V_{sc}. Additionally, R_{int} decreased as the cell voltage decreased due to the decrease of R_p^a and R_p^c. For both tests it was found that $R_{int}=R_{ext}$ at V_{max} as suggested by Logan et al. [5]. For $V<V_{max}$ it is shown that $R_{int}>R_{ext}$ and for $V>V_{max}$ it is shown that $R_{int}<R_{ext}$.

A comparison of the impedance spectra in Figs. 1 and 3 with those in Figs. 5 and 6 indicates that R_s^{cell}, i.e. the impedance at the highest frequencies, was higher in the tests with an applied cell voltage (Figs. 5 and 6) than R_s obtained for test at the OCPs of the anode and the cathode (Figs. 1 and 3). This result is due to the fact that R_s^{cell} includes the resistance R_Ω of the solution between the anode and the cathode as well as the membrane resistance R_m. In order to obtain an estimate of the experimental value of R_m the potential difference between two references electrodes in the anode and cathode compartment and the current flowing through the cell were measured at V_{max}, V_1 and V_{sc} for 3 h in buffer, lactate and MR-1 anolyte. Fig. 9a shows the potential difference data, while Fig. 9b shows the current–time curves. The potential difference in the absence of current flow is due to small differences in the potential of the two Ag/AgCl reference electrodes. The potential difference increases as a cell voltage is applied between the anode and cathode because of the contributions of the resistance R'_Ω of the electrolyte between the two reference electrodes and R_m. This resistance value $R'_\Omega+R_m$ was calculated based on the potential difference in the absence and presence of current flow (Fig. 9a) and the value of the current at the end of the test (Fig. 9b). The results of these calculations in Table 5 demonstrate that $R'_\Omega+R_m$ decreased slightly with increasing current and the sum of these components is very small compared to R_{int} (Table 4).

4. Summary and conclusions

EIS has been found to be a convenient tool for the determination of the electrochemical properties of the anode and the cathode of a MFC. EIS data have been collected for several MFC operational conditions including different anode solutions and various applied potentials. In all cases the impedance spectra followed a simple one-time-constant model (OTCM) in which the solution resistance R_s is in series with a parallel combination of the polarization resistance R_p and the electrode capacitance C. Analysis of the impedance spectra for the anode showed that addition of MR-1 to a solution of buffer and lactate greatly increased the rate of the lactate oxidation at the OCP^a of the anode. The large decrease of OCP^a in the presence of MR-1 increased the cell voltage of the MFC and its power output. The fact that R_p^a is much larger than R_p^c shows that in the evaluated MFC system, the rate of oxygen reduction at the cathode is much larger than the rate of lactate oxidation at the anode.

Measurements of impedance spectra for the MFC at different cell voltages allow the determination of the internal resistance R_{int} of the MFC. R_{int} was lower by about a factor 100 in the presence of MR-1 and decreased with decreasing cell voltage since both R_p^a and R_p^c decrease with increasing current flow. It was found that at V_{max}, the MFC produces the maximum power, and $R_{int}=R_{ext}$.

Potential measurements between the two reference electrodes and current measurements at different cell voltages have been used to obtain an estimate of the membrane resistance R_m of about 5 Ω.

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