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Comparison of Various Techniques to Characterize a Single Chamber Microbial Fuel Cell Loaded with Sulfate Reducing Biocatalysts

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Abstract: *A single-chamber microbial fuel cell (SCMFC) with a carbon supported Pt-cathode for the oxygen reduction reaction (ORR), and loaded with a sulfate reducing bacterial consortium as biocatalyst in the anodic chamber was characterized by polarization by variable resistance (VR) and linear sweep voltammetry (LSV) methods. From VR a whole cell configuration maximum volumetric power of 92.5 mW m^{-3} was attained at a current density of 459 A m^{-3} and voltage of 0.202 V. The LSV method of whole cell configuration gave a higher maximum power density of 197.5 mW m^{-3} at current density of 696 mA m^{-3} at the potential of 0.284V; this disagreement was ascribed to possible reduction of power and potential overshoot with the LSV. There was a fair agreement between internal resistance values of whole cell configuration determined by VR and electrochemical impedance spectroscopy (EIS): 2225 and 2307 Ω, respectively. Yet, internal resistance measured by LSV was 30% lower for the whole cell configuration. Both LSV and EIS show the advantage of reduced potential overshoot; yet, EIS provides more detailed information on equivalent circuit of the cell and resistance contributions of the electrodes, electrolyte and membrane. Further cyclic voltammetry tests gave midpoint potential of -0.215 V vs saturated calomel electrode, a value close to those reported for bacterial cytochromes involved in extracellular electron transfer processes. It is concluded that in spite of particular advantages of some techniques over others, the combination of electrochemical methods can be very valuable for shedding light and internal checking of the main characteristics of a microbial fuel cell.*

Keywords: internal resistance, linear sweep voltammetry, microbial fuel cell, polarization, sulfate-reducing bacteria

1. INTRODUCTION

A microbial fuel cell (MFC) is a promising renewable energy source; in the anodic chamber the microorganisms anoxically oxidize the organic compounds and release electrons and protons. Electrons are channeled to the anode that acts as external electron sink. Protons are released in organic matter oxidation, and they migrated through proton exchange membrane to the cathode. There, the transported protons react with the oxygen producing water in the presence of carbon-supported Pt nanoparticles [1,2]. Collected electrons are directed to an external circuit where there is a resistor or a device to be powered, in this way direct current is produced. The internal resistance (R_{int}) is one of the main characteristics of a MFC, because high values tend to result in low power output of the cell. On the other hand, according to Jacobi's theorem of maximum power delivered by an electromotive force, an MFC fitted with an external resistance equal to its internal resistance will give its maximum power output [3].

There are a variety of factors that can affect the overall performance of a MFC [2,4-6]. Biocatalyst used is one of them [7]. A few works with MFC using sulphate reducing bacteria have been reported [8,9]. Recently, our Group has observed a 13 fold substantial improvement in volumetric power (P_v) of a MFC using sulphate reducing bacteria, compared to methanogenic and aerobic inocula [4].

On the other hand, estimates of the maximum power generated

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ABBREVIATIONS

A	configuration of the cell with three electrodes, with a reference electrode (Saturated calomel electrode-SCE) inserted near the anode (anode – working electrode, the cathode acts as counter electrode
AMC	assemblage (sandwiched) anode-membrane-cathode
EIS	electrochemical impedance spectroscopy
C	configuration of the cell with three electrodes. with a SCE reference electrode inserted near the anode, the cathode-working electrode, anode acts as counter electrode
CV	cyclic voltammetry
LSV	linear sweep voltammetry
MFC	microbial fuel cell
OCP	open circuit potential
ORR	oxygen reduction reactions
P_v	volumetric power
QPE_a	double layer capacitance of the anode
QPE_c	double layer capacitance of the cathode
R_a	anodic resistance by EIS
R_A	anode internal resistance by LSV, anode configuration
R_c	cathodic resistance by EIS
R_C	cathode internal resistance by LSV, cathode configuration
R_{ext}	external resistance
R_{int}	internal resistance
$R_{int,W}$	whole cell configuration internal resistance
R_{int-A}	anode cell configuration internal resistance
R_{int-C}	cathode cell configuration internal resistance
R_m	membrane plus electrolyte resistance by EIS
SCMFC	single chamber microbial fuel cell
VR	variation of resistance method
W	configuration of the cell with two electrodes, the anode acts as working electrode and the cathode as reference and counter electrode

in an MFC and the internal resistance can depend upon the technique used to obtain the polarization curve [10-12]. In the variable resistance (VR) method the circuit resistance is varied at fixed time intervals, ranging from 10s to 24h. An alternative approach is sweeping the potential at different scan rates using a technique called linear sweep voltammetry (LSV)[13,14].

There have been very few studies comparing the above mentioned techniques [12,14]. In one of them, [14] it was found that power production with scan rates higher than 0.1 mV/s produced higher power densities than those with the VR method. A common problem often encountered when evaluating polarization curves is "power overshoot" [12,15-17],. Power overshoot refers to the response of the system at high current densities (past the maximum power) in a power density curve where the cell voltage and current

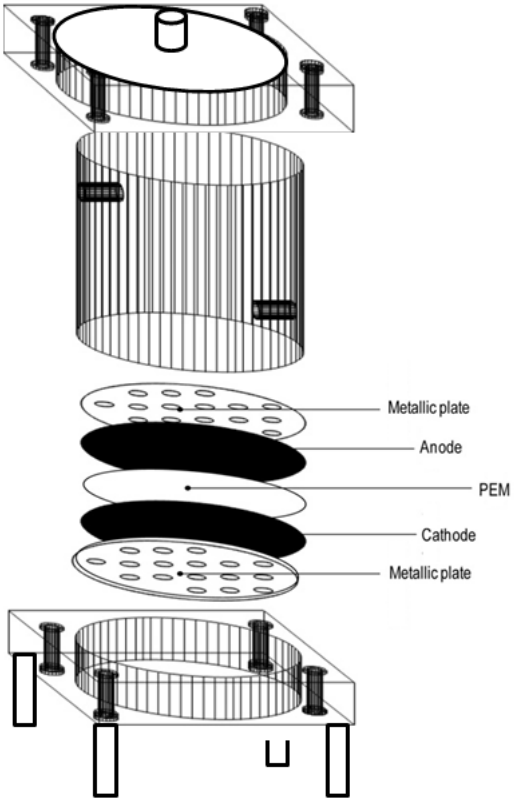

Figure 1. Schematic diagram of the vertical single chamber microbial fuel cell

drop very quickly resulting in a doubling back of the power density curve, producing lower power than previously measured for the lower current densities. One hypothesis on the cause of this power overshoot is that as the current resistance is decreased the bacteria on the anode are unable to produce sufficient current at lower voltages [16]. However, it does not seem to be a correlation in the literature between the magnitude of current density and power curve shape [14]. Here our focus was to compare the various techniques like VR, LSV, EIS and cyclic voltammetry (CV) for determining internal resistance, polarization behaviour, and bacterial activity for the characterization of a MFC loaded with a sulphate reducing inoculum.

2. EXPERIMENTAL

2.1. Construction of single chamber vertical MFC

The MFC consisted of a vertical cylinder built in Plexiglass 9cm long and 5.6cm internal diameter (Fig. 1.). An assemblage of proton exchange membrane (PEM) was fitted at the bottom of the cell. For brevity, this 'sandwich' arrangement was abbreviated as AMC (for Anode-proton exchange Membrane- Cathode.) The AMC consisted of (from inside to outside) a circular, perforated stainless steel plate 1 mm thickness covered below with a Toray flexible carbon-cloth sheet, a proton exchange membrane (Nafion 117), a flexible carbon-cloth containing 0.5mg cm^{-2}Pt catalyst (10wt%/C-ETEK) as cathode, and a perforated plate of stainless steel 1 mm thickness. The area of the circular electrodes was 0.0023 m^2.

2.2. Model extract (fuel) and biocatalysts

The cells were loaded with 7 mL from a model extract similar to that produced in the anaerobic fermentation of solid wastes and 193 mL of a sulphate-reducing inoculum (liquor from a suspended-growth sulphate reducing bioreactor.) The model extract consisted of a mixture of the following substances (in g/L): acetone, ethanol, acetic, propionic, and butyric acids (4 each), mineral salts such as $NaHCO_3$ and Na_2CO_3 (3 each) and K_2HPO_4 and NH_4Cl (0.6 each). Organic matter concentration of model extract was ca. 25 g chemical oxygen demand (COD)/L, whereas the initial organic matter concentration in the cell content was 1120 mg COD/L. The initial pH of the solution was 7.66 and the conductivity was 20 mS/m.

2.3. Characterization of single chamber vertical MFC

2.3.1. Variable resistance method

The MFC was loaded with substrate and inoculum as described in section 2.2. MFC was batch-operated for 7 h at 35 ºC. First, the MFC was operated at open circuit for 1 h. Afterwards, the external resistance R_{ext} was varied from 10Ω to 1MΩ and vice versa. After this, the cell was set to open circuit condition for 1 h in order to check the adequacy of the procedure (values of initial and final open circuit voltages should be close). Monitoring of both the voltage and current intensity was carried out, according to procedures suggested elsewhere [17,18]. The voltage was measured and recorded with a Multimeter ESCORT 3146A. The current intensity (I) was calculated by Ohm`s law:

$$I=E/R \quad (1)$$

The delivered power was obtained as the product of the current intensity times the voltage, that is:

$$P=I \times E \quad (2)$$

Two types of measurements were done, one using a whole cell, two-electrode configuration (W), and the other using a three-electrode configuration with a reference electrode (Saturated calomel electrode-SCE) inserted near the anode (anode– working electrode, cathode acts as counter electrode, named as A configuration; and cathode- working electrode, anode acts as counter electrode, named as C configuration). The whole cell measurements were done using the cathode as the working electrode and the anode as the reference and counter electrodes [19].

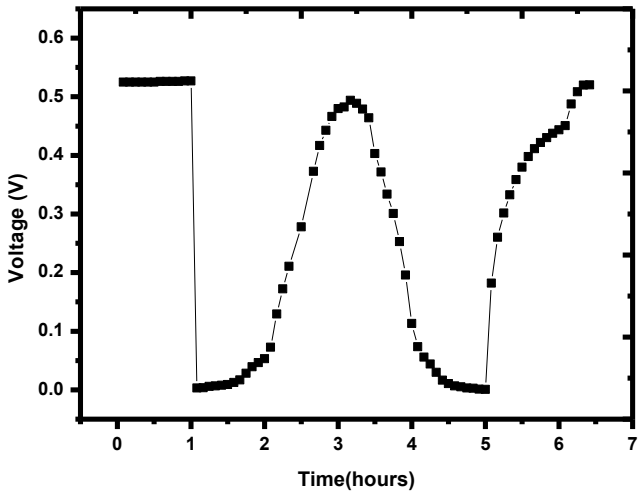

Figure 2. Voltage vs. time curve behavior of single chamber microbial fuel cell

2.3.2. Linear sweep voltammetry

Linear sweep voltammetry (LSV) was run at the recommended scan rate of 1mV s^{-1} starting from the measured open circuit potential [1] using a 273A Potentiostat/Galvanostat from EG&G Princeton Applied Research. Two types of measurements were done, one using a whole cell, two-electrode configuration, and the other using a three-electrode configuration with a reference electrode (Saturated calomel electrode-SCE) inserted near the anode similarly to what was described above.

2.3.3. Electrochemical Impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) measurements were performed in order to monitor the internal resistance of the SCMFC at the open circuit potential (V_{OCP}) and the two applied voltages, i.e., Vo = 0 mV and V100 = 100 mV. Similarly to what

Table 1. Characteristics of the single chamber microbial fuel cell using various techniques

Technique		I_{max} (mA/m^3)	P_{max} (mW/m^3)	E_{max} (vs SCE*)	R_a	R_m	R_c	R_{int}
Variable Resistance Method	Anode	551±1	61±4	0.11±0.01	1726±2	NA	NA	NA
	Cathode	68 ±1	5.1±0.2	0.076±0.01	NA	NA	5652±6	NA
	Whole Cell	459±2	93±1	0.20±0.01	NA	NA	NA	2225±16
Linear Sweep Voltammetry (1mV/s)	Anode	1341±7	240±3	0.18±0.01	496±5	NA	NA	NA
	Cathode	989±18	79±2	0.08±0.01	NA	NA	539±9	NA
	Whole Cell	696±3	198±6	0.28±0.01	NA	NA	NA	1601±3
EIS	Anode	NA	NA	NA	932±2	1.6±0.15	461.5±10	1395±9
	Cathode	NA	NA	NA	1299±13	2.3±0.06	408±2	1709±6
	Whole Cell	NA	NA	NA	1938 ±7	2±0.3	367.9±2	2307±9

*Saturated calomel electrode ; $R_{int}=R_{anode}+R_{membrane}+R_{cathode}$; NA- not applicable

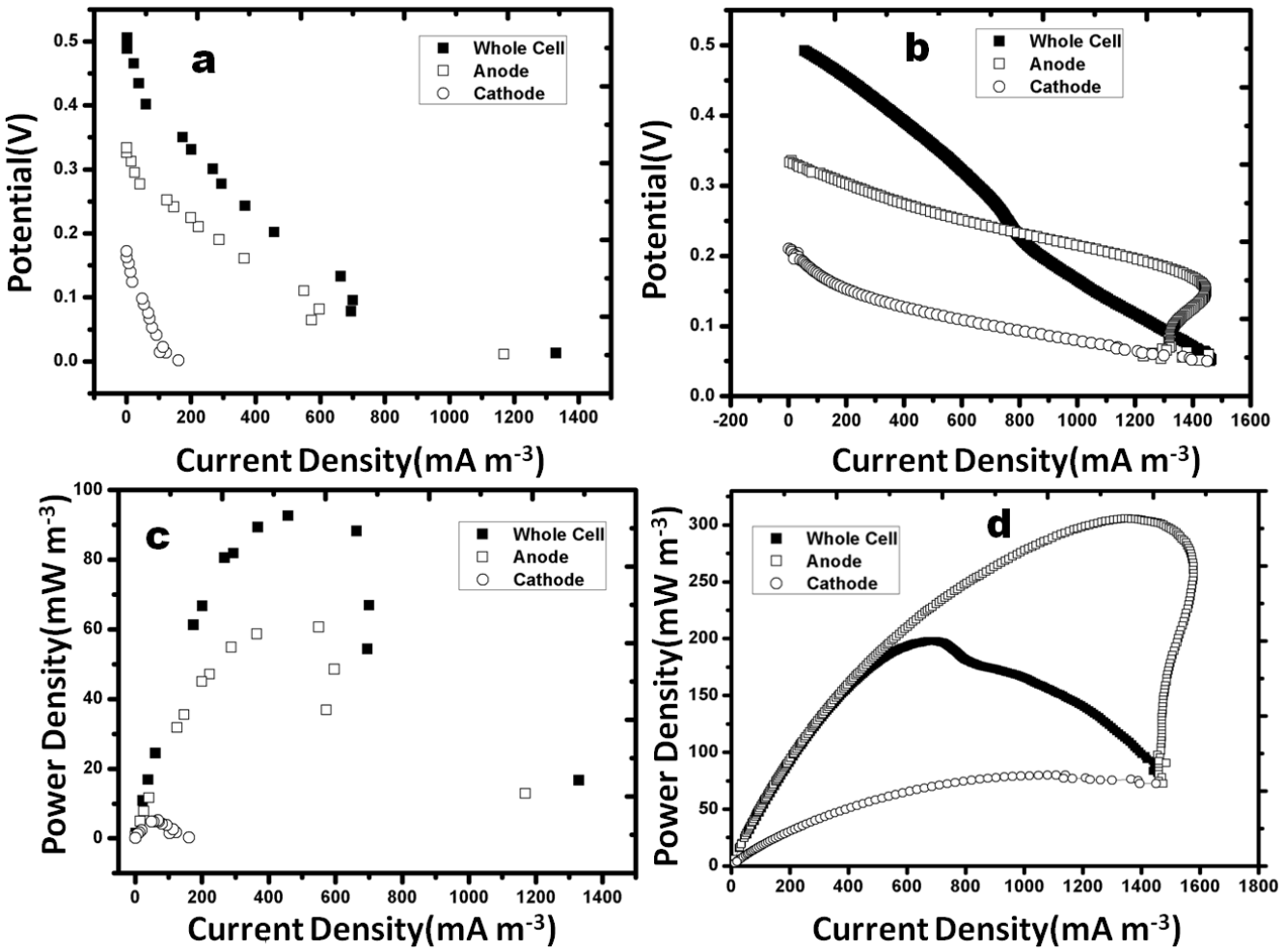

Figure 3. Evaluation of single chamber microbial fuel cell: (a) polarization curve-VR method; (b) polarization curve-LSV method; (c) volumetric power curve-VR method; (d) volumetric power-LSV method.

was described above, were done with configurations W, A, and C. The frequency range was 100 kHz-1mHz, the amplitude of the signal perturbation was 10mV.

2.3.4. Cyclic voltammetry

Cyclic voltammetry (CV) studies were performed to monitor the bacterial activity on the electrode surface with a 273A Potentiostat/Galvanostat from EG&G Princeton Applied Research. The working electrode was the anode. The reference electrode was a saturated calomel electrode (SCE) that was in contact with the cell liquor through a saline bridge known as Luggin capillary tube placed closely to the working electrode. The counter electrode was the cathode. The scan rate was 20mV/s.

3. RESULTS AND DISCUSSION

The single chamber MFC was operated at open circuit voltage for 1 h and a maximum voltage of 0.527 V was attained. Afterwards, the R_{ext} was varied from 10Ω to 1MΩ and backwards. After this, the cell was again kept at OCP conditions for 1 h to check that the maximum voltage was attained again (Fig. 2), that is, 0.525 V. This feature validated the application of the VR to our cell. The comparison of VR and LSV methods was performed on three different configurations designated whole cell (W), Anode (A) and Cathode (C) as detailed in Table.1. With the VR method, the whole cell system achieved the maximum power density ($P_{VR\text{-}max}$) of 93 ± 0.3 mW/m^3 (per volume) at the current density (IVR-max) of 459 ±2 mA/m^3 and the potential (EVR-max) of 0.202 ± 0.01 V (Fig. 3.). The LSV method in whole cell configuration yielded a maximum power density ($P_{LSV\text{-}max}$) of 198 ±6 mW/m^{3}at the current density ($I_{LSV\text{-}max}$) of 696 ±3 mA/m^3 and the potential ($E_{LSV\text{-}max}$) of 0.284 ±0.003 V. In a whole cell configuration of the SCMFC, $P_{LSV\text{-}max}$ was two-fold the value of the $P_{VR\text{-}max}$, whereas $E_{LSV\text{-}max}$ was 29 % higher than $E_{VR\text{-}max}$. One possible reason for the higher power density observed with the LSV method is that with this technique the long times between switching circuit loads during a fed-batch cycle for biological systems are avoided or eliminated [20] and it may reduce the power overshoot of the system by rapidly measuring the potential at the steady state [12,14]. The discrepancy between the anode and cathode polarization values estimated in Table 1 could be partially explained by the time sequence of

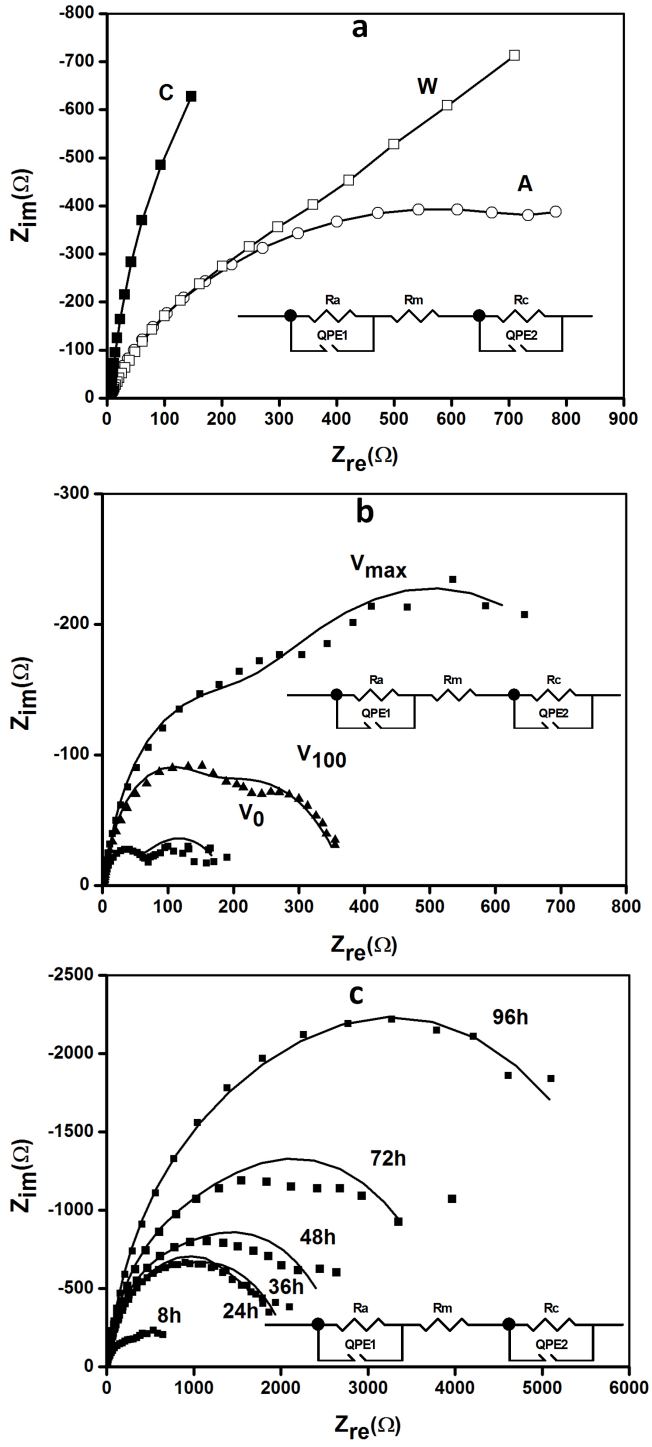

Figure 4. Spectra and equivalent circuits of the microbial fuel cell: (a) EIS spectra of the cell at three different configuration (whole cell: W; anode configuration: A; cathode configuration: C). Inset in this figure shows the electric circuit that fits the impedance spectra. (b) EIS spectra of three different applied cell voltages at V_{OCP}, V_{100} = 100 mV, and V_0 = 0 mV ;(c) Evolution of internal resistance of the microbial fuel cell with time, W configuration and open circuit potential conditions.

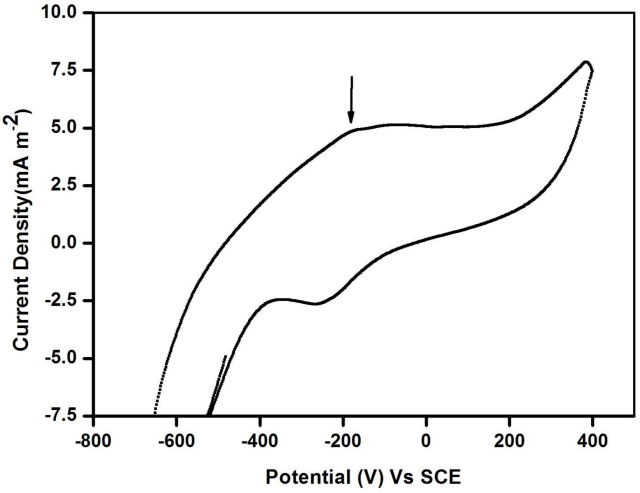

Figure 5. Cyclic voltammetry of sulphate reducing inoculum, arrow shows the peak potential similar to the bacterial cytochrome activity could be involve in exo-electron transfer process.

these experiments. The anode polarization test was conducted immediately after loading the sulphate reducing inoculum and substrate to the cell; afterwards, the cathode polarization experiment was carried out.

The polarization curves from VR are shown in Fig. 4(a). The corresponding internal resistance values for each configuration tested in Fig. 4(a) are shown in Table 1; they were 2225, 1726 and 5652 Ω for W, A, and C configuration, respectively.

The polarization curves from LSV are shown in Fig. 4(b). The corresponding resistance values for each configuration in Fig. 4(b) are depicted in Table 1; they were 1601, 496, and 539 Ω for the W, A, and C configurations, respectively. Internal resistance of the whole cell determined with LSV was nearly 30% lower than that of VR method. It seems that the value obtained with LSV could be the most reliable since this technique reduces power and potential overshoot.

Fig. 4a shows the EIS spectra of the SCMFC corresponding to the three configurations W, A, and C. The Inset exhibits the equivalent electric circuit that fits the impedance spectra. The values were obtained by fitting the Nyquist data with an equivalent circuit shown in Table 1 (Fig. 5(a)). The internal resistance of the MFC is defined as the sum of the elementary resistances, i.e., $R_{int}=R_{anode}$ (R_a)+ $R_{cathode}$(R_c)+$R_{membrane}$ (R_m). QPE_c and QPE_a in the equivalent circuit are related to the double layer capacitance of the cathode and anode respectively. The internal resistance of W configuration (R_{int-W}) was 2307± 9 Ω. This value was very close to that measured by VR, i.e., 2225±16 Ω although it was higher than the value determined by LSV (1601±3 Ω). The major contribution to the internal resistance determined by EIS was from the anode in the three configurations tested, as it is shown in Table.1.

The parameters of the electrical circuits that best fitted the impedance spectra are listed in Table. 2. At OCP the cell an R_{int-W} of 884±2 Ω. At the applied potential of 100mV (V_{100}) and 0 mV (V_0) the values of R_{int-W} were substantially lower, i.e., 372±7 and 194±6Ω, respectively (Table 2). The contribution of the anodic

resistance alone accounted for 70 to 80% of the total internal resistance value (Table 3). The cathode resistance R_c was the second resistance of the circuit in importance. On the other hand, R_m was almost negligible and kept approximately constant with applied voltage. In all cases the R_{int} decreased with the applied cell voltage and increasing the current flow, in agreement with results reported elsewhere [18, 21].

In order to monitor the internal resistance of the system with respect to time and likely to bacterial growth behaviour, several EIS spectra were acquired at different times under OCP condition (Fig. 4(c)). In this test the microbes were not subjected to selective pressures in the sense of preferent growth of electrochemical active bacteria since no net electron transfer processes occur at OCP [13].

Fitted parameter values at different time are listed in Table 3. In general, R_{int-W} increased with time; an exponential equation fitted the resistance increase (Eq. 3 below), formally similar to the exponential growth of microorganisms.

$$R_{int}(t) = a * exp(b * t); \quad R^2 = 0.9829 \qquad (3)$$

Where t is time (h), a is the fitted initial resistance equal to 1122 Ω; b is an exponent equal to 0.0181 (1/h).

Yet, final increases of the resistance value with time could also be due to loss of microbial activity such as that experienced in the declining phase [22]. It could be assumed that the kinetics of the anode is slower than that of the cathode [3, 23]. Therefore, we assigned the highest value of the fit to R_a. In this regard, the R_a determined by EIS for the W configuration is 932 Ω (Table 1), which is close to the regression value 1122 Ω in Eq 3.

Cyclic voltammetry tests revealed oxidation and reduction peaks at -0.180 and -0.250 V (Fig. 5). This pattern may reflect the redox activity on the surface of the anode material. The midpoint potential deduced from the CV was -0.215 V vs SCE. This value was close to the cytochrome redox potential described by C.Aubert et al. (1998)[24]. It is likely that the extracellular electron transfer process might be carried out by cytochrome of sulphate reducing bacteria and deliver the electron to the electrode surface.

Table 2. Parameter values obtained by electrochemical impedance spectroscopy at three different applied cell voltages (V_{OCP}, V_{100} = 100 mV, V_0 = 0 mV) and cell in whole configuration W.

Applied Cell Voltage (mV)	R_{anode}(Ω)	$R_{cathode}$(Ω)	$R_{membrane}$(Ω)	R_{int-W}[a](Ω)
OCP[b] (mV)	736±4	148±2	1.331±0.001	884±2
V_{100}[c] (mV)	255±3	115±4	1.326±0.001	372±7
V_0[d] (mV)	151±3	41±2	1.299±0.006	194±6

Notes: [a] $R_{int-W}=R_{anode}+R_{cathode}+R_{membrane}$; [b] open circuit potential; [c] 100mV; [d] 0mV

Table 3. Values of overall internal resistance obtained by electrochemical impedance spectroscopy at different times of operation, cell at OCP conditions and whole configuration W.

Time (hours)	R_{anode}(Ω)	$R_{cathode}$(Ω)	$R_{membrane}$(Ω)	R_{int-W}[a](Ω)
8	736±4	148±1	1.331±0.001	884±6
24	1604±3	493±2	1.342±0.002	2100±6
36	1657±2	483±2	1.347±0.001	2147±1
48	2199±1	533±1	1.351±0.05	2740±2
72	3421±2	616±4	1.358±0.002	4048±9
96	5527±1	822±1	1.409±0.006	6361±11

Notes: [a] $ren'_{t-W}=R_{anode}+R_{cathode}+R_{membrane}$

4. CONCLUSION

A single-chamber microbial fuel cell (SCMFC) loaded with a sulfate reducing bacterial consortium as biocatalyst in the anodic chamber was characterized by the variable resistance (VR) and linear sweep voltammetry (LSV) methods, as well as other techniques. From VR a whole cell configuration maximum volumetric power of 92.5 mW m^{-3} was attained at a current density of 459 mA m^{-3} and potential of 0.202 V. The LSV method of whole cell configuration gave a higher maximum power density of 197.5 mW m^{-3} at the current density of 696 mA m^{-3} and the potential of 0.284 V; this disagreement was ascribed to reduction of power and potential overshoot with the LSV.

There was a fair agreement between internal resistance values of whole cell configuration determined by VR and electrochemical impedance spectroscopy (EIS): 2225 and 2307 Ω, respectively. Yet, internal resistance measured by LSV was 30% lower for the whole cell configuration.

Both LSV and EIS show the advantage of reduced potential overshoot; yet, EIS provides more detailed information on equivalent circuit of the cell and resistance contributions of the electrodes, electrolyte and membrane. Information from cyclic voltammetry tests showed a midpoint potential of -0.215 V vs saturated calomel electrode, a value close to those reported for bacterial cytochromes involved in extracellular electron transfer processes. It is concluded that in spite of particular advantages of some techniques over others, the combination of electrochemical methods can be very valuable for shedding light and internal checking of the main characteristics of a microbial fuel cell.

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