Performance of microbial electrolysis cells with bioanodes grown at different external resistances

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ABSTRACT

Bioelectrochemical systems need an anode with a high abundance of exoelectrogenic bacteria for an optimal performance. Among all possible operational parameters for an efficient enrichment, the role of external resistance in microbial fuel cell (MFC) has gained a lot of interest since it indirectly poises an anode potential, a key parameter for biofilm distribution and morphology. Thus, this work aims at investigating and discussing whether bioanodes selected at different external resistances under MFC operation present different responses under both MFC and microbial electrolysis cell (MEC) operation. A better MEC performance (i.e. shorter start-up time, higher current intensity and higher H2 production rate) was obtained with an anode from an MFC developed under low external resistance. Quantitative real-time polymerase chain reaction (qPCR) confirmed that a low external resistance provides an MFC anodic biofilm with the highest content of Geobacter because it allows higher current intensity, which is correlated to exoelectrogenic activity. High external resistances such as 1,000 Ω led to a slower start-up time under MEC operation.

Key words | external resistance, microbial electrolysis cell (MEC), microbial fuel cell (MFC), quantitative real-time PCR (qPCR)

INTRODUCTION

Bioelectrochemical systems (BESs) are an emerging technology focused on converting waste into energy (microbial fuel cells, MFCs) or added-value chemical compounds (microbial electrolysis cells, MECs). BESs rely on the anode enrichment with exoelectrogenic bacteria, also called anode respiring bacteria. These bacteria have the ability to transfer the electrons from their metabolism to an external solid anode. These electrons flow through an electrical circuit to a cathode (Logan 2009), where a reductive reaction occurs. In an MFC, where oxygen reduction takes place on the cathode, the overall process is spontaneous and electricity is produced (Logan 2008). On the other hand, in an MEC, additional energy supply is required, since the reductive cathodic process is different from oxygen reduction, for example, hydrogen production (Tartakovsky et al. 2009; Ruiz et al. 2015).

Optimizing MFC performance to obtain maximum power output at affordable costs has been the research goal of many authors so far. Many different factors, from reactor configuration to medium composition, have been studied (Borole et al. 2009; Kiely et al. 2011; Velasquez-Orta et al. 2011). Among them, the role of the external resistance in MFCs has gained a lot of interest due to its significant effect on biofilm characteristics. In this frame, Jung & Regan (2011) showed that the use of different external resistances modifies the anodic potential, which is equivalent to changes in the anode’s capacity to accept electrons. These changes influence the competition between exoelectrogenic and non-exoelectrogenic bacteria, as, for example, methanogens. Zhang et al. (2011) stated that the external resistance affected MFC performance and, also, the structural and morphological characteristics of anodic biofilms. Rismani-Yazdi et al. (2011) demonstrated that changes in the external resistance affected not only the MFC performance but also the microbial metabolism and the relation between planktonic and anode-attached biofilm. Conversely, Lyon et al. (2010) evaluated the effect of external resistance on power production and microbial community structure and observed that power production was independent of external resistance even though the internal biofilm...
structure varied. Finally, Premier et al. (2011) showed that MFC performance could be increased by using a real-time dynamic control strategy that modified on-line the external resistance to match the internal MFC resistance estimated with power curves.

These previous works show how the choice of a certain external resistance influences several key factors that affect the final MFC performance in terms of power output in MFC. The choice of an external load equal to the internal cell resistance leads to an optimum MFC operation (Premier et al. 2011). On the other hand, the external resistance indirectly poises an anode potential, a key parameter in BESs, since it determines the energy gain for the bacteria, and thus enhances the growth of different anode microbial populations and determines the biofilm morphology. In fact, many authors control the anode potential of BESs using a potentiostat (Aelterman et al. 2008; Wagner et al. 2010; Batlle-Vilanova et al. 2014), which is a rather expensive piece of equipment. In contrast, the use of external resistances is a low-cost procedure to regulate the anode potential and the current. While the effect of external resistance on MFC performance has already been described, the response of microbial communities grown with different external resistances when operated as MEC is yet to be studied.

The start-up of an MEC can be conducted from an already running MFC (the most usual scenario) or from anaerobic sludge. In the latter case, there are two options: operating with a fixed anodic potential (using a potentiostat) or with a fixed applied potential (using a power source). Again, the potentiostat is an expensive option, but the start-up with a power source may be slower and prone to failure due to the lack of biofilm and to the uncontrolled anodic potential. Inoculating an MEC with an already running MFC can avoid the use of a potentiostat due to the already developed exoelectrogenic biofilm. Hence, this work aims at investigating and discussing whether bioanodes selected at different external resistances under MFC operation present different responses when operated as MEC with a fixed applied potential.

**METHODS**

**MFC and MEC operation**

Three cube-shaped air-cathode MFCs were used in this work. Each MFC was a 28 mL methacrylate cylindrical vessel provided with a lateral aperture (3.8 cm diameter), where the cathode was fitted. The cathode consisted of a graphite fiber cloth (3.8 cm diameter, 7 cm² total exposed area) coated with platinum (5 mg Pt/cm², ElectroChem Inc.) in the catalytic layer and a polytetrafluoroethylene diffusion layer which permitted oxygen diffusion into the cell while preventing water leakage (Cheng et al. 2006). It was placed 2.5 cm apart from the anode. The anode was a graphite fiber brush (20 mm diameter × 30 mm length; 0.18 m²) made with fibers (diameter 7.2 μm, PANEX33 160 K, MillRose Company, Mentor, OH, USA) connected with a titanium wire core.

The anode was inoculated by mixing (in a volume ratio 1:1) fresh medium and media from an already working Inoculum-MFC (with an external resistance of 12 Ω), built as described in Ribot-Llobet et al. (2014) using a 400 mL glass vessel with a lateral aperture (6.3 cm diameter) for the air-cathode assembling. For each cube-shaped air-cathode MFC, the external resistance to connect both electrodes was different: 12 Ω (MFC12), 220 Ω (MFC220) and 1,000 Ω (MFC1000).

The MFCs achieved stable operation around day 60, when similar performance was observed in consecutive cycles. Subsequently, the configuration was changed to MEC by transferring the MFCs, anodes to three different MECs as detailed in Montpart et al. (2014) and with the application of a constant potential of 0.8 V. Both MFCs and MECs were operated in batch mode. The fresh medium was a 100 mM phosphate buffer with the following components in 1 L of deionized water: NH₄Cl (0.41 g), mineral media (5 mL), 1 mL of 4 g L⁻¹ FeCl₂ stock solution, and 0.5 mL of 37.2 g L⁻¹ Na₂S·9H₂O stock solution, and fed with 30 mM of acetate. The mineral media had the composition previously described in Parameswaran et al. (2009).

**Chemical and electrochemical analysis**

Acetate was analyzed by gas chromatography (Agilent Technologies, 7820-A, Santa Clara, CA, USA) using a flame ionization detector with helium as carrier gas. H₂ and CH₄ were also analyzed with the same gas chromatograph equipment but using a thermal conductivity detector with argon as carrier gas, as described in Ruiz et al. (2013). Chronoamperometric analyses were performed to evaluate MEC performance using a Multi Autolab system (Ecochemie, Utrecht, The Netherlands). The anode was set as the working electrode and the cathode was used as both the auxiliary and the reference electrode. The anode potential (vs. cathode potential) was set at 13 levels ranging between 250 mV and 850 mV in steps of 50 mV. Each potential was set for 300 s to allow current intensity to stabilize. The last data point corresponding to each potential was used to build the curve Current intensity vs. Electrode potential. Similar chronoamperometric measurements were used to
build polarization curves for MFCs (Cell potential vs. Current intensity). In this case, the anode potential (vs. cathode potential) was set at 17 levels from ~675 mV (anode open circuit potential) to ~−20 mV. The applied potentials were negative, because it is equivalent to using different external resistances. Finally, power curves (Power vs. Current intensity) were calculated from the polarization curves as the product between potential and current intensity.

Electrochemical calculations

Coulombic efficiency (CE) was calculated as in Equation (1):

$$\text{CE} = \frac{\text{Coulombs recovered as current intensity}}{\text{Coulombs in substrate}} = \frac{\int_{t_0}^{t_f} I dt}{F \cdot b_{Ac} \cdot V_L \cdot \Delta c \cdot M^{-1}}$$ (1)

where $t_0$ and $t_f$ are the initial and final time of an experiment, $\Delta c$ is the change in acetate concentration during the experiment ($g$ acetate·L$^{-1}$ cell), $M$ is the molecular weight of acetate (59 g·mol$^{-1}$), $b_{Ac}$ is the number of e$^{-}$ transferred per mole of acetate (8 mol e$^{-}$·mol$^{-1}$ acetate), $F$ is Faraday’s constant (96,485 C·mol$^{-1}$·e$^{-}$), $I$ is the current intensity and $V_L$ is the volume of liquid in the reactor (L).

Energy recovery of the MEC was calculated as the amount of energy contained in the produced hydrogen with respect to the electrical input ($r_E$) (Equation (2)) and to the electrical input and the energy content of the substrate ($r_{E+S}$) (Equation (3)).

$$r_E = \frac{n_{H_2} \cdot \Delta H_{H_2}}{\int_{t_0}^{t_f} (I \cdot E_{ap} - I^2 \cdot R_{ext}) dt}$$ (2)

where $n_{H_2}$ are the moles of produced hydrogen, $\Delta H_{H_2}$ is hydrogen heat of combustion (−285.83 kJ·mol$^{-1}$), $E_{ap}$ is the applied voltage (V) and $R_{ext}$ is the external resistance used for monitoring (Ω).

$$r_{E+S} = \frac{n_{H_2} \cdot \Delta H_{H_2}}{n_S \cdot \Delta H_S + \int_{t_0}^{t_f} (I \cdot E_{ap} - I^2 \cdot R_{ext}) dt}$$ (3)

where $n_S$ are the moles of consumed acetate and $\Delta H_S$ is the heat of combustion of acetate (−870.28 kJ·mol$^{-1}$).

Quantitative real-time polymerase chain reaction

Quantitative hydrolysis probe based real-time polymerase chain reaction (qPCR) was used to quantify exoelectrogenic proteobacteria Geobacter as a member of the Fe(III)-reducing Geobacteraceae family. qPCR was performed in a LightCycler 480 instrument (LC480; Roche, Basel, Switzerland) using the corresponding primers GEO561F (5′-GCCATGCACCCWCT-CW-3′) and GEO825R (5′-GGTCGAGACCGTGGTCTTCAA-3′) and the Gbc1 Taqman probe (5′-AGCACCACACCGGCT-GGA-3′) previously described (Stults et al. 2001; Cummings et al. 2003). Each reaction mixture of 20 μL was prepared using the LightCycler 480 Probe Master kit (Roche Diagnostics) with final concentration 100 nM, hydrolysis probes (final concentration 100 nM), 2X LC480 Probe Master and 2 μL of template DNA. Geobacter was quantified as described by Rago et al. (2015a). All DNA templates were analyzed in duplicate. A detailed description of the quantitative standard curves generation can be found in Rago et al. (2015).

RESULTS AND DISCUSSION

MFC operation with different external resistances

Three MFCs were inoculated by mixing (1:1) the effluent from an already working MFC (with an external resistance of 12 Ω) and fresh medium. Each MFC was operated in batch cycles (5 days per cycle) using a different external resistance: 12 Ω (MFC12), 220 Ω (MFC220) and 1,000 Ω (MFC1000). Current intensity and power values obtained from the first five batch cycles for the three MFCs are shown in Figure 1.
A higher current intensity was obtained under lower external resistance, in agreement with previous literature reports (Jung & Regan 2011; Rismani-Yazdi et al. 2011). Regarding CE, higher values were obtained for lower external resistances: MFC$_{12}$ (74%), MFC$_{220}$ (53%) and MFC$_{1000}$ (23%). Finally, the lowest power output was obtained at lower external resistances despite its high current intensity. This observation was previously discussed in the literature. Zhang et al. (2011) stated that this lower power output might be due to the significant ohmic losses resulting from void spaces in the interior of the biofilm. Zhang et al. (2011) also demonstrated that the use of an external resistance lower than the optimum value generates a reduction of the electrical conductivity within the biofilm, which has a higher active biomass but also a higher exopolysaccharide content. On the other hand, the highest power output was obtained from MFC$_{220}$ (MFC$_{220}$: 718 $\mu$W, MFC$_{12}$: 86 $\mu$W and MFC$_{1000}$: 279 $\mu$W) due to the closeness between the external resistance and the cell internal resistance. The internal resistances of the MFCs were estimated through polarization curves, obtaining values around 100 $\Omega$ for MFC$_{250}$ and MFC$_{1000}$, and slightly higher for MFC$_{12}$ (118 $\Omega$ for MFC$_{12}$; 101 $\Omega$ for MFC$_{250}$ and 95 $\Omega$ for MFC$_{1000}$). Therefore, the experimental data in this work corroborate that an optimal external resistance has to be used to obtain the highest sustainable current generation and power output. In this sense, many efforts in on-line optimization of the external resistance have been reported in the literature (Logan 2008; Pinto et al. 2011; Molognoni et al. 2014).

Transference of MFC anodes to MEC

After 60 days of MFC operation, the anodes of MFC$_{12}$, MFC$_{220}$ and MFC$_{1000}$ were transferred to three different MECs (MEC$_{12}$, MEC$_{220}$ and MEC$_{1000}$) with 0.8 V of applied voltage between anode and cathode provided by a power source. The current intensity values obtained from the three MECs during 4 weeks are shown in Figure 2. The anodes obtained from MFC$_{12}$ and MFC$_{220}$ (i.e. inoculated with lower external resistances) were immediately adapted to the new configuration. The maximum current intensity was achieved from the first
cycle in MEC$_{12}$ and from the second cycle in MEC$_{220}$. The cycle-length of each batch was lower in MEC mode than in MFC mode (2 vs. 5 days) since the intensity in MEC was higher and the cycles had the same initial amount of acetate. On the other hand, the anode from MFC$_{1000}$ needed more than 1 week (five MEC batch cycles) to obtain its best performance. Although the initial current intensity produced by MEC$_{12}$ was the highest, it decreased around 15% throughout the 25 days of MEC operation, whereas the current intensity produced by MEC$_{220}$ and MEC$_{1000}$ did not decrease after the start-up period. Despite the 15% decrease in current intensity for MEC$_{12}$, this cell provided the best performance and faster adaptation to the MEC operation. The volume of hydrogen produced was measured for each MEC at the end of each of the last two batch cycles. Hydrogen production rate in MEC$_{12}$ (1.54 ± 0.13 LH$_2$ L$^{-1}$ REACTOR$^{-1}$ d$^{-1}$, $n = 2$) was slightly higher than that in MEC$_{220}$ (1.34 ± 0.02 LH$_2$ L$^{-1}$ REACTOR$^{-1}$ d$^{-1}$, $n = 2$) or MEC$_{1000}$ (1.1 ± 0.3 LH$_2$ L$^{-1}$ REACTOR$^{-1}$ d$^{-1}$, $n = 2$) in agreement with the trend in current intensity values. Moreover, in all cases, the CE was around 90%.

Common MEC performance indexes were calculated for the three MECs. The values obtained are in agreement with those found in the literature for similar systems (Ruiz et al. 2015) and indicate that a positive energy recovery is obtained when considering the electrical input. The energy recovery ($r_E$) was 145 ± 9% for MEC$_{12}$, 158 ± 13% for MEC$_{250}$ and 153 ± 27% for MEC$_{1000}$. The energy recovery obtained considering the substrate internal energy and the electrical input ($r_{E,I}$) was 54 ± 7% for MEC$_{12}$, 55 ± 9% for MEC$_{250}$ and 54 ± 6% for MEC$_{1000}$. These results do not show a clear trend among the different MECs in steady-state operation.

Supplementary Figure S1 (available with the online version of this paper) shows the chronoamperometric measurements from the three MECs after 4 weeks of operation. For a certain applied potential, higher intensity values were obtained from the anode operated with lower external resistance. For example, at an applied potential of 0.8 V, the highest current intensity was obtained from MEC$_{12}$ (7.7 mA) and from MEC$_{220}$ (6.5 mA), while in MEC$_{1000}$ (4.5 mA) it was 41% lower than that obtained for MEC$_{12}$.

Regarding the microbiological analysis, Figure 3 compares the Geobacter quantification by qPCR from the inoculum and the anodes of MFC and MEC. Black: 12 Ω, light grey: 220 Ω, dark grey: 1,000 Ω.

Figure 3  | qPCR results of Geobacter (mean of triplicate values ± standard deviation) in the inoculum and anodes of MFC and MEC. Black: 12 Ω, light grey: 220 Ω, dark grey: 1,000 Ω.

![Figure 3](image_url)

but only 1.48 × 10$^6$ gene copies/mg were obtained from MFC$_{1000}$. Planktonic Geobacter concentration in the inoculum sample was lower compared with anodic biofilm samples obtained from the MFCs (around 20 times in the case of MFC$_{12}$ and MFC$_{220}$, and two times for MFC$_{1000}$). Then, the development of a biofilm with a high content of Geobacter was possible in all the MFC anodes, although the best development was observed at the lowest external resistances. This higher Geobacter concentration under lower external resistances agrees with the fact that the CE under those resistances was higher.

The amount of Geobacter in MECs was higher than in MFCs. No clear trend was detected between MEC anodic biofilm samples, in contrast to MFCs: after 4 weeks of operation all MECs had almost the same amount of Geobacter, between 4.38 × 10$^7$ (MEC$_{12}$) and 4.92 × 10$^7$ gene copies/mg (MEC$_{1000}$), regardless of the external resistance used in MFC operation. Furthermore, it was demonstrated that the applied potential and the anoxic conditions of MEC reactors favored Geobacter growth in the anodic biofilm, which resulted in a concentration more than double with respect to the MFCs’ anodic biofilm. The improved operational conditions with respect to MFC (total absence of oxygen and applied voltage) also justified the increased CE around 90% in all three MECs.

Considering all the results presented, this work shows that the shortest start-up time for an MEC is obtained using a very low external resistance in MFC (12 Ω). Although MFC$_{12}$ shows the lowest power output, it also shows the highest current intensity, which results in a better development of the anodic biofilm (Figure 3). This allows a very fast adaptation to MEC operation: MEC$_{12}$ is able to achieve a current intensity very close to its maximum
value in just one cycle. In addition, after the start-up period, MEC_{12} achieves the highest current intensity compared with MEC_{220} and MEC_{1000} (Figure 2) and also the maximum current intensity at different applied potentials (Supplementary Figure S1). This observation is related to published reports aiming at maximizing current intensity in MFC by optimizing external resistance (Aelterman et al. 2008; Pinto et al. 2011; Molognoni et al. 2014). An MFC operating at high current intensity will result in better results when moved to MEC mode.

The utilization of a 220 Ω resistance also provides good performance, although the current intensities are slightly lower in both MFC and MEC. High resistances such as 1,000 Ω are not recommended because Geobacter growth is more limited (lower current intensity), which leads to a slower start-up time under MEC operation. Nevertheless, under steady-state conditions, any of the three resistances led to an anodic biofilm with a high content of Geobacter, near 5 \times 10^7 gene copies/mg.

CONCLUSIONS

An anode inoculated under low external resistance in MFC mode (12 Ω) showed better performance when moved to MEC mode (i.e. it gave higher current intensity and showed higher H2 production rate) than other MFCs inoculated under higher external resistances (220 and 1,000 Ω). qPCR confirmed that the MFC under lowest external resistance had the highest content of Geobacter. Low external resistances resulted in higher current intensity, which is correlated with exoelectrogenic activity. Lower external resistances also resulted in a faster transition to stable MEC operation, since the anodic biofilm was able to drive higher current intensities. However, long-term MEC operation resulted in anodic biofilms with similar Geobacter content of around 5 \times 10^7 gene copies/mg in spite of external resistance used in the previous MFC mode.

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