# Effect of Type of Inoculum and Application of $Ru_xMo_ySe_z$ on Microbial Fuel Cell Performance

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**ABSTRACT**

This research aimed at evaluating the effect of inoculum type and the application of $Ru_xMo_ySe_z$ as a cathode catalyst on the treatment and bioelectricity production of a microbial fuel cell fed with recalcitrant, municipal leachate. The device was an air-cathode, two-face microbial fuel cell fitted with graphite flakes as anodic material (*MFC-G*). The cathode was painted with $Ru_xMo_ySe_z$ at a dose of 0.5 mg/cm$^2$. The inocula assayed in our work were a plain sulphate-reducing inoculum (*In-SR*), an enrichment in Mn(IV)-reducing bacteria (*In-*$E_{Mn(IV)}$), and two enrichments in Fe(III)-reducing bacteria, namel, *In-*$E_{Fe(III)-S}$ and *In-*$E_{Fe(III)-SR}$.

Each face (I and II) of the *MFC-G* was characterized by separate (I and II), in series and parallel connection. We found that parallel connection of electrode faces lead to significantly lower values of the internal resistnace. In the batch operation where the cells were operated with the faces connected in parallel and loaded with an external resistance of 100 Ω, enrichment of the inocula had a significant, positive effect of cell performance. The average volumetric powers $P_{V\text{-}ave}$ observed were 4 376, 9 555, 11 249, and 13 303 mW/m$^3$ for the *In-SR*, *In-*$E_{Mn(IV)}$, *In-*$E_{Fe(III)-SR}$, and *In-*$E_{Fe(III)-S}$, respectively. The high $P_{V\text{-}ave}$ registered with the enriched inocula in our work could be attributed to the synergism of increased concentrations of exoelectrogenic bacteria as well as the high total anodic surface area by the use of granular graphite that could have facilitated the electron transport to the anode. The first issue was confirmed by molecular characterization of enriched inocula. In general, values of $P_{V\text{-}ave}$ obtained with the chalcogenide catalyst were 30-40% lower than those registered with Pt catalyst. Yet, the cost of the chalcogenide is 80% lower than that of platinum. We conclude that the application of inocula enriched in Fe(III) and Mn (IV)-reducing bacteria significantly improved the performance of cells that used $Ru_xMo_ySe_z$ as a cathodic catalyst for the ORR

*Keywords:* ***$Ru_xMo_ySe_z$ cathodic catalyst, leachate, microbial fuel cell, enriched inocula***

## 1. Introduction

A microbial fuel cell (MFC) is an electro-biochemical reactor capable of directing converting organic matter into electricity [1-2]. Platinum has been commonly used as a catalyst of the oxygen reduction reaction (ORR) in MFCs. Yet the high cost of an MFC is mainly due to the high price of this noble metal. This, in turn, could deter the commercial MFC applications. So, the development of new materials with high catalytic properties to perform oxygen reduction is presently a task of great importance [3-6]. One of the challenges in microbial fuel cells research consists of the application of new electrochemically active catalytic materials (such as RuxMoySez [7]) as alternative electrocatalysts to replace the extensive use of the more expensive platinum. Vazquez-Larios et al. [7] evaluated the application of bimetallic chalcogenide RuxMoySez, as an ORR catalyst and two anodic materials on the performance of two MFCs fed with synthetic substrate. The power delivered by cell the with fitted graphite triangular pieces and chalcogenide catalyst was 43% inferior to that of a similar cell with Pt although the cost of the first catalyst is significantly lower than that of Pt., i.e., 73% lower.

On the other hand, municipal leachate is an aggressive effluent with relatively high concentration of organic matter [8,9]. Leachate from sanitary landfills is of concern in Mexico City, since very recently the Bordo Poniente mega landfill has been closed and it is known that it generates large amounts of both fresh and aged leachates. So far, the available information on treatment of municipal leachate in MFC loaded with inoculum enriched in Mn (IV)-reducing bacteria is still scarce. Previous works have demonstrated the feasibility of using leachate as substrate in MFCs. Yet, the powers delivered were in the low-to-mid side of the range. Greenman et al. [10] demonstrated that it was possible to generate electricity and simultaneously treat landfill leachate in MFC columns, 1.35 mW/m-2 and 43 % BOD removal. Gálvez et al. [11] operated three MFCs hydraulically connected in series for simultaneous leachate treatment and electricity generation. The system when configured into a loop was able to remove 79% of COD and 82% of BOD5 after 4 days. Ganesh & Jambeck [12] operated to treat landfill leachate in cylindrical single air-cathode without inoculation; they observed a volumetric power (PV) of 699 mWm-3 and 74% COD removal. Tugtas et al. [13] investigated treatment of anaerobically pre-trated landfill leachate in a investigated in batch and continuous-flow two-chambered MFCs. They reported a PV of 2 482 mWm-3 and 90 % COD removal. On the other hand, Puig et al. [14] evaluated an air-cathode MFC fed with landfill leachate, the PV was 344 mWm-3 whereas the COD removal was 70 %. It can be seen that, in general, volumetric powers obtained in MFC fed with leachate were in the low-to-mid part of the power range.

This research aimed at evaluating the effect of inoculum type and the application of $Ru_xMo_ySe_z$ as a cathode catalyst on the treatment and bioelectricity production of a microbial fuel cell fed with recalcitrant, municipal leachate.

## 2. Experimental

### *2.1.Microbial fuel cell architecture*

The *MFC-G* consisted of a horizontal cylinder built Plexiglass 90 mm long and 57 mm internal diameter. The opposing faces of the cylindrical shell were fitted with corresponding sets of an assemblage of (inside to outside) proton exchange membrane (Nafion 117), a Toray flexible carbon-cloth containing 1 mg/cm$^2$ $Ru_xMo_ySe_z$ (20wt%/C) and a perforated plate of stainless steel 1 mm thickness. Each assemblage was corresponded with anodes made of granular graphite and a graphite rod as collector (80 mm long and 5 mm diameter). The average separation between cathode-anode in *MFC-G* was 17.5 mm. The anode chamber volume was 100 mL. All the cathodes in cell were in direct contact with atmospheric air on the perforate perforated metallic plate side. When the cathodic biocatalyst was the chalcogenide, the cathode had a loading of 1.0 mgcm$^{-2}$ $Ru_xMo_ySe_z$ 20 wt% dispersed in Vulcan carbon XC-72 as it was mentioned above. The catalytic ink was prepared by mixing 11.1 µLcm$^{-2}$ Nafion® 5 wt% and 333.3 µLcm$^{-2}$ of ethanol and the resulting suspension was sprayed onto the *PEM* of a home fabricated electronic semiautomatic device. Afterwards, the *PEM* was pressed the by hot pressing (4.4 kg cm$^{-2}$) at 120°C for 3 min to improve adherence of catalyst to the membrane [15,16].

### *2.2. Catalyst synthesis and characterization*

The $Ru_xMo_ySe_z$ catalyst was synthesized by decarbonylation of transition-metal carbonyl compounds in organic solvent, under refluxing [17,18]. The $Ru_xMo_ySe_z$ catalyst was synthesized by reacting 0.07 mM $Ru_3(CO)_{12}$ (Aldrich) with 0.20 mM $Mo(CO)_6$ (Strem) and 0.20 mM of elemental selenium (Strem) in a chemical reactor containing 150 mL of 1,6-hexanediol for 3 hours at 230 °C. The un-reacted precursors and the organic reaction medium were eliminated by several washes using organic solvents, and dried overnight at room temperature [19].

The catalyst was composed of uniform agglomerates of nanocrystalline particles with an estimated composition of $Ru_6Mo_1Se_3$, embedded in an amorphous phase. Tafel slopes for the *ORR* remained invariant with temperature at -0.116 V $dec^{-1}$ with an increase of the charge transfer coefficient in $d\alpha/dT = 1.6\times10^{-3}$, ascribed to an entropy turnover contribution to the electrocatalytic reaction. The apparent activation energy was 45.6 ± 0.5 $kJmol^{-1}$. The catalyst generated less than 2.5% hydrogen peroxide during oxygen reduction [18,19].

*2.3. Municipal leachates*

The cells were loaded with 10 mL of municipal leachates sampled from the Mexico City landfill "Bordo Poniente" stage 4 (Etapa 4). The characterization of the leachate is given in Table 1. The relatively high organic matter content and high value of $BOD_5$/COD ratio indicated that the leachate is biodegradable, and likely not very aged [6]. Interestingly, we expected a lower pH consistent with fresh leachate [9]. That was not the case. It is known that the landfill is emplaced in a site whose soil is sodic-saline soil with pH as high as 11 [9,19]. The local soil was likely used to cap the landfill cells during the daily operation of the landfill, possibly releasing sodium salts (carbonate, bicarbonate) as well as hydroxides that increased leachate pH. This explanation is supported by the high values of the electrolytic conductivity of the leachate (Table 1).

**Table 1.** Characteristics of municipal leachate.

| **Parameters** | **Value** |
|---|---|
| pH | 8.26 ± 0.02 |
| Conductivity (mS/cm) | 36.7 ± 0.1 |
| Total Kjeldahl nitrogen (g/L) | 2.9 ± 0.03 |
| $SO_4^{2-}$ (g/L) | 0.281± 0.01 |
| COD (g/L) | 12.3± 0.5 |
| $BOD_5$ (g/L) | 10.6± 0.2 |
| $BOD_5$/COD | 0.86 |

*2.4. Sample collection and enrichment of inoculum*

The inoculum enrichment was obtained with three serial transfers, described below. Sample from soil was collected in the CINVESTAV-IPN to a depth 2 m that was transferred to anaerobic bottle. The soil sample was moved to the laboratory without oxygen contact, and 5 g of soil sample was suspended in 50 mL of anaerobic saline solution. 5 mL of sample was transferred to 50 mL metal-reduction medium with acetate, Mn (IV) or Fe (III). Duplicate enrichments were incubated at 30 °C for 15 d or 9 d for Mn (IV) and Fe (III), respectively, in the dark condition. The enrichment procedure was repeated 3 times.

Metal-reduction medium consisted of ($gL^{-1}$): 2.5 $NaHCO_3$, 0.25 $NH_4Cl$, 0.6 $NaH_2PO_4.H_2O$, 0.1 KCl, 10 mL vitamin solution and 10 mL mineral solution (Lovley & Phillips,[21]). The $MnO_2$ was synthesized by slowly adding a solution of $MnCl_2$ (30 mM) to basic solution of $KMnO_4$ (20 mM) which was stirred with a magnetic stir bar. This procedure is similar to a previously described technique (Lovley & Phillips, [21]). The Fe(III) oxide was synthesized as follows: a solution 0.4 M of $FeCl_3.6\ H_2O$ (pH adjusted to 7.0 with 10 M of NaOH) was added, according to the technique described by Lovley & Phillips [21]. The $MnO_2$ was synthesized by slowly adding a solution of $MnCl_2$ (30 mM) to basic solution of $KMnO_4$ (20 mM) which was stirred with a magnetic stir bar. This procedure is similar to a previously described technique (Lovley & Phillips, [21]).

*2.5. Electrochemical characterization of the microbial fuel cells*

Potential sweep experiments were carried out from open-circuit cell voltage ($E_{OC}$), to the final potential of 0.02 V at a scan rate of $1mVs^{-1}$, performed in a potentiostat/galvanostat Voltalab model PGZ402 [22,23]. Values of $R_{int}$ were estimated from the slopes of corresponding regression lines selected in the linear range of the polarization curves. The current ($I_{MFC}$), power ($P_{MFC}$) and volumetric power ($P_V$) were calculated as previously described [24]. The power density (surface area) was normalized to the projected cathode surface area (surface power density $P_S$).

The cells were loaded with 80 mL of enriched in Mn(IV)-reducing bacteria and 20 mL of actual municipal leachate. The initial biomass concentration in the cell inoculum was *ca.* 1 300 mg $VSSL^{-1}$.

*2.6. Batch operation of the microbial fuel cells*

The batch *MFCs* (with two different inocula) were loaded with municipal leachate and inoculum enriched in Mn(IV)-reducing bacterian inoculum enriched in Fe (III)-reducing bacteria, sulphate reducing and without inoculum. The organic matter concentration in *MFCs* was *ca.* 2 g $CODL^{-1}$. The cells were loaded with 80 mL of enriched in Mn(IV)-reducing bacteria; the initial biomass concentration in the cell inoculum was *ca.* 1 000 mg $VSSL^{-1}$. The cells were operated for two Periods; in Period I the cells were run until a decrease organic matter concentration was observed. At the end of Period I, the cell was loaded with new municipal leachate (*ca.* 3.5 g $CODL^{-1}$) although the electrodes and membrane as well as the microbial community remained the same, and further operated for what we denominated In Period II.

At the start of Period I the cells were operated for 24 h to open circuit voltage. Afterwards the cells were batch-operated for a total 250 h, at ambient temperature (23°C average) without mixing. The circuit of each *MFC* was fitted with a corresponding external resistance of 100 Ω and $Ru_xMo_ySe_z$ catalyts.

The main variable responses of this experiment were the average volumetric power ($P_{V\text{-}ave}$), the efficiency of organic matter removal ($\eta_{COD}$), and the coulombic efficiency ($\eta_{Coul}$).

*2.7. Analytical methods and calculations*

The COD and VSS of the liquors of sulphate-reducing seed bioreactor and cells were determined according to the Standard Methods [25]. Manganese (Mn II) contents were analyzed by the method of Brewer and Spencer [26] as modified by Armstrong *et al.* [27] whereas the presence of Mn (IV) was assessed with a benzidine acetate reagent [28].

The $\eta_{COD}$ was calculated as

$$\eta_{COD}(\%) = \frac{(COD_i - COD_f)}{COD_i} \cdot 100 \quad (1)$$

where $COD_i$ initial chemical oxygen demand and $COD_f$ final chemical oxygen demand.

The volumetric power was estimated with Eq. 2 below

$$P_V = \frac{E_{MFC}^2}{V_{MFC} \cdot R_{ext}} \quad (2)$$

where $E_{MFC}$ is the voltage, $R_{ext}$ is the external resistance, and $V_{MFC}$ is the cell volume. The average value during the batch operation was calculated by numerical integration *versus* time using the Simpson's rule [29].

The $\eta_{Coul}$ is the ratio between the actual amount of produced electrons (CRS) to the electrons that could be produced from the substrate (CTS), as it follows:

$$\eta_{Coul}(\%) = \frac{CRS}{CTS} \cdot 100 \quad (3)$$

$$CRS = \int_0^t I_{MFC}\, dt \quad (4)$$

$$CTS = \frac{F_i \cdot b_{COD} \cdot (COD_i - COD_f) \cdot V_{MFC}}{M_{COD}} \quad (5)$$

where *F*: Faraday's constant (96 485 Coulombs $mol^{-1}$ $e^-$), $b_{COD}$: number of moles of electrons harvested from the COD (4 mol $e^-$ per mol of COD), $COD_i$: initial COD (g $L^{-1}$), $COD_f$: final COD (g $L^{-1}$), $V_{MFC}$: *MFC* operation volume (L), $M_{COD}$: COD's molecular weight (32 $gmol^{-1}$)

## 3. Results and discussion

### *3.1.Electrochemical characterization of microbial fuel cells*

The first set of experiments consisted of the evaluation of the effect of the type of inoculum (*In-*$E_{Fe(III)\text{-}S}$, *In-*$E_{Fe(III)\text{-}SR}$, *In-*$E_{Mn(IV)}$ and *In-SR*) on the electrochemical characteristics of the MFC-G. Each face of the MFC-G was characterized by separate (I and II), in series, and in parallel electric arrangements.

Parallel connection of faces increased the maximum volumetric power $P_{V\text{-}max}$ up to 14 521, 15 825, 16 359 and 9293 $mWm^{-3}$ (Figure 1), compared with series connection 10 377, 12 778, 10 685 and 6 842 $mWm^{-3}$. Parallel connection significantly decreased the $R_{int}$ of the cells and almost doubled volumetric power. The $P_{V\text{-}max}$ for the MFC-G (*In-*$E_{Fe(III)\text{-}S}$, *In-*$E_{Fe(III)\text{-}SR}$, *In-*$E_{Mn(IV)}$ and *In-SR*) when faces were connected in series and parallel were higher than those reported by Ortega-Martinez *et al*. [30], Puig *et al.* [14] and Ieropoulos *et al.* [31]. Our $P_{V\text{-}max}$ was superior to that reported by Ortega-Martinez *et al.* [28] for the characterization of a novel, multi-face parallelepiped MFC (Pt for ORR); this cell was fitted with a 'sandwich' cathode-membrane-anode assemblage in five of their faces. When the 5 faces of the MFC-P were connected in series and parallel, the $P_{V\text{-}max}$ achieved values of 62 and 570 $mWm^{-3}$, respectively. On the other hand, Puig *et al.* [14] also characterized an air-cathode MFC loaded with landfill leachate and estimated a $P_{V\text{-}max}$ of 278.2 $mWm^{-3}$. Ieropoulos *et al.* [31] compared the performance of two small size MFCs connected in parallel using acetate as the substrate and a seed that consisted of anaerobic sludge; the characterization studies gave a $P_{V\text{-}max}$ of 860 $mWm^{-3}$.

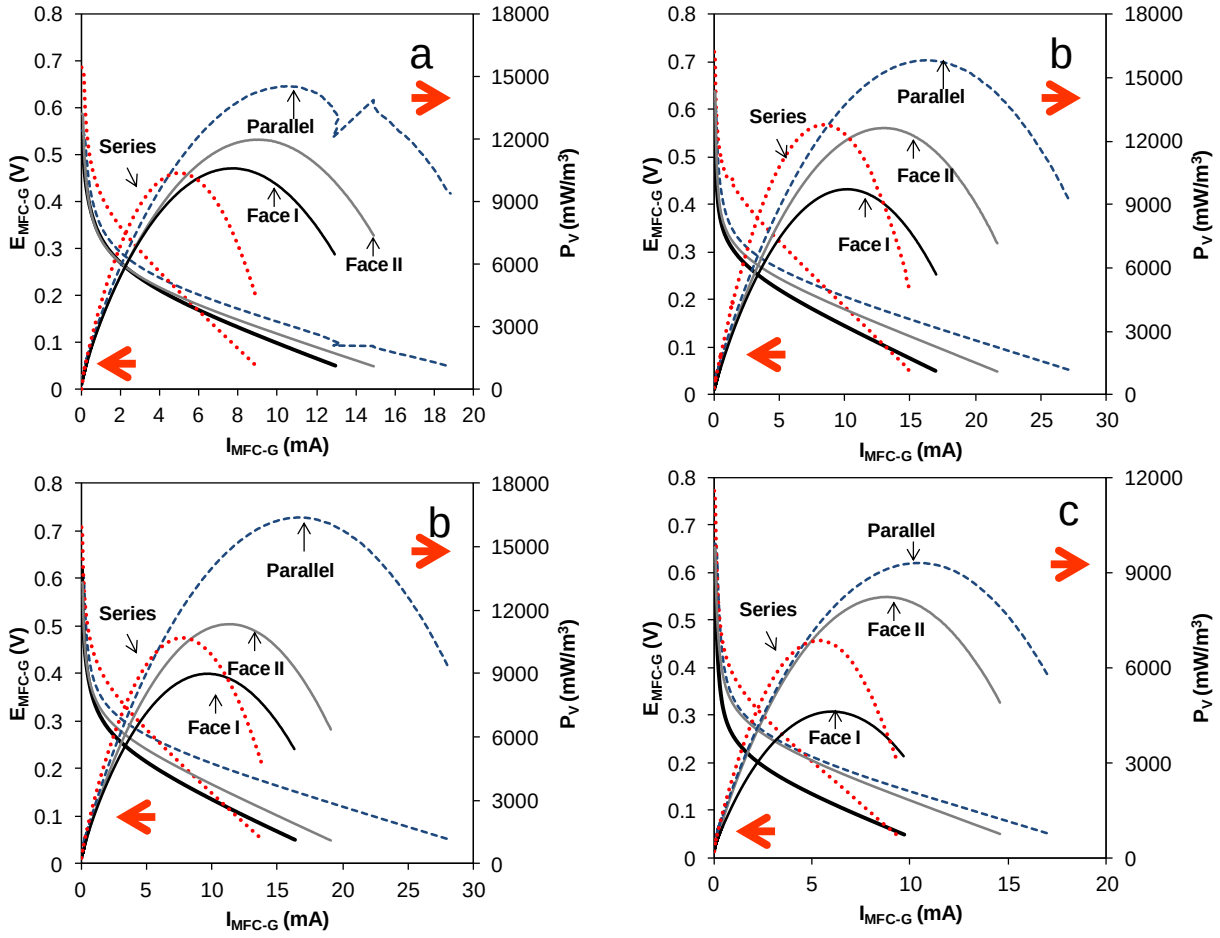

Fig. 1. Polarization curves by linear sweep potential studies in electrochemical characterization of microbial fuel cells, a) *In-$E_{Fe(III)-S}$*, b) *In-$E_{Fe(III)-SR}$*, c)*In-$E_{Mn(IV)}$* and d) *In-SR*. Keys: convex curves stand for volumetric power, read on the right axis; descending parabolic curves represent the E-I relationship, read on the left axis; curve in blue-hyphen stand for parallel connection of the two faces of the cell; red-dot stands for series connection of the faces; continuous gray corresponds to face II alone, and continuous black stands for face I alone.

The relatively higher $P_{V\text{-}max}$ in our MFCs could be attributed to the increase of the total electrode surface area by the application of granular graphite, and this, in turn, could have improved the electron transfer microbe-to-anode process [7]. Also, our work demonstrated that parallel connection of cell faces was more appropriate. On the other hand, the increase of power could also be ascribed to the use of the enriched inoculum. It is known that dissimilatory metal reducing bacteria are capable of the reduction of soil metal oxides such as $MnO_2$, FeOOH and very often exhibit exoelectrogenic (electrochemical activity) properties [32-34]. This, in turn, is related to improved electron transfer to anode and improved cell characteristics and performance [32,33]

*3.2. Batch operation of microbial fuel cells loaded with actual leachate and enriched inoculum*

Figure 2 shows the time course of cell potential (MFC-G; either inocula *In-$E_{Fe(III)-S}$*, *In-$E_{Fe(III)-SR}$*, *In-$E_{Mn(IV)}$* , *In-SR* or without inoculum ) when their two faces were connected in parallel. The batch runs lasted for *ca.* 250 h. The MFC were connected to $R_{ext}$ of 100 Ω. The gray area in Fig. 2 shows that the maximum, open circuit potential (the 24 h and without a resistance in the external circuit). Two periods or cycles of electricity generation with 2.0 g/L municipal leachate were carried out (Period I and Period II in Fig. 3).

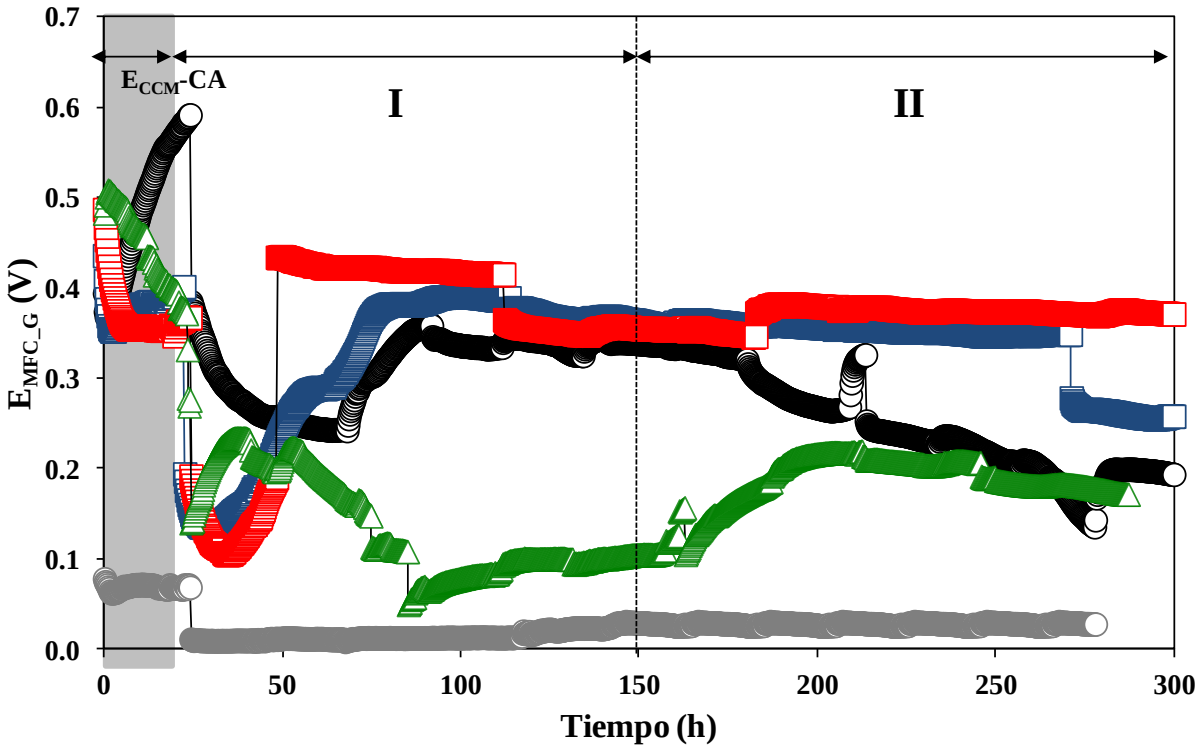


Fig. 2. Time course of voltage outputs of *MFC-G* cell using a inoculum enriched in Fe (III)-reducing bacteria (either one soil origin, *In-$E_{Fe(III)-S}$* and sulphate-reducing origin, *In-$E_{Fe(III)-SR}$*), inoculum enriched in Mn (IV)-reducing bacteria (*In-$E_{Mn(IV)}$*), inoculum sulphate reducing (In-SR) and without inoculum.

The $P_{V\text{-}ave}$ obtained in the cells for the different inocula (*In-*$E_{Fe(III)\text{-}S}$, *In-*$E_{Fe(III)\text{-}SR}$, *In-*$E_{Mn(IV)}$ , *In-SR* and without inoculum) were 13 303, 11 249, 9 556, 4 377 and 53 $mWm^{-3}$. The highest average $P_V$ in our cells were higher than that reported by Puig et al. [14] and Vazquez-Larios *et al.* [24]. Puig *et al.* [14] treated landfill leachate in an air-single MFC fitted with Pt catalyst, for two periods. Indeed, in their Period I the organic matter content of the influent was 0.507 g COD/L and they registered a very low $P_{V\text{-}ave}$ of 6.1 $mWm^{-3}$. In Period II they increased the organic matter concentration up to 8.51 g COD/L and they found a $P_{V\text{-}ave}$ in the range 106 to 344 $mWm^{-3}$ respectively. Vazquez-Larios *et al*. [24] operated a two-face MFC whose main features were the assemblage or sandwich' arrangement of the anode-PEM-cathode; during the batch operation of the cells loaded with a model extract typical of leachate from the hydrogenogenic fermentation of organic solid wastes and a sulphate-reducing inoculum, the $P_{V\text{-}ave}$ was low to moderate, 479 $mWm^{-3}$.

## 4. Summary and perspectives

The values of $P_{V\text{-}ave}$ obtained with the chalcogenide catalyst were 30-40% lower than those registered with Pt catalyst in MFC loaded with municipal leachate as fuel. Yet, the cost of the chalcogenide is 80% lower than that of platinum.
The application of inocula enriched in Fe(III) and Mn (IV)-reducing bacteria significantly improved the performance of cells that used $Ru_xMo_ySe_z$ as a cathodic catalyst for the ORR. In general parallel connection of electrode faces significantly decreased the $R_{int}$.
Finally, the RuxMoySez can be an attractive alternative cathodic catalyst for MFCs that generate electricity from leachate.

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