Analysis of Microbial Diversity of Inocula used with a Five-Face Parallelepiped and Standard Microbial Fuel Cells

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ABSTRACT

This work had a double purpose: *(i)* to study the effect of inoculum (sulfate-reducing (SR) and enriched (E) inocula on the characteristics of one-chamber standard microbial fuel cell (MFC-S) and parallelepiped cell and *(ii)* to analyze the bacterial communities in cells operated with either SR or E.

The MFC-P consisted of a parallelepiped built in plexiglass with a liquid volume of 270 mL. Five faces of this cell were fitted with 'sandwich' cathode-membrane-anode assemblages (CMA). The internal resistances were 400 and 84, and 230 and 292 Ω; and volumetric powers were 655 and 1800 mW/m^3, and 5804 and 1772 mW/m^3 for the faces connected in series and parallel and the MFC-P loaded with SR and E, respectively. Anode density powers were 18.4 and 50 mW/m^2, and 209 and 64 mW/m^2 for the faces connected in series and parallel and the MFC loaded with SR and E, respectively. The MFC-S of 150 mL consisted of one-chamber plexiglass cell with electrodes separated 7.8 cm. The values of R_{int} were 4602 and 1593 Ω, and volumetric powers were 52 and 76 mW/m^3, for the MFC-S loaded with SR and E, respectively.

Regarding the enrichment of the microbial inoculum, we could achieve a 419 mM Fe^{+2} concentration at the end of the 3rd and last transfer; this was an evidence of the increase of either the Fe(III) reducing microflora, or Fe(III)-reducing activity, or both. Furthermore, 100 mM Fe^{+2} concentration was achieved on day 4 of the first transfer, while this concentration in last transfers was achieved in just the first day of incubation. There was a significant difference in community composition between both inocula. *Clostridia* predominated in the community of the biofilm derived from the SR inoculum; this class is believed to be responsible for the direct electron transfer to the anode according to other literature reports. Predominant microbes in the biofilm derived from the E belonged to *Deferribacteres* class; this class is known to contain c-type cytochromes. Current evidence suggests that a series of c-type cytochromes associated with the inner membrane, the periplasm, and the outer membrane might interact to transfer electrons to the outer membrane surface. Shannon indices were 1.27 and 1.38 for the community derived from SR and E inoculum, respectively.

As conclusion, parallel connection of cell faces significantly improved the electrochemical characteristics of the cell. E inoculum was better than SR on the performance of the MFCs as well as, performance of MFC-P was better than MFC-S performance, feed with both inoculua. Also, the SR-derived community was sligthtly diverse than the E-

derived one. Both communities harbored microbes that are electrochemically active.

Key words: enriched inoculum, internal resistance, microbial fuel cell.

1. Introduction

A microbial fuel cell (MFC) is a promising technology for generating electricity directly from biodegradable compounds using bacteria under anaerobic conditions [1,2]. The power generation in a MFC is still insufficient for the practical applications. In order to improve theMFC performance, efforts have been made to improve reactor configuration and to enrich more electrochemically active bacteria [3-10].

The actual voltage output of an MFC is less than the predicted thermodynamic ideal voltage due to irreversible losses; this limits MFC performance. The three major irreversibilities that affect MFC performance are: activation losses, ohmic losses, and mass transport losses. These losses are defined as the voltage required to compensate for the current lost due to electrochemical reactions, charge transport, and mass transfer processes that take place in both the anode and cathode compartments. The electrochemical limitations on the performance of MFC are due to the internal resistance (R_{int}) [11,12]. The primary component of R_{int} is ohmic resistance, which can be further divided into the electrolytic resistance and ohmic resistance of electrodes, and the transfer resistance electrodes. The R_{ohmic} is dominated by the R_{ion} associated to the electrolyte(s) resistance [12.13]. The low voltage has been a large obstacle in energy recovery from MFCs as this voltage is too low to be used directly for many practical applications. For example, a single light emitting diode (LED) requires a minimum voltage of 2 V [14], a single MFC can produce a maximum working potential of only 0.3–0.7 V because of thermodynamic constraints. Thus, effective methods of boosting MFC voltages are needed. Several approaches have been used to increase MFC voltages [15]. One approach is to produce voltage (and/or current) high enough for practical application, therefore, the connection of multiple MFC units in series and/or parallel is necessary [16]. In series linking of six MFCs, Aelterman *et al.* (2006) [17] produced a usable output (2.2 V at 228 W/m3), but this system used a hexacyanoferrate cathode that is not suitable for large scale wastewater treatment. Shimoyama *et al.* (2008) [18] reported a more implementable system, consisting of a series of cassette electrodes with air cathodes.

On the other hand, optimizing the growth conditions for the electrochemically active bacteria in the anode is also an important consideration for improving the performance of MFCs [3]. A fuel cell type electrochemical device can be used to enrich a microbial consortium using wastewater as the electron donor [19]. Molecular techniques are now widely applied to assess the diversity of microbial communities by analyzing the 16S rDNA sequence [20]. MFC systems can be a tool for selecting key electrochemically active bacteria (EAB) for cell inoculum, although recent research has been focussed on ex-cell enrichment procedures, that is, inoculum enrichment in flasks by serial transfers and other methods before loading to the MFC. The differences in bacterial populations between the enriched cultures may also be due to the types of fuel cells used for the enrichment studies.

The aims of this work were to to characterize an design and characterize a novel, multiface parallelepiped MFC (MFC-P) in the perspective of decreasing the internal resistance (R_{int}) and increasing volumetric power (P_v) output,

and to characterize one-chamber standard microbial fuel cell (MFC-S), both loaded with either sulphate-reducing inoculum (SR) and enriched inoculum (E) in order to compare the performance with different inocula and different cells, and to analyse with molecular ecological techniques the bacterial community in MFCs and differences in the bacterial population between MFC fed with sulphate-reducing inoculum and enriched inoculum.

2. Experimental

2.1 Characterization of MFC-S and MFC-P loaded with SR and E inocula

2.1.1. Physical models of the cells

MFC-P consisted of a parallelepiped built in plexiglass with a liquid volumen of 1 000 mL (Figure 1A). Five faces of this cell were fitted with 'sandwich' cathode-membrane-anode assemblages (CMA). Each CMA (from inside to outside) consisted of an anode made of Toray carbon cloth, the proton exchange membrane (Nafion 117), and the cathode made of flexible carbon-cloth containing 0.5mg/cm^2 Pt catalyst (Pt 10 wt%/C-ETEK, and a perforated plate of stainless steel 1 mm thickness.

On the other hand, a standard cell of 150 mL MFC-S was fitted with a circular anode made of stainless steel plate 1 mm thickness with a Toray flexible carbon-cloth sheet placed in one circular face and a cathode in the opposing face made of (from inside to outside): proton exchange membrane (Nafion 117), a Toray flexible carbon-cloth painted with Pt catalyst, and a perforated plate of stainless steel 1 mm thickness. Separation between electrodes was 7.8 cm (Figure 1B).

The MFC-P had a ratio ξ = 19.1 (1/m) whereas the corresponding value of the standard MFC-S was 12.9 (1/m).

2.1.2.Model Extract and Biocatalystst

The cells, MFC-S and MFC-P, were loaded with 7 and 15 mL, respectively, from a model extract [21-23]. The model extract was concocted with a mixture of the following substances (in g/L): acetic, propionic and butyric acids (4 each) as well as acetone and ethanol (4 each) and 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 COD/L. The cells, MFC-S and MFC-P, were loaded with 143 and 255 mL, respectively, of biocatalyst (SR and E inocula). SR inoculum was obtain from a sulphate-reducing, mesophilic, complete mixed, continuous bioreactor. The bioreactor had an operation volume of 3 L and was operated at 35°C in a constant temperature room. The bioreactor was fed at a flow rate of 150 mL/d with an influent whose composition was (in g/L): sucrose (5.0), Acetic acid (1.5), $NaHCO_3$ (3.0), K_2HPO_4 (0.6), Na_2CO_3 (3.0), NH_4Cl (0.6), plus sodium sulphate (7.0). The initial COD and biomass concentration in the MFC-S liquor were ca. 1 450 mg O_2/L and 1100 mg VSS/L, the concentration and biomass in the MFC-P liquor were ca. 1600 mg O_2/L and 1400 mg VSS/L.

The enriched inoculum was obtained with serial transfers. Sediment sample was suspended in nitrogen filled pressure tubes containing media with ferric citrate (55 mM) as electrons acceptor and sodium acetate (2 M) as electrons donor. The tubes were incubated at 30°C for 7 days in the dark conditions. The enrichment procedure was

repeated 3 times [24].

The bioreactor with enriched inoculum had an operation volumen of 1.5 L was operated at 35°C in a constant temperature room. The bioreactor was fed at a flow rate of 75 mL/d an influent whose composition was (in g/L): Sodium acetate (2.0), $NaHCO_3$ (1.8), Na_2CO_3 (0.5), Na_2SeO_4 (0.1). The initial COD and biomass concentration in the MFC-S liquor were ca. 950 mg O_2/L and 800 mg VSS/L, the concentration and biomass in the MFC-P liquor were ca. 1000 mg O_2/L and 900 mg VSS/L.

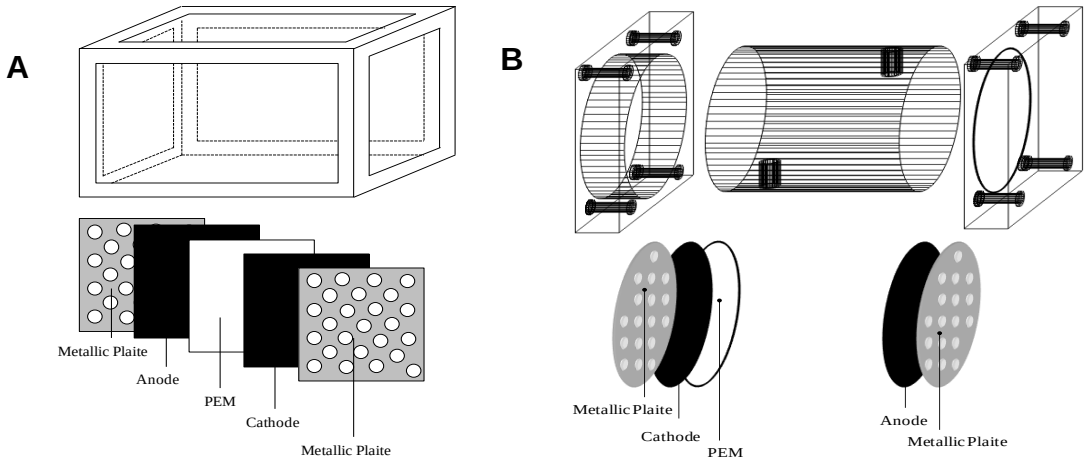

Figure 1. Schematic diagrams of microbial fuel cells: (A) type P (parallelepiped cell), and (B) type S (standard design).

2.1.3 Determination of internal resistence of the cells

The internal resistance of cells was determined using the polarization curve method, by varying the external resistance (R_{ext}) and monitoring both the voltage and the current intensity, according to procedures suggested by Clauwaert *et al.* (2007) [25] and Logan *et al.* (2006) [1]. For the MFC-P, characterization was first carried out with the five faces connected in series and second with faces connected in parallel. In brief, MFCs were loaded with substrate and inocula as described above. MFCs were batch-operated for 8 h at room temperature. The circuit of the MFCs were fitted with an external variable resistance. In this regard, we carried out the polarization curve of the MFCs, relating mathematically the cell voltage (E_{MFC}) and current intensity (I_{MFC}) against the external resistance value, forwards and backwards regarding the external resistance (R_{ext}) values. *Ab initio*, the MFCs were operated at open circuit for 1 h. Afterwards, the R_{ext} was varied from 100 Ω to 100 KΩ and viceversa. After this, the cell was set to open circuit conditions for 1 h in order to check the adequacy of the procedure (values of initial and final open circuit voltages should be close). The voltage was measured and recorded with a multimeter. The current was calculated by the Ohm's Law as indicated below.

2.1.4 Analytical methods and calculations

The COD and VSS of the liquor of sulphate-reducing seed bioreactor and cell were determined according to the Standard Methods [26]. The current intensity I_{MFC}, the power P_{MFC} and the power density P_{An} were determined according to [2].

The power per unit volume or volumetric power P_V was calculated as follows:

$$P_V = \frac{E^2_{MFC}}{V_{MFC} \cdot R_{ext}} \quad (1)$$

where R_{ext} is the external resistance, E_{MFC} is the voltage, and V_{MFC} is the cell volume.

2.1.5 Dermination of Fe(III) reduction

Fe(III) reduction activity was determined using a previous method [27]. A half mL of culture was aseptically sampled with a syringe and mixed with 1 mL HCl solution (0.5 N). This mixture was reacted for 15 min. at room temperature, and then centrifuged for 5 min. The micture of supernatant (0.1 mL) and ferrozine solution (1mL, 1 g/L in 50 mM HEPES buffer) was reacted for 15 min before measurement of optical density at 562 nm using a spectrophotometer. The fresh medium was used as a control sample instead of culture. Ferrous ethylenediammonium sulfate tetrahydrate ($C_2H_{10}N_2O_4SFeSO_4 4H_2O$) was used to make a standard Fe(II) solution [24].

2.2 Analysis of bacterial community

The biofilm formed on the carbon cloth electrodes from the anodes was used for DNA extraction using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc. Carlsbad, CA) according to the manufacturer's instruction. Total genomic DNA was used as template for PCR amplification of approximately 1500 pb of 16S rDNA with a forward primer (27f, 5´-AGAGTTTGATCCTGGCTCAG-3´) and a reverse primer (1492r, 5´-GGTTACCTTGTTAACGACTT-3´) [28]. The PCR products were purified and cloned into TOPO TA cloning vector pCR2.1 according to the manufacturer´s instructions (Invitrogen, Carlsbad, CA). Then they were transformed into competent cells of *E. coli* XL1-Blue by electroporation. White transformants were transferred to plates containing LB broth (25ug/mL kanamycin and 200ug/mL ampicillin), grown overnight at 37°C. Plasmids were isolated using High Pure Plasmid kit (ROCHE, Indianapolis, IN) subsequently clones were digested (2 h, 37°C) with *EcoR1* (BioLabs, New England) for the presence of inserts.

2.2.1 Sequencing and phylogenetic analysis

Inserts were sequenced on the sense and antisense stands at the Instituto de Biotecnologia de la Universidad Nacional Autonoma de Mexico using a Taq FS Dye Terminator cycle fluorescence-based sequencing with an automated capillary sequencer (Perkin Elmer, model 3130xl, Applied Biosystems). The sequencing reaction was performed using M13F-pUC (5´-GTTTTCCCAGTCACGTTGTA-3´) and M13R-pUC (5´-TTGTGAGCGGATAACAATTTC-

3´). 16s RNA gene sequences of aproximately 1500 nucleotides retrieved from each clone were assembled and edited using Bioedit. All sequences were further analyzed with Bellerephon chimera check program and with BLAST program (National Center for Biotechnology Information) [29] to determine the closest available database sequences. Multiple sequence aligments were performed using ClustalW and MEGA 5.0 software. Phylogenetic analyses were performed aligned sequences by the Neighbor-Joining algorithm with Kimura 2 parameter distance and bootstrapping of 1000 replicates in the Phylip program.

2.2.2 Calculations of ecological indices

Shannon-Weaver diversity index has been a popular index in the ecological literature [30]. Shannon-Weaver index is defined as:

$$H' = -\sum_{i=1}^{S} (p_i \log p_i) \qquad (2)$$

where:

p_i = is the proportion of characters belonging to the *i* type of letter in the string of interest. In ecology, p_i is often the proportion of individuals belonging to the *i* species in the dataset of interest.

Pielous evenness index is a measure of a biodiversity which quantifies how equal the community is numerically [31]. This index [32] is defined as:

$$J' = \frac{H'}{\ln S} \qquad (3)$$

where:

H´= is the number derived from the Shannon-Weaver index

S´= is the total Lumber of species

J´= is contrained between 0 and 1

Poggi´s divergence index is defined as.

$$\Delta p = \frac{(n'_A + n'_B)}{(n_A + n_B)} \qquad (4)$$

where:

n_{AB}= number of bands that are present both in lane A and lane B

n'_A= number of bands of lane *A* absent in lane *B*

n'_B = number of bands of lane *B* absent in lane *A*

n_A= total number of bands in lane A

n_B= total number of bands in lane B

3. Results and discussion

3.1 Characterization of MFC-S loaded with SR and E

The polarization curves and the power variation with current intensity of the MFC-S loaded with SR and E are shown in Figure 2A and Figure 2B, respectively.

The internal resistences were calculated as the slopes of the sets of aligned points of the corresponding polarization curves; the values were 4602 and 35 Ω for the MFC loaded with SR and E, respectively (Table 2).

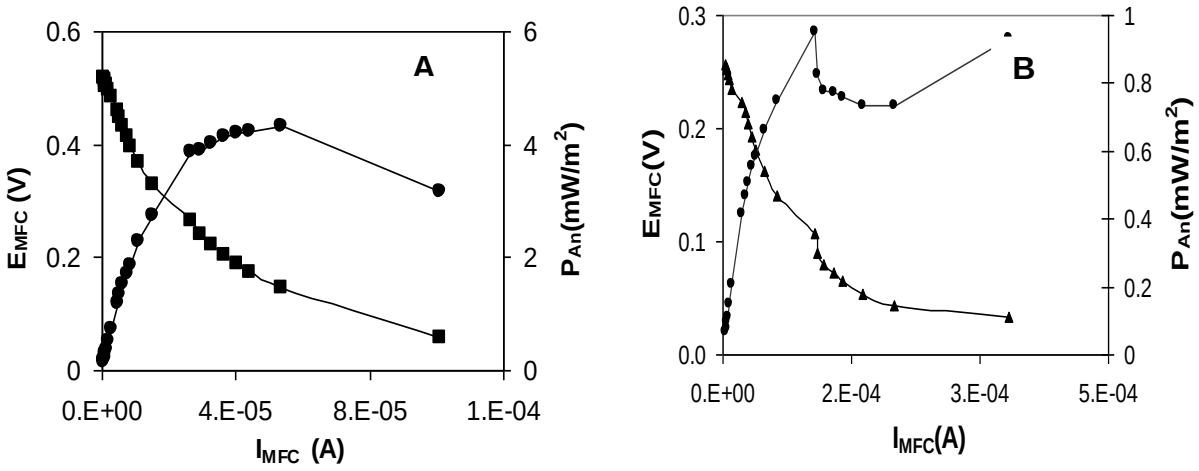

Figure 2. Polarization curves of MFC-S: (A) loaded with SR and (B) loaded with E.

Maximum volumetric powers P_V in MFC loaded with SR and E were 52 and 76 mW/m^3, respectively, and anode density powers P_{An} of the MFC loaded with SR and E were 4.3 and 6.4 mW/m^2, respectively. When MFC was loaded with E, the voltage was 0.65 V; this voltage is higher than MFC load with SR (0.52 V). All the other response variables in MFC-S loaded with E were higher than MFC-S loaded with SR (Table 1).

Table 1. Effect of inocula SR and E on characteristics of a standard microbial fuel cell

Parameter	MFC-S SR	MFC-S E
R_{int} (Ω)	4602 ± 40	1593 ± 5
$P_{An\text{-}max}$ (mW m^{-2})	4.3 ± 0.7	6.4 ± 1.2
$P_{V\text{-}max}$ (mW m^{-3})	52 ± 14	76 ± 21
$I_{MFC\text{-}max}$ (mA)	0.10 ± 0.1	0.33 ± 0.1
$E_{MFC\text{-}max}$ (V)	0.52 ± 0.05	0.65 ± 0.04
$P_{MFC\text{-}max}$ (mW)	0.008 ± 0.002	0.015 ± 0.03

E inoculum was better than SR inoculum on the performance of MFC-S, this could be due that the electrochemically

active bacteria in MFCs are thought to be iron-reducing such as *Shewanella* and *Geobacter* species [33-34]. A number of bacteria have been isolated with the ability to use Fe(III) as a terminal electron acceptor [35-36]. Although there is evidence that a soluble electron carrier is involved in the electron transfer to the water-insoluble electron acceptor [37-38], direct contact between the bacterial cells and the electron acceptor is required for the dissimilatory Fe(III) reduction [35,39]. Among the Fe(III)-reducers, *Shewanella putrefaciens* [40] and *Geobacter sulfurreducens* [41] are known to localize the majority of their membrane-bound cytochromes on the outer memebrane; and the former is electrochemically active [42-43].

On the other hand, performance of MFC-S loaded with E inoculum was superior to performance of MFC-S loaded with SR inoculum (46% higher $P_{V/max}$, 65% lower Rint), these results are similar to those reported by Wang *et al*. (2010) [44] who developed a rapid selection method to enrich for a stable and efficient anodophilic consortium for MFCs. They compared the characteristics of their MFC inoculated with the enriched consortium with those of cell inoculated with original biofilm or activated sludge. They found that power density achieved with the enriched consortium (226 mW/m^2) was higher (by 10%) then those of the original biofilm (209 mW/m^2) and activated sludge (192 mW/m^2).

In general, in our work performance of MFC-S with both inocula was relatively modest. This could be due to the architecture of cell wich has a spacing inter-electrode of 7.8 cm, therefore the internal resistance values are high and power densities are low. The significant decrease of R_{int} with decrease of inter-electrode distance is consistent with previous experiments on the effect of electrode spacing on internal resistance of MFC [45-48].

3.2 Characterization of MFC-P loaded with SR and E inocula

The polarization curves and the power variation with current intensity of the MFC-P loaded with SR inoculum and connected in series and parallel, are shown in Figure 3A and Figure 3B, respectively. The figures 4A and 4B show the variation with current intensity of the MFC-P loaded with E inoculum and connected in series and parallel, respectively.

The values of R_{int} were 400 and 84 Ω for the MFC-P loaded with SR inoculum and connected in series and parallel, respectively, and 292 and 130 Ω for the MFC-P loaded with E, connected in series and parallel, respectively In particular, the proportion of R_{int} decrease in our work was similar to that reported elsewhere [11, 29]. Also, the effect of using enriched inoculum was significantly beneficial.

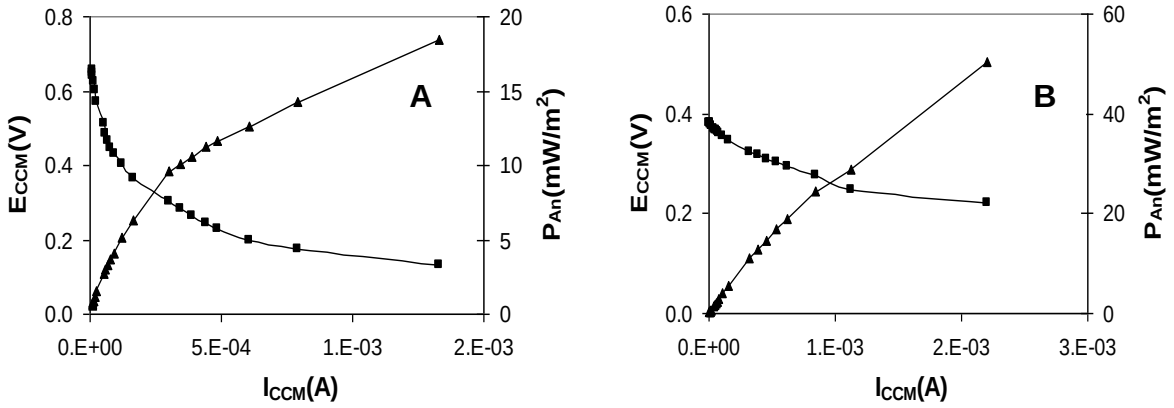

Figure 3. Polarization curves of MFC-P: (A) connection in series and (B) connection in Parallel.

Maximum volumetric powers P_V in MFC-P loaded with SR inoculum and connected in series and parallel were 655 and 1800 mW/m^3, respectively, and anode density powers P_{An} of the MFC-P connected in series and parallel were 18.4 and 50 mW/m^2, respectively. During the connection in series, the voltage was 0.66 V; this voltage was almost double of that obtained when the MFC-P was connected in parallel (0.34 V). All the other response variables in MFC-P connected in parallel were higher than MFC connected in series (Table 2).

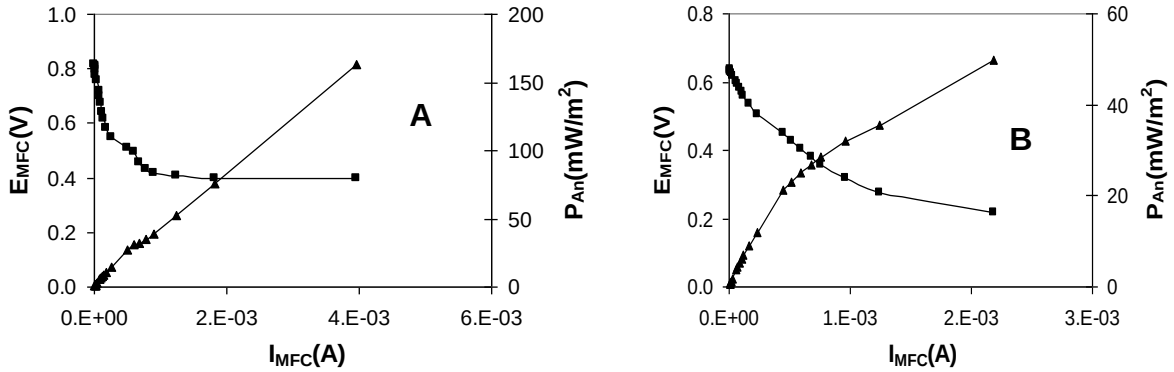

Figure 3. Polarization curves of MFC-P loaded with E: (A) connection in series and (B) connection in parallel.

On the other hand, the values of maximum volumetric powers in MFC-P loaded with E inoculum and connected in series and parallel, respectively, maximum volumetric powers were 1772 and 5 804 mW/m^3, and anode density powers P_{An} were 64 and 209 mW/m^2. When MFC-P loaded with E was conected in series, the voltage was 0.82 V; this voltage is higher than MFC-P connected in parallel (0.64 V). All the other response variables in MFC-P loaded with E connected in series were higher than MFC-P connected in parallel (Table 2).

Table 2. Characterization of a MFC-P using SR and E inocula and connected in series and parallel

Parameter	MFC-P Series SR	MFC-P Parallel SR	MFC-P Series E	MFC-P Parallel E
R_{int} (Ω)	400 ± 40	84 ± 5	292 ± 40	130 ± 5
$P_{An\text{-}max}$ (mW m^{-2})	18 ± 0.7	50 ± 1.2	64 ± 0.7	209 ± 1.2
$P_{V\text{-}max}$ (mW m^{-3})	655 ± 14	1800 ± 21	1772 ± 14	5804 ± 21
$I_{MFC\text{-}max}$ (mA)	1.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	3.4 ± 0.1
$E_{MFC\text{-}max}$ (V)	0.66 ± 0.05	0.34 ± 0.04	0.82 ± 0.05	0.64 ± 0.04
$P_{MFC\text{-}max}$ (mW)	0.19 ± 0.02	0.49 ± 0.03	0.49 ± 0.002	1.5 ± 0.03

Parallel connection decreased the R_{int} by presumably increasing the cross sectional area for ion flow.

Energy loss in the series connection is known to be caused by lateral ion cross-conduction between electrodes; this phenomenon is common when fuel cell arrays sharing the same electrolyte are connected in series to increase voltage output [16]. Parallel conection of multiple electrodes of MFC-P signficantly increased $P_{V\text{-}max}$ compared to that of the MFC connected in series. Also, multiple MFC-P can be connected in series, forming a stacked system in order to increase the voltage. However, when this is done the stack usually undergoes voltage reversal, resulting in a dramatic decrease of stack voltage [15].

Parallel connection decresed not only the internal resistence by increasing the cross sectional area for ion flow, but also possibly diminished the electrode overpotential by increasing the total electrode suface area.

On the other hand, series connection showed an inverse trend to those in the parallel connection, with one order of magnitude higher resistence. Energy loss in the series connection is known to be caused by lateral ion cross-conduction between electrodes [16].

The relatively low values of P_{An} obtained in this work could be due to the be the surface area electrode material, the effect of the larger anode surface area on power was show with several material such as plain graphite, carbon cloth, graphite foam; this effect was relatively insignificant by adding graphite granules or using graphite fiber brushes in the MFC. Those material increased the surface area [4,8].

Parallel conection of multiple electrodes of MFC-P signficantly increased $P_{V\text{-}max}$ compared to P_V of MFC-P connected in series (Table 2).

On the other hand, E inoculum was better than SR inoculum on the MFC-P performance, this could be due to the the presence of iron-reducing bacteria are, probably electrochemically active bacteria. Chang *et al.* (2006) [49] defined EAB as bacteria that possess the ability to transfer electrons from oxidized fuel (substrate) to a working electrode without mediators. Dissimilatory metal-reducing bacteria (DMRB), wich are capable of the reduction of soil metal oxides, are known EAB species, including *Geobacter* and *Shewanella* spp. It was shown that the anode electrode in MFCs served as the electron acceptor for growth and metabolism of EAB, which are capable of current production in the absence of a mediator.

3.3 Analysis of bacterial community

3.3.1 Enrichment procedure

The figure 4 show the results of serial transfers procedure. A concentration of 100 mM Fe^{+2} was achieved on day 4 at the stage inoculation, while this concentration in the other stages is achieved on the first day.

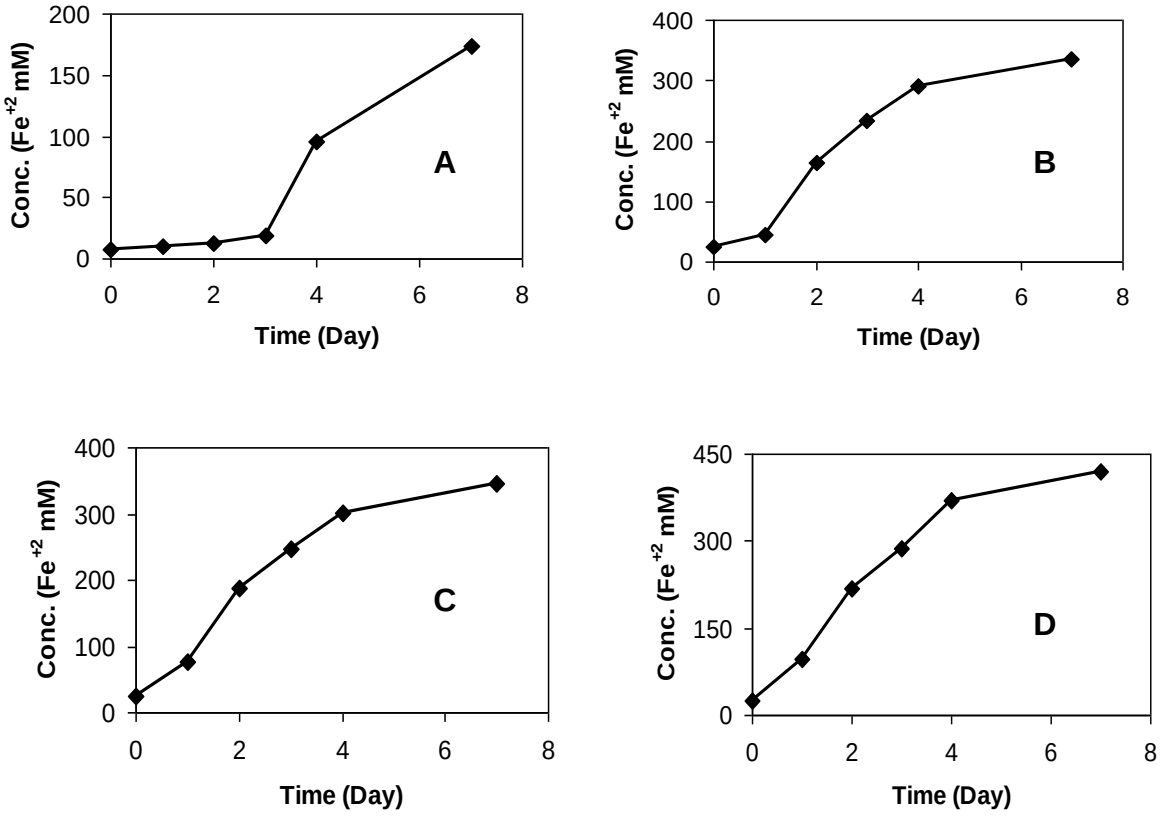

Figure 2. Serial transfers procedure. (A) Inoculation, (B) First Transfer, (C) Second Transfer, (D) Third Transfer.

Table 3 show Fe^{+2} initial and final concentration in serial transfers procedure. We could achieve a 419 mM Fe^{+2} final concentration in the last transfer, this value is more than twice what was obtained in the final inoculation stage. All this is an evidence that the enrichment procedure was successful.

Tabla 3. Concentrarion of Fe^{+2} mM during serial transfer procedure

Stage	Initial Fe^{+2} mM	Final Fe^{+2} mM
Inoculation	7.3	173
First Transfer	25.5	336.5
Second Transfer	25.5	345.7
Third Transfer	25.9	419.9

Our results are similar to those reported by Hyun *et al*. (1999) [24] who isolated a Fe(III)-reducer from the natural enviroment. Soil samples were collected from various paddy fields and enriched with ferric citrate as a source of Fe(III) under anaerobic conditions. The final enriched culture showed the highest Fe(III)-reduction activity. Bacterial growth was coupled with oxidation of lactate and pyruvate to Fe(III)-reduction, final concentration Fe^{+2} was 192.3 mM and 231.9, respectively, this results were obtained after 4 days

Table 4 shows the bacterial population obtained from biofilm of the MFC-S loaded with sulphate-reducing inoculum and enriched inoculum. *Clostridiales bacterium* was present in both inocula.

The major clones amplified from biofilm of the MFC-S loaded with sulphate-reducing inoculum were: *Clostridia* (42%), 98% identity with *Clostridiales bacterium*; *δ-Proteobacteria* (16%), 99% identity with *Desulfovibrio desulfuricans*; *Firmicutes* (16%), 96% identity with *Alkaliphilus oremlandii*, and an *Uncultured bacterium* (26%) 93% identity with *uncultured bacterium*.

The major clones amplified from biofilm of the MFC-S loaded with sulphate-reducing inoculum were: *Deferribacteres* (25%), 97% identity with *Geovibrio ferrireducens*; *Deferribacteres* (25%), 97% identity with *Geovibrio Thiophilus*; *Deferribacteres* (25%), 97% identity with *Denitrovibrio acetiphilus*, and a *Clostridia* (25%) 98% identity with *Clostridiales bacterium*.

There was a significant difference in community composition between both inocula. *Clostridia* predominated in the community of the biofilm of the MFC fed with sulphate reducing inoculum, whereas in the the biofilm of the cell loaded with enriched inocula the predominant microbes belonged to *Deferribacteres* class.

Table 3. The bacterial diversity on biofilm of MFC loaded with different inocula.

Inocula	Similar relatives (clones)	Identity (%)	Abundance (%)	Phylum (class)
Sulphate-reducing	*Desulfovibrio desulfuricans (1)*	100	16	*δ-Proteobacteria*
	Clostriales Bacterium (3)	99	42	*Clostridia*
	Alkaliphilus oremlandii (1)	96	16	*Firmicutes*
	Uncultured Bacterium (2)	93	26	*Uncultured bacterium*
	Geovibrio ferrireducens (1)	97	25	*Deferribacteres*

Enriched	*Geovibrio Thiophilus (1)*	97	25	*Deferribacteres*
	Denitrovibrio acetiphilus (1)	97	25	*Deferribacteres*
	Clostridiales Bacterium (1)	98	25	*Clostridia*

Our results were similar to those observed by Fung *et al*. (2006) [50], who enriched electrochemically active bacteria in a MFC using glucose and glutamate (copiotrophic conditions); their enriched population consisted of *γ-Proteobacteria* (36.5%), followed by *Firmicutes* (27%) and *δ- Proteobacteria* (15%). Logan and Regan (2006) [51], observed that the bacterial communities that develop in MFC show great diversity, ranging from primarily *δ-Proteobacteria*, that predominate in sediments MFCs to communities composed of α- , β-, *γ*- or *δ-Proteobacteria*, Firmicutes and uncharacterized clones in other types of MFCs. On the other hand *Geovibrio ferrireducens*, *Geovibrio thiophilus* and *Denitrovibrio acetiphilus* are known to contain c-type cytochromes [52]. Current evidence suggests that a series of c-type cytochromes associated with the inner membrane, the periplasm, and the outer membrane might interact to transfer electrons to the outer membrane surface.

The bacterial population in the anodic biofilms of our cell was not as rich as found in other types of inocula. For instance, diversity given by Shanon-Weaver index [30] was 1.27 and 1.38 and the species evenness given by Pielou´s evenness index [31] was 0.66 and 0.71, for the sulphate reducing and enriched inoculum, respectively (Tabla 4). These values mean that diverstiy of inocula was relatively low and the evenness was low-to-moderate, respectively.

Tabla 4. Ecological indices of the biocatalysts

Inoculum	**Shanon-Weaver**	**Pielou´s evenness**	**Divergence index of Poggi**
SR	1.27	0.66	0.75
E	1.38	0.71	

4. Conclusion

MFC-P whose main features were the assemblages or ‘sandwich’ arrangement of the cathode-memebrane-anode and the extended surface area of electrodes (higher ξ) exhibited a performance significantly superior to that of a similar cell (standard cell) where the electrodes were separated. The characterization experiments showed that the MFC-P lead to significant reduction of cell internal resistances compared to the standard cell. The improvement in P_V was ascribed to the combined effects of increased ξ and decrease of R_{int}.

The R_{int} of the MFC-P loaded with both inocula was significantly lower than that of the standard cell; this result was ascribed to both the changes in cell architecture and decrease of the inter-electrode distance.

Our results confirm the advantages of the ‘sandwich’ assemblage of CMA over separated electrodes, and demonstrate the convenience of parallel connection of faces in multi-face MFC-P in order to further abate the

internal resistance of the new design cell and increse volumetric power P_V.

The enrichment procedure was successful and indicates the presence of iron-reducing bacteria. Enriched inoculum improved the characteristics of both types of cells used in this work.

On the other hand, we demostrated the successful application of molecular ecological techniques to analyze bacterial diversity, direct 16S rDNA analysis showed low species richness and low-to-moderate evenness. Microbial community anchored in the MFC consisted primarily of *Clostrdiiales bacterium* and *Desulfovibrio desulfuricans*, the last one is a member of *δ*-subdivision of Proteobacteria. These bacteria are recognized to be capable of exocellular electron transfer collectivelly defined as a community of "exoelectrogens". Geovibrio ferrireducens, Geovibrio thiophilus and Denitrovibrio acetiphilus are known to contain c-type cytochromes, current evidence suggests that a series of c-type cytochromes associated with the inner membrane, the periplasm, and the outer membrane might interact to transfer electrons to the outer membrane surface.

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Abbreviations

CMA	'sandwich' arrangement cathode-membrane-anode
COD	chemical oxygen demand

E	enriched inoculum
E_{MFC}	MFC voltage
EAB	electrochemically active bacteria
I_{MFC}	current intensity
$I_{MFC-max}$	maximum current intensity
LED	light emiting diode
MFC	microbial fuel cell
P_{An}	power density
P_{An-max}	maximum power density
P_{MFC}	MFC power
P_V	volumetric power
P_{V-max}	maximum volumetric power
PCR	Polymerase chain reaction
PEM	proton exchange membrane
R_{ext}	external resistance
R_{int}	internal resistance
R_{ohmic}	ohmic resistance
R_{ion}	Ionic resistence
SR	Sulphate-reducing inoculum
V_{MFC}	MFC operation volume
VSS	volatile suspended solids