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Performance of planar and cylindrical carbon electrodes at sedimentary microbial fuel cells

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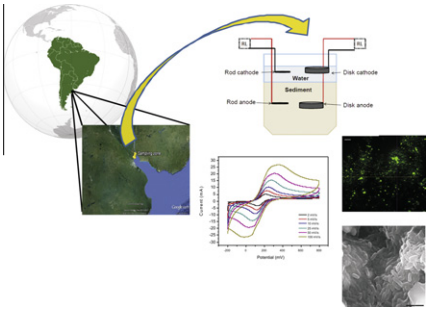
HIGHLIGHTS

- Mud obtained from zones surrounded by marshy vegetation performs better (current).
- Current production seemed to be controlled by diffusion processes.
- The addition of acetate decreased microbial diversity and increased biofilm development.
- An external carbon source (acetate) increased the power generation rate.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper presents data obtained using an indigenous microbial community contained in anaerobic sediments (mud) collected from the shore of the Río de La Plata River (South America). After the sedimentary microbial fuel cells were assembled the evolution of current and power vs. time was studied. Two types of commercially available graphite materials were used as electrodes, which differ mainly in shape and size. In some experiments, an external carbon source (acetate) increased the power generation rate. The maximum power density observed in the aforementioned condition was 19.57 ± 0.35 and 8.72 ± 1.39 mW/m^2 using rod and graphite disk electrodes, respectively. The better performance of the rod electrodes can be explained, at least in part, by an enhanced rate of mass transport by radial diffusion. DGGE fingerprints were used to study the electrogenic community growing over the electrodes.

1. Introduction

In a microbial fuel cell (MFC) microorganisms are used to generate an electrical current. The bacteria oxidize organic matter while some of the electrons are transferred to the anode. An external circuit (which may or may not contain a load resistance) leads the electrons to the cathode producing an electrical current. At the cathode, oxygen is reduced into water. Reimers et al. (2001) were the first to develop the concept of sedimentary microbial fuel cell (SMFC), also known as benthic microbial fuel cell (BMFC), showing that the power generation can be sustained just by the oxidization of organic matter naturally present in sediments. In a SMFC, while an anode is embedded in an anaerobic sediment (marine or River sediment, rice paddy fields and other aquatic environments rich in organic matter) a cathode is exposed in the aqueous phase over the sediment usually saturated with oxygen (Bond et al., 2002;

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Holmes et al., 2004; Tender et al., 2002) and the main applications described are as alternative renewable power source for autonomous sensors, environmental monitoring devices, and military tactical surveillance sensors, for real-time data acquisition from remote locations (Donovan et al., 2008; Holmes et al., 2004; Tender et al., 2002). Furthermore, SMFCs have a potential application in places with a lack of electric infrastructure, although it is also a fact that the density of energy produced by MFCs, and especially by SMFCs, is very low. Some authors have proposed that these biosystems must enhance their performance (W/m^3) not less than 100 times to have large-scale applications (Rabaey et al., 2005). Various research groups have applied a different focus (on microbial genetic, artificial selection, engineering, materials, etc.) in order to attain this goal in the medium or long term.

Some bacteria can transfer electrons directly to an extracellular terminal electron acceptor, such as a metal oxide, without the presence of a redox mediator (Logan et al., 2005). These bacteria have been called exoelectrogens (Logan, 2008a), electrochemically active or anodophilic bacteria (Chang et al., 2006), and the most well-known and studied organisms are in the genera *Geobacter* and *Shewanella,* both gammaproteobacteria (Bond et al., 2002; Marsili et al., 2008).

Nevertheless, the precise electron mechanism is nowadays controversial (Gorby et al., 2006; Logan et al., 2005; Marsili et al., 2008; Reimers et al., 2006). SMFCs have operative and technological advantages compared to other current bioenergetics processes. First of all, they enable the direct conversion of organic matter to current with high efficiency (over 95% of the released electrons by the oxidation of the organic matter can be turned into electricity) (Reimers et al., 2001). Secondly, they may work in a wide range of environmental conditions and also low temperatures (Reimers et al., 2006).

The aim of this study was to evaluate the bioelectric response (current and power generation), and its relation with the diversity of the bacterial community in the anode when the sediments from the Río de La Plata River were used as a source of both, organic matter and electrogenic microbial communities. Also, the performance of two different graphite electrode geometries have been examined in lab-scale freshwater SMFCs to study in which way the shape and size control the production of current and power. Microscopic techniques were used to confirm the presence of electrode-attached bacteria which were further, evaluated using DGGE. This is the first time that an electrochemical characterization of a SMFC is made using sediments of the Río de la Plata River.

2. Methods

2.1. Sediments sampling

The sediment was collected from the shore of the Río de la Plata River on the intertidal zone. This estuary is strongly affected by the marine tide; hence the sampling was made at low tide. Two sampling zones were defined: a silt-sandy beach without marshy vegetation, referred to as ''zone nv'' and abundant marshy vegetation, referred to as ''zone v'', populated just by the ''rush'' (*Schoenoplectus californicus*).

In order to get the samples an excavation was made taken care of maintain the soil structure. The samples collection was made in those places where the oxidation reduction potential (ORP) and the color of the sediment indicated anoxic conditions (ca. 15 cm). Surface water was also collected in the same location. Measurements as pH (Hanna Instruments HI 98103), oxidation reduction potential (ORP electrode Oakton), and sediment temperatures were done *in situ*.

Samples were deposited in clean polycarbonate boxes, which were filled up avoiding the formation of air chambers. Samples were packed in a cooler and then transported to the laboratory. During the following 2 h after the samples were taken, the SMFCs were set up.

For the determination of the content of organic carbon and the denaturing gradient gel electrophoresis (DGGE) analysis sediment samples were taken using sterile polycarbonate tubes and stored at −20 °C until processing. The determination of organic carbon was performed using the Walked-Black method (Walkley and Black, 1934).

2.2. Construction and operation of lab-scale SMFCs

Anoxic sediments were used to fill up 1 L beakers up to 3/4 of its total volume. The anodes were completely embedded horizontally in the sediment at a distance of 7 cm below the surface, while the cathodes were suspended (also horizontally) in the overlying freshwater at a distance of 5 cm from the sediment surface.

The overlying water was taken from the sampling sites, and was continuously bubbled with air using an aquarium air-pump in order to maintain saturated oxygen conditions. Water lost by evaporation was replaced with double osmosis water. Sediments were equilibrated under open circuit condition for 48 h. Afterwards the anode was connected to the cathode via a fixed external load of 4.6 kΩ. All SMFCs were operated at room temperature (25 °C).

Three different SMFCs were made: non-current control SMFCs, made by adding formaldehyde to a final concentration of 5% v/v ($SMFC_f$; $n = 2$); SMFCs amended with sodium acetate to a final concentration of 1.7 g/L ($SMFC_{sa}$; $n = 3$); and SMFCs made without exogenous addition ($SMFC_{wea}$; $n = 3$).

2.3. Electrodes

Graphite disks and rods were used as electrodes in these experiments. The graphite disks (La Casa del Grafito SRL, Bs. As., Argentina, purity 99.8%) had a surface area of ca. 102.5 cm^2; the graphite rods (Staedler Mars Carbon pencil leads) denomination 7B (87:7:5, graphite:clay:wax, wt.%) had a diameter of 2 mm and a surface area of ca. 2.26 cm^2 (given the manual construction procedure, the actual area of each individual electrode was used for calculations). All current and power production values were normalized to the anode surface geometrical area; as both type of electrode (disk and rod) were polished and cleaned in the same way before use, we assume the geometrical and effective areas to be similar (see Supplementary Information (SI), Fig. A-1). The corresponding cathode superficial areas were also similar.

2.4. Data acquisition and calculations

Potential (E) was daily measured by a multimeter with a data acquisition system (UNI-T, Uni-Trend Technology, China). Current (i) was calculated as $i = E/R$, where R is the external circuit resistor (R_L, load resistor), that can be easily replaced. Power (P) was calculated as $P = iE$. The power density (P_D) and current density (J) values are the P and i values normalized by the anode total surface area. The maximum operating current density (J_{max}) was obtained from the curves of current density versus time.

Every 15 days polarization curves were made. The external load was disconnected 24 h before the polarization curve experiments were made. Measuring the E values while varying the R_L applied to the SMFCs (values between 100 kΩ and 100 Ω) enabled the construction of power density curves; the E value at each R_L was recorded when reaching a pseudo-steady state, as described by Logan et al. (2006). The maximum power density (P_{Dmax}) was obtained from these curves. At the beginning of the experiment, the open circuit potential (OC) was measured.

2.5. DGGE analysis

DNA was extracted from electrodes and sediment samples by a modified phenol–chloroform procedure involving physical disruption of cells with bead mills. A fragment spanning the V3 region of 16S rDNA (positions 341 and 534 in *E. coli* numbering) was amplified from genomic DNA. The forward primer had a 40 bp GC-rich sequence to prevent complete denaturation during DGGE (Muyzer et al. 1993). Amplification reactions were performed under the following conditions: 5 min at 95 °C, followed by 35 cycles that consisted in a 95 °C denaturation step for 45 s, 55 °C annealing for 45 s, 72 °C extension for 1 min and a final extension of 5 min at 72 °C. Reactions were performed in a total volume of 50 μL. The PCR mixture used contained 20 ng of DNA template, 5% dimethyl sulfoxide (DMSO), 0.5 μM of each primer, 50 μM of each deoxynucleoside triphosphate, 3 mM $MgCl_2$, 1 × reaction buffer ($MgCl_2$ free), 1.25 U of Taq DNA polymerase (Invitrogen), and 0.19 μg/mL of bovine serum albumin.

The DGGE system (CBS Scientific, DelMar, CA) was used as specified by the manufacturer. Electrophoresis was performed in 1 × TAE (20 mM Tris acetate, 10 mM sodium acetate, and 0.5 mM EDTA) at a constant temperature of 65 °C and a potential of 65 V for 16 h. After electrophoresis, gels were soaked for 30 min in SYBR Gold nucleic acid stain (Invitrogen, 1:10,000 dilution in Tris–acetic acid–EDTA; pH 8.0). The stained gels were immediately photographed on an UV transillumination table combined with a camera module and imaging system (Gbox, Syngene). Gel image data were stored in TIFF files and analyzed by using the GelCompar II software, version 5.1 (Applied Maths, Sint-Martens-Latem, Belgium). The similarity between DGGE profiles was calculated by using the band-matching Dice coefficient with optimization at 0.5% and tolerance level at 0.5%. Cluster analysis was performed by using the unweighted pair-group method with arithmetic averages to produce a similarity dendrogram. Bands of interest were excised and DNA eluted with 25 μL of water overnight at 4 °C. The resulting solution (2 μL) was used as target DNA for a subsequent PCR amplification for sequencing with bacterial primers F341 and R534GC. The purity and correct running position of each fragment was confirmed by further DGGE.

PCR products were sequenced at the Macrogen sequencing facility (Seoul, Korea). The sequences were compared to GenBank sequences using Blast algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results and discussion

3.1. Characterization of the sample zone

The sampling areas of the sediment of the Río de la Plata River included a place with no vegetation (zone nv) and a place near the roots of marsh vegetation (zone v) in order to study the effect of plant exudates over current production. The anoxic sediments from zone nv had a pH 6.83 ± 0.06 ($\bar{x} \pm SD$) with $n = 3$, a redox potential of −464 ± 10 mV ($n = 3$), and the organic carbon content was 1.05 ± 0.23% p/p ($n = 3$). The anoxic sediment from zone v had a pH 6.96 ± 0.03 ($n = 3$), a redox potential of −460 ± 12 mV ($n = 3$), and the organic carbon content was 1.50 ± 0.24% p/p ($n = 3$). The temperature of the sampled sediments was 18 °C.

We do not observe any evident difference between both sediments regarding color, granulometry or water content between the two sampled zones. Apparently the two sampling zones have similar characteristics except for the amount of organic carbon present in each one of them.

To study the performance (current production) of the sediments from both sampling areas, two $SMFC_{wea}$ (without any reagent addition) were prepared. Disk anodes were connected via a fixed R_L of 4.6 kΩ to disk cathodes. We observed that the current output in the $SMFC_{wea}$ with sediments from zone nv was approx. 30% lower than the one from zone v.

The maximum operating current density (J_{max}) production (continuous operation, fixed R_L of 4.6 kΩ) was between 1.30 and 1.48 mA/m^2 in the $SMFC_{wea}$ made with the zone v sediments (values obtained between days 19 and 32). The J_{max} production were between 0.37 and 0.54 mA/m^2 in the $SMFC_{wea}$ made with the zone nv sediments (values obtained between day 5 and 28).

In both cases the J was followed by a gradual decrease (Fig. 1). This is consistent with the fact that the time needed to reach the maximum current density depends on the origin of sediments (marine or freshwater), the content of organic matter in them and other operating conditions, such as oxygen availability at the cathode (Holmes et al., 2004). In this experiment, all the named factors are maintained as constants, with the exception of the content of organic matter and probably the microbial community related to each zone (nv or v).

The deterioration in the performance of the $SMFC_{wea}$ observed when it was operated longer than 28 day may be due to a depletion of organic matter in the surroundings of the anode, given that the performances of these systems depend on the microbial metabolism which would be limited by the speed at which organic molecules may diffuse from zones far away from the anode (Bond et al., 2002; Holmes et al., 2004).

These results are in concurrence with other authors' hypothesis that consider that in zones surrounded by marshy vegetation the plants provide a continuous input of organic matter to the submerged soil. During the growing season organic carbon enters the soil as rhizodeposits (De Schamphelaire et al., 2008).

The maximum current density output in samples from the zone nv was smaller than those from the zone v, this phenomena could be related to the microbial mass and diversity in each starting material, given that more microbial activity is expected when the interaction with the rhizosphere exists. In a SMFC, this flow of energy can be exploited by oxidizing the substrates derived from living plants and the rhizodeposits directly on the anode through a series of microbiological catalyzed reactions.

Given that we obtain a higher current density with the zone v sediment, we concentrate our efforts using this type of sediment. The following data informed here were obtained using the zone v sediments.

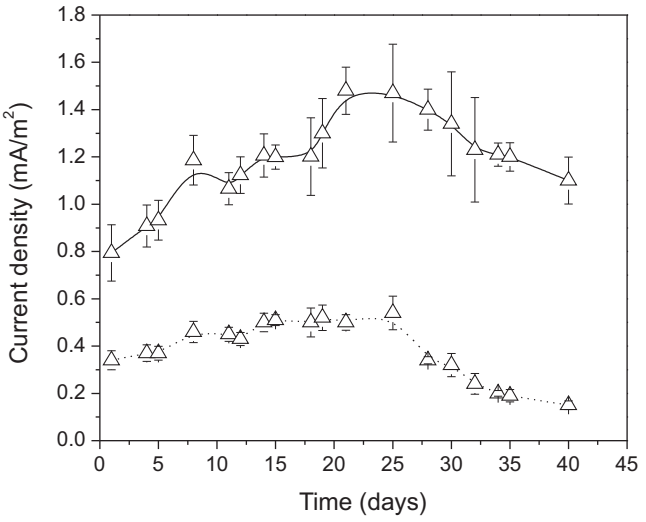

Fig. 1. Current density vs. time measured in the zone v $SMFC_{wea}$ (solid line) and zone nv $SMFC_{wea}$ (dot line) ($n = 3$) with the disk electrodes connected to a $R_L = 4.6$ KΩ.

3.2. Current output of $SMFC_{sa}$ and $SMFC_{wea}$

Three different types of zone v SMFCs were made to study the current production using a $SMFC_f$ as a negative control. Given that the performance of the SMFCs may be deteriorating due to the depletion of the organic matter in the surroundings of the anode, we searched for a probable effect of an exogenous addition of a carbon source as sodium acetate, one of the carbon sources used by most of the anodophilic bacteria (Chang et al., 2006).

In order to study the effect of the geometry and size of the electrodes, two pairs of electrodes were used in each SMFC. An anode disk electrode was connected to a cathode disk electrode, and the same was made for the rod electrodes.

The J_{max} production (with a fixed R_L of 4.6 kΩ) in the $SMFC_{wea}$ ($n = 3$) was 1.36 ± 0.13 mA/m^2 measured with the pair of disk electrodes (days 8–15). The J stayed at an average of 1.10 mA/m^2 within the 95 days the test lasted. The rod electrodes placed in the $SMFC_{wea}$ achieved a J_{max} production of 6.93 ± 0.54 mA/m^2 (days 8–15) remaining in approx. 3.53 mA/m^2 afterwards (Fig. A-2).

The J_{max} production using the $SMFC_{sa}$ ($n = 3$) with the disk electrodes was 22.67 ± 6.38 mA/m^2 (days 22–36) and with rod electrode was 72.28 ± 6.37 mA/m^2, (days 18–36). Then J values were maintained at approx. a 5.39 mA/m^2 for the disk electrodes and at a 23.72 mA/m^2 for the rod electrodes. After both systems reached the maximum values between 20–40 days, the J value decreased sharply reaching minimal values at day 50 and 92 with the disk and the rod electrodes respectively (Fig. 2).

Given these results we could speculate that the addition of an external carbon source resulted in an increased current density measured with both electrodes and confirm that the sodium acetate addition promoted a higher performance in the $SMFC_{sa}$ prepared with mud of the Río de la Plata River. This is the first time that the sediments from this river are electrically characterized as SMFC. We are also proving for the first time that the electrical performance is limited by microbial substrate availability, using either rod or disk electrodes.

Furthermore, sodium acetate can be used to support the growth of dissimilatory metal reducing bacteria capable of extracellular electron transfer, as *Geobacter* spp. and *Shewanella* spp. Although lactate is preferred as electron donor for *Shewanella*, it can also grow also using acetate or pyruvate (Tang et al., 2009).

The best results in current output were obtained using rod electrodes, which may be related more probably with its geometry, allowing better diffusion of substances from and to the electrode. To study if diffusive phenomena could explain the differences between electrodes, cyclic voltammetries were made at different scan rate in quiescent solutions, using ferricyanide as a tracer. From the results we found that linear or planar diffusion is predominant in disk electrodes, whereas radial diffusion or mixed mechanism of mass transport involving both radial and planar diffusion is important in rod electrodes (See Fig. A-4). The peaks for ferricyanide/ferrocyanide couple were almost unmodified in both types of electrodes showing that the materials are probably very similar. Furthermore, the graphite rod is a good and interesting candidate for lab-scale SMFCs electrodes since they are inexpensive, easily accessible, produced in massive amounts, and has good mechanical properties.

3.3. Power output of $SMFC_{sa}$ and $SMFC_{wea}$

Power-current properties of $SMFC_{sa}$ and $SMFC_{wea}$ (Fig. 3) indicate the optimal i or E ranges at which each fuel cell can be operated to maximize the P generation. With the $SMFC_{sa}$ ($n = 3$) disk electrodes we saw P_{Dmax} of 8.72 ± 1.39 mW/m^2 (day 53), which then decreased gradually up to half the maximum. The P_{Dmax} measured with the rod electrodes was 19.57 ± 0.35 mW/m^2, obtained at day 39 (Fig. 3A).

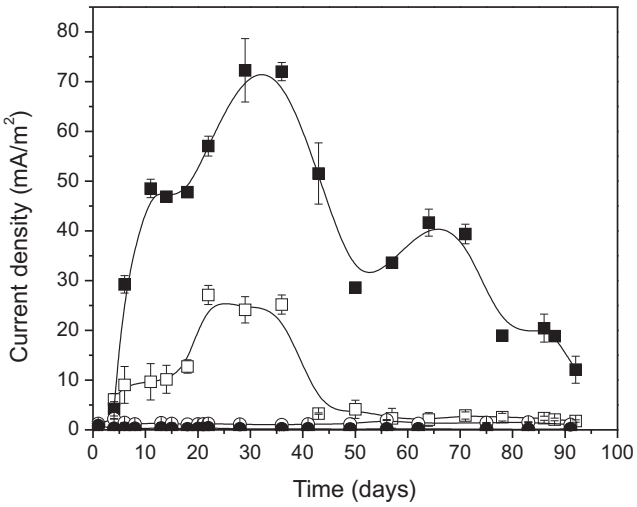

Fig. 2. Current density vs. time measured in SMFCs either with disk electrodes (open symbols) or rod electrodes (closed symbols), (□) $SMFC_{sa}$/disk electrodes; (■) $SMFC_{sa}$/rod electrodes; (○) SMF_f /disk electrodes, (●) SMF_f /rod electrodes using $R_L = 4.6$ KΩ.

The results obtained with the $SMFC_{wea}$ ($n = 3$) were less suitable. The disk electrodes yielded a P_{Dmax} of 0.36 ± 0.03 mW/m^2 and the rod electrodes, 2.20 ± 0.35 mW/m^2. The behavior of both electrodes P_D was similar up to day 25 and then decreased in time (Fig. 3B).

The addition of an external carbon source had an effect on the P_D production as higher values were obtained with both electrode types in the $SMFC_{sa}$.

3.4. Current and power output of the $SMFC_f$

As some of the current production at a given MFC may be related to inorganic reactions that can occur spontaneously at the anode surface, formaldehyde was used as a way to extinguish active microbial life, and also to avoid any further microbial resistance form, such as spores, from germinating and developing given that this non-microbial related effect could be very important at a SMFCs, where the composition of the sedimentary materials is not controlled.

The $SMFC_f$ was assembled with the zone v mud ($n = 2$). The continuous operation power ($R_L = 4.7$ kΩ) achieved with this construction was 0.20 ± 0.02 mW/m^2 for the disk electrodes and 0.27 ± 0.13 mW/m^2 for the rod electrodes during the entire experiment. These values were approximately constant for both electrodes until the experiment was finished at day 91. At the beginning of the experiment the OC was 10 mV and the J_{max} was 0.148 ± 0.01 mA/m^2. After leaving the $SMFC_f$ to mature for 91 days, the OC obtained was 17.10 mV and the J delivered from the rod electrodes was 0.167 ± 0.01 mA/m^2. The results obtained with the disk electrodes were quite similar showing a very low J production, related with non-biological reactions (data not shown).

Therefore, the method of the sediments sterilization by adding formaldehyde allowed us to confirm that both, i and E generated in the $SMFC_{sa}$ and $SMFC_{wea}$, correspond to the native bacterial community of the sediments and their growth over the electrodes and in the electrodes surrounding sediment.

3.5. Polarization curves and power density curves

The real potential of a real fuel cell is usually below its equilibrium potential due to different types of losses. Three different

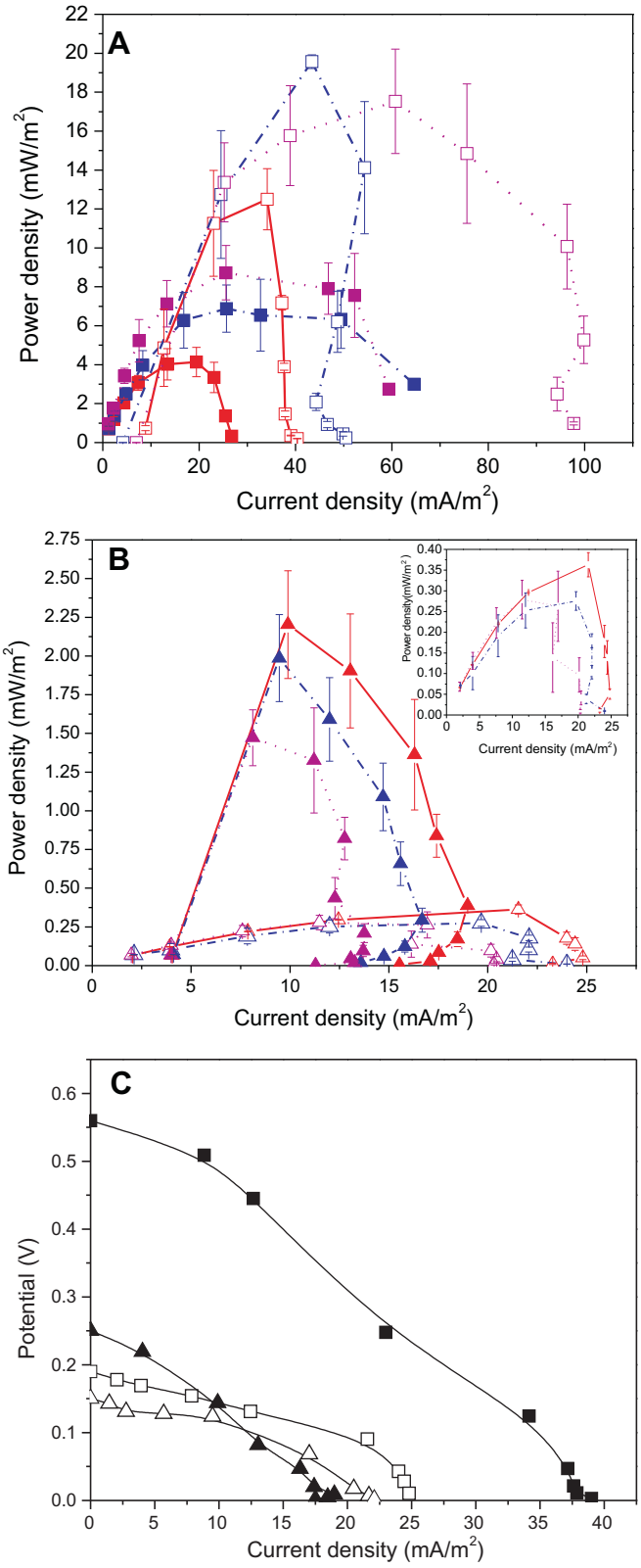

Fig. 3. Power density curves from the $SMFC_{sa}$ (A) and $SMFC_{wea}$ (B) made after 25 (red solid line), 39 (blue dash-dot line) and 53 (magenta dot line) days. The inset in B is the disk electrodes data rescale. (C) Comparison of polarization curves from the $SMFC_{sa}$ (square symbols) and $SMFC_{wea}$ (triangle symbols). Disk electrodes (open symbols) and rod electrodes (close symbols) in A, B and C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

types of losses can be distinguished as: ohmic, activation and concentration losses (Logan, 2008b). In order to compare the results of the different SMFCs with rod or disk electrodes, potential and polarization curves were made for all SMFCs.

The polarization curve depicted in Fig. 3C from the rod and disk electrodes in the zone v $SMFC_{sa}$ and $SMFC_{wea}$, presents E as a function of J obtained during the stable phase of power generation (day 25) for resistances varying from 100 kΩ to 100 Ω.

The results obtained with the rod electrodes show that the initial step of E decrease for low J depicted in Fig. 4 suggested that the $SMFC_{sa}$ had high activation losses (Logan et al., 2006; Lovley, 2006). For a J higher than 10 mA/m^2 approx. the curve slope decreased considerably, which indicated the prevalence of ohmic losses. After analyzing the value of each curve slope (linear regression) from the $SMFC_{wea}$ and $SMFC_{sa}$, we saw that the slope was higher in the $SMFC_{sa}$ suggesting that activation and ohmic losses in this cell are higher.

The polarization curve of the disk electrodes was quite similar to the one obtained with the rod electrodes in the case of the $SMFC_{sa}$ and $SMFC_{wea}$. Prevalence of ohmic losses was also observed. In the $SMFC_{sa}$ curve the slope decreased more smoothly than in the case of rod electrode for J higher than 20 mA/m^2, which would indicate that ohmic losses are probably lower than in disk

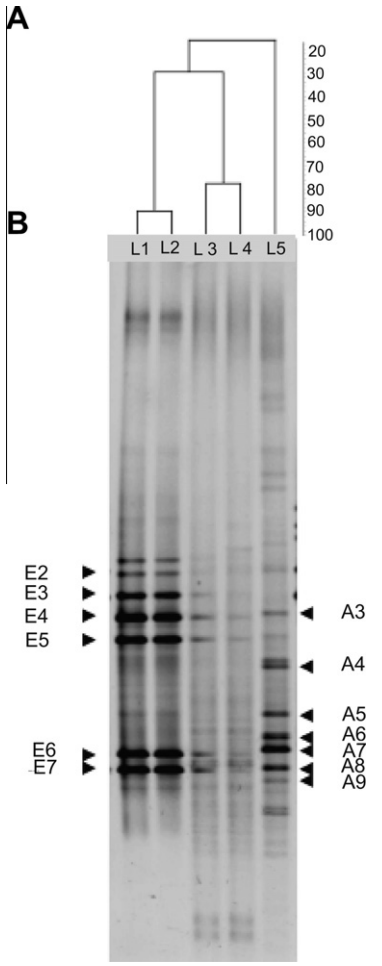

Fig. 4. (A) Dendrogram constructed with UPGMA based in DICE coefficient showing community composition similarity. (B) Bacterial communities DGGE analysis corresponding to amended electrode duplicates (L1) and (L2), sediment duplicates (L3) and (L4), and non-amended electrode (L5).

electrodes. In both cases the slope changes in the area of higher J show involve concentration losses (Fig. 3C).

From the polarization curves (using the data shown in Fig. 3C), the internal resistance (R_{int}) was estimated for the different systems we assayed. The obtained values were mainly related to the type of electrode used; $SMFC_{wea}$ and $SMFC_{sa}$ using rod electrodes shown a R_{int} of ca. 25.6 kΩ/cm^2, whereas disk electrodes shown lower R_{int}, about 4.8 kΩ/cm^2. The values were relatively high, as expected in these mud based systems.

Disk and rod electrodes were used and compared in this study since both materials can be suited for different applications. Disk electrodes have been more used in SMFC *in situ*, *i.e.*, natural environments. On the other hand, rod electrodes are suitable for SMFC studies at lab-scale since their size is sufficiently small to be studied by SEM and confocal microscopy (Figs. A-3 and A-4). Rod electrodes are easy to break to obtain subsamples, and their relatively small area is suitable for electrochemical studies using normal low-current potentiostats.

The comparison we show, where both electrode types are immersed in the same SMFC, is worthy and confirms an effect demonstrated by other authors, *i.e.*, the difficulty to extrapolate results obtained with small surface and different geometry electrodes to results obtained with big ones. As the electrode size goes down, relatively more electricity (A/cm^2) can be harvest from it. Though power density has been used frequently as a way to compare different sized electrodes and very different MFCs set-ups, but recent work has shown that this is not truly correct; the smaller the electrodes, typically the better the current and power performances, expressed in function of anode area. This is usually true when the analysis is realized over the current-limiting electrode or other limiting steps, which can include anode or cathode reaction, membrane transport, or others; diffusion, "edge effect" and other unknown phenomena are postulated responsible for this effect (Dewan et al., 2008). The previously mentioned work shows that when electrodes plates are used, a P_D relationship of ca. 1.7 can be calculated, whereas the relation we found using disk and rod electrodes ($SMFC_{sa}$) was of 2.2 (similar areas were compared). More work is needed to study the size, shape and other phenomena in order to completely explain this scale-up problem and to try to reach, in big electrodes, the same level in function of area obtained in small sized MFCs. Unfortunately, the electrode shape, which is very important in order to model or compare the diffusion effect, is not quite detailed informed in the literature.

Using disk electrodes in marine sediments, Tender et al. (2002) noted a P_D of 28 mW/m^2, which is slightly higher than our observation with $SMFC_{sa}$ and rod electrodes. In ocean cold seeps, Reimers et al. (2006) observed a P_{Dmax} 34 mW/m^2 during day 20 and 31 of the operation, and the P_{Dmax} decreased to 6 mW/m^2 during days 103 and 114. We should note that the maximum power refers to the power obtained by polarization curves experiments, which may not be sustainable in the long term. The continuous operation power in our $SMFC_{sa}$ (disk electrode) was approx. 3.3 mW/m^2 with (R_L of 4.6 kΩ, days 19–94) and for this extended period showing that design can produce energy for a long time (data not shown). Our results with freshwater sediments provide a power comparable with the value deployed in a marine environment (Reimers et al., 2006).

The power density of approx. 19 mW/m^2 with the rod electrodes can be compared with other works, such as for example the values obtained by Nielsen et al. (2008), who using carbon fiber electrodes between a laminating of glass fiber and a framework of plexiglas obtained an initial value of 32 mW/m^2, and a potential decrease to 10 mW/m^2 after 80 days. Tender et al. (2002) using graphite electrodes similar to ours obtained about 20 mW/m^2.

These two authors placed electrodes in the coastal margins of Rivers in the U.S. It should be noted that these sediments generally contain between 2–3% of organic carbon (dry weight); and the constant renewal of carbon in sediments by such SMFC can be used to sustain the generation of energy indefinitely.

3.6. Bacterial community analysis of the sediment and the biofilms

Several studies have focused on the colonization of the MFC anodes with different environmental inocula sources. Bacterial communities' analysis of naturally-colonized anodes have shown a great diversity of electrochemically active taxa (Bond et al., 2002; Borole et al., 2009; Jung and Regan, 2007; Phung et al., 2004). DGGE has proved to be a useful tool for monitoring the development and composition of microbial communities in bioelectrochemical systems (Kim et al., 2011; Zhang et al., 2011). In this study, the DGGE fingerprints and band intensities for biofilm samples collected from the electrode surfaces indicated that bacterial communities were similar between SMFC duplicates. Cluster analysis based on Dice similarity grouped patterns of the electrode and inoculum duplicates (Fig. 4A). While both supports received the same inoculum, the band pattern of the electrode without external carbon source was different from those of the acetate-fed electrodes and from that of the inoculum, though showing more similarity with the latter. Considering the reproducibility exhibited between amended electrodes, the observed differences in bacterial community composition with non-amended electrode could be interpreted as resulting from the electron donor used. Prevailing conditions in the SMFCs seems to select some of the dominant populations from the sediment, while other populations are lost or greatly diminished in number. Similar results were reported by Phung et al. (2004) for oligotrophic MFCs inoculated with river sediment. According to DGGE profiles, communities present in the SMFCs presented a small amount of dominant taxa (Fig. 4B). The Shannon diversity index for amended electrode biofilms was in average 3.07 and for non-amended electrodes 3.26, showing a greater diversity than amended electrodes. Several of the most prominent bands were excised and sequenced (Table 1).

DGGE community profiling of amended SMFCs anodes showed the dominance of sequences most similar to *Shewanella* spp. (bands E4 and E7), *Pantoea* spp. (bands E3 and E6), *Pseudoalteromonas* spp. (band E5), and to the Antarctic bacteria R-11381 (band E2).

Typically, bacterial communities' analysis of electrogenic biofilms in MFCs fed with acetate has shown dominance of *Geobacter*-related species (Bond et al., 2002; Chae et al., 2009; Kiely et al., 2011). While, the anaerobic character of members of the genus *Geobacter* has been questioned recently (Methé et al., 2003), these species may be outcompeted by *Shewanella* spp. in our experimental conditions. Oxygen diffusion from the cathode could explain the absence of *Geobacter*-related sequences since *Shewanella* species are facultative anaerobes well-known to have exoelectrogenic activity. Pure cultures of *S. oneidensis* (formerly *S. putrefaciens*) were used previously for MFCs studies (Kim et al., 2002). Moreover, *Shewanella* spp. closely related to *S. affinis* and *Pseudoalteromonas* spp. have been reported as dominant members of the anodic bacterial community from a MFC inoculated with marine sediment and fed with cysteine (Logan et al., 2005). *Pantoea* spp. has been reported as a dominant member of anodic biofilm community in a tubular MFC (Kim et al., 2011) and in a two chamber MFC (Zhang et al., 2011).

The microbial community in the biofilm of the $SMFC_{wea}$ was dominated by different species of the *Bacillus* genera (bands A3, A5, A6, A7, and A8) and one faint band associated with a clone sequence from a rice SMFC anode. Firmicutes belonging to the *Bacillus* genus have been reported previously to be dominant members of anodic communities in a propionate-fed MFC (Chae et al., 2009). A recent study of bacterial community dynamics in a sucrose-fed tubular longitudinal MFC has found a temporal shift in the

Table 1
Summary of DGGE band sequences.

Band	Closest cultivated isolate	Isolation source	Similarity (%)
A3	*Bacillus megaterium* strain SMC-1/09	Saliva sample	99
A5	*Bacillus niacini* strain EAS2-16	Soil crust	97
A6	*Bacillus soli*	Soil	97
A7	*Bacillus* sp. WPCB169	Freshwater	99
A8	*Bacillus barbaricus* strain TDSAS2-8	Mangrove sediment	100
A9	Uncultured bacterium clone MFC-B129	Rice MFC anode	100
E2	*Antarctic bacterium* R-11381	Lake microbial mat	97
E3	*Pantoea ananatis* strain DF-2	Rice crop	100
E4	*Shewanella putrefaciens*	Faeces of fish	98
E5	*Pseudoalteromonas* sp. Bsw21495C	Sea water	97
E6	*Pantoea* sp. B232	Rainbow trout skin	98
E7	*Shewanella oneidensis* MR-1	Intestinal tract of carp	100
E8	*Pantoea agglomerans* strain P20-14	Plant habitat	100
E9	*Citrobacter freundii* strain ARY2	River water	99
E10	*Pseudidiomarina sediminum*	Sand samples	96

dominant species from gamma-proteobacteria and Bacteroidetes to Firmicutes (Kim et al., 2011). Similar results have been reported for an acetate-fed MFC, in which a shift from communities dominated by proteobacteria to communities dominated by Firmicutes occurred at the end of the operation (Aelterman et al., 2006). However, it has been demonstrated that a *B. subtilis* strain was able to produce an anodic biofilm in a MFC with electrochemical activity via excreted redox mediators (Nimje et al., 2009). Moreover, it has been shown that some Firmicutes genera are responsible for power generation in a thermophilic MFC (Wrighton et al., 2008). Considering these results, it seems likely that the electron transfer to the electrodes was conducted by the dominant *Bacillus*-related species detected. It remains to analyze whether the dominance of *Bacillus* within the community is the result of a succession process from Proteobacteria to Firmicutes, as it was demonstrated by other authors in different MFC systems.

We believe that obtaining a higher current/power yield in the $SMFC_{sa}$ than in the $SMFC_{wea}$, is due to the fact that the acetate, as an easily metabolizable carbon source, promotes bacterial growth in the mud and over the anodes (originating a change in the oligotrophic status of the sediments). The addition of acetate (even if it is not the preferred carbon source for *Shewanella*), has promoted a greater dominance of this genera in the $SMFC_{sa}$. Although lactate is preferred as electron donor for *Shewanella*, it can also grow using acetate or pyruvate (Tang et al., 2009).

4. Conclusions

This study demonstrated that the external carbon source addition increased the size of the electrogenic community present in the sediments used, therefore enhancing the SMFC current and power production, while the diversity was diminished. The geometry and size of the electrodes have an influence on the current and power generation, may be due to a more efficient mass transport on the surface of the rod electrode, or other effects. Given the shape of the voltammograms, a mixed mechanism involving both radial and planar diffusion rather than a single radial diffusion seem more probable to be occurring in this electrode type. In future designs the electrodes geometry and size will be considered for the development of more efficient systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2012.09.060.

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