

Microbial Fuel Cells Applied to the Metabolically Based Detection of Extraterrestrial Life

Ximena C. Abrevaya¹, Pablo J.D. Mauas¹, and Eduardo Cortón²

Abstract

Since the 1970s, when the Viking spacecrafts carried out experiments to detect microbial metabolism on the surface of Mars, the search for nonspecific methods to detect life *in situ* has been one of the goals of astrobiology. It is usually required that a methodology detect life independently from its composition or form and that the chosen biological signature point to a feature common to all living systems, such as the presence of metabolism.

In this paper, we evaluate the use of microbial fuel cells (MFCs) for the detection of microbial life *in situ*. MFCs are electrochemical devices originally developed as power electrical sources and can be described as fuel cells in which the anode is submerged in a medium that contains microorganisms. These microorganisms, as part of their metabolic process, oxidize organic material, releasing electrons that contribute to the electric current, which is therefore proportional to metabolic and other redox processes.

We show that power and current density values measured in MFCs that use microorganism cultures or soil samples in the anode are much larger than those obtained with a medium free of microorganisms or sterilized soil samples, respectively. In particular, we found that this is true for extremophiles, which have been proposed as potential inhabitants of extraterrestrial environments. Therefore, our results show that MFCs have the potential to be used for *in situ* detection of microbial life. Key Words: Life detection—Microbial metabolism—Microbial fuel cell—Extremophile—Archaea—Astrobiology. Astrobiology 10, 965–971.

1. Introduction

THE SEARCH FOR LIFE on other planets has been a main objective of a number of space missions over the course of the last 30 years. Many astrobiological investigations attempt to identify methods that could be applied to the detection of life or evidence of life beyond the confines of Earth. The first set of biological extraterrestrial experiments were those of the Viking mission in 1976 that searched for evidence of metabolism as a signal of putative microbial life. Three life-detection tests were performed inside the Viking landers to look for the production or absorption of volatile compounds in samples of martian soil: the Pyrolytic Release experiment, which tested the incorporation of radioactive CO₂ and CO into the organic fraction of a soil sample; the Labeled Release experiment, which used radiorespirometry for the detection of metabolism or growth by using radioactive nutrients labeled with ¹⁴C; and the Gas Exchange experiment, which was particularly designed to detect signals of metabolism by measuring compositional changes in the atmosphere above the soil sample after the addition of a nutrient medium. Most of these ex-

periments sought to identify activity driven by a process of chemical or even biological origin. In fact, the Pyrolytic Release experiment found that a small proportion of gas was converted into organic material, which was not reproduced in the control experiment; the Gas Exchange experiment detected increases in several biogenic gases above the soil; and the Labeled Release experiment identified the presence of radioactive gases, which were absent in the control experiment (Klein *et al.*, 1976; Klein, 1977).

However, the gas chromatography–mass spectrometry chemical analysis that was additionally performed failed to find traces of organic compounds in the martian soil samples, which is indicative of the absence of life. The overall general consensus was that positive results obtained from biological tests were due to the presence of strong oxidizing agents on the martian surface rather than activity from biological origin. Various investigations reviewed the controversy that followed these findings (Klein, 1977, 1979; Ponnampertuma *et al.*, 1977; Owen, 1979; Navarro-González *et al.*, 2006; Biemann, 2007).

At present, current approaches use several new techniques and employ a wide variety of biomarkers for the detection

¹Instituto de Astronomía y Física del Espacio (IAFE), UBA-CONICET and Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina.

²Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA, and CONICET, Buenos Aires, Argentina.

of different biosignatures (Kounaves *et al.*, 2002; Schweitzer *et al.*, 2005; Sims *et al.*, 2005; Parnell *et al.*, 2007; Suo *et al.*, 2007; Tang, 2007).

The discovery of extremophiles and the capacity of microorganisms to adapt to different physicochemical conditions and exploit versatile metabolic pathways has helped to focus research toward the development of new methods for *in situ* search and detection of microbial activity in extreme environments on Earth or in extraterrestrial settings. One such method involves the use of microelectrodes for the measurement of pH, mineral content, or natural gradients of gases, all of which provide information about metabolic activity (Horneck, 2000).

Although there is no general consensus as to the definition of life, it is usually assumed that life produces signatures that can be detected and quantified to a certain degree. Even though most investigations are directed toward the detection of life as we know it on Earth, the methods with which to detect biosignatures in extraterrestrial settings should be the least Earth-centric possible (Conrad and Nealon, 2001). To produce reliable results, the search method must be repeatable, sensitive enough to detect life (Conrad and Nealon, 2001; Kounaves *et al.*, 2002; Nealon *et al.*, 2002; Schweitzer *et al.*, 2005), and have the capacity to (1) distinguish between biotic and abiotic processes, (2) be applied on other rocky planets, (3) perform a fairly rapid scan of a large spatial region, (4) detect life independently from its composition or form.

A biosignature should be a distinguishable and common feature of all living systems (Nealon *et al.*, 2002). In particular, one of the most indicative signatures of life is that of metabolic activity, which is common to all organisms. Through metabolism, organisms have the capacity to convert energy from the environment into biologically usable energy. This process involves chemical oxidation-reduction (redox) reactions in which an electron donor is oxidized and the released electrons are accepted by an electron acceptor that becomes reduced. Also, electron flow across a cellular membrane is used to generate an electrochemical gradient that will result in the synthesis of adenosine triphosphate, ATP, the main molecular unit of chemical energy storage and transfer in the cell. The biologically driven redox gradients and electron flow involved in these reactions can be detectable and are measurable. Regardless of the high metabolic diversity found in terrestrial microorganisms, all types of metabolism of which we are aware use the same basic processes of redox flow accompanied by electron transfer (Guzman and Martin, 2009; Srinivasan and Morowitz, 2009).

The capability of organisms to carry out this process is the basis of microbial fuel cells (MFCs), electrochemical devices originally designed for electricity production. They work in a similar way to a battery and are usually composed by a cathode and anode separated by a cation exchange membrane. An MFC could be described as a fuel cell in which the anode is submerged in a medium (the anolyte) that contains microorganisms which, as part of their metabolic process, oxidize organic material, releasing electrons and protons. The electrons are captured by the anode, which is made with a corrosion-resistant conductor material, travel through an external conductor, and reach the cathode, thereby closing the circuit. In the cathode, the reduction reactions can be achieved by contact with an oxidizing agent, like oxygen or ferricyanide, among others. The protons liberated in the

proximity of the anode could reach the cathode through a polymer electrolyte membrane (such as Nafion) to complete redox reactions. This electron flow, which is proportional to metabolic and other redox processes in the anode region, produces a current that can be easily measured if an adequate resistor is incorporated in the electric circuit. In this way, MFCs couple the metabolism of a microorganism to an electrical circuit.

The first description of MFCs is attributed to Potter (1911), who worked with cultures of yeast and *E. coli*. Later, they were rediscovered by Bennetto (1984; see also Allen and Bennetto, 1993). At present, there is considerable work being done with MFCs, with multiple combinations of electrode materials and microorganisms (see the reviews by Rabaey and Verstraete, 2005; Bullen *et al.*, 2006; Davis and Higson, 2007). In particular, Miller and Oremland (2008) used extremophilic bacteria as biocatalysts and suggested the use of MFCs as life detectors.

Since extremophiles live in habitats with extreme physicochemical conditions, which are considered as analogues of extraterrestrial environments, they have been proposed as potential inhabitants of other planets (Cavicchioli, 2002). In particular, the haloarchaea are extremophilic microorganisms that live in hypersaline environments (3–5 M NaCl) such as salt lakes, marine salterns, or salt ponds. They have a special importance in astrobiology because they were also found entrapped in salt deposits in ancient evaporitic rocks such as halites (Grant *et al.*, 1998; Grant, 2004; Fendrihan *et al.*, 2006). Since the same structures were identified in SNC martian meteorites (Gooding, 1992; Zolensky *et al.*, 1999; Whitby *et al.*, 2000; Stan-Lotter *et al.*, 2004), it is thought that such organisms could be present on Mars or on other planets that harbor saline environments (Sims *et al.*, 1997; Grant, 2004; Fendrihan *et al.*, 2006).

In the present study, we investigated the capacity of MFCs to detect the presence of metabolism and their potential use to search, *in situ*, for extraterrestrial life. We show that the method of detection examined here satisfies all the requirements previously mentioned. In particular, we report new results derived from our use of a culture of an archaeon, which has not been previously employed as a biocatalyst in MFCs. We also used a mixed community of microorganisms from humus soil. Therefore, our conclusions were obtained based on representatives of the three domains of life—Archaea, Bacteria and Eukarya—which are taxonomically and metabolically diverse.

2. Materials and Methods

2.1. Microbial fuel cell design and operation

Microbial fuel cells used in this study (Fig. 1) were made of polystyrene cylinders sealed at one end with a Nafion membrane (DuPont, Wilmington, DE) that acts as a barrier of separation but allows proton exchange between the media contained within the cylinder and the media to be analyzed for redox activity outside the cylinder. The cathodic compartment, the catholyte, was situated inside the cylinder and contained a solution made of ferricyanide (8.4 g L^{-1}) dissolved in an appropriate buffer solution, according to the sample to be measured. Outside the cylinder, the anodic compartment behaved as an anolyte and contained a sample of the microorganisms to be studied (culture or soil), which, in

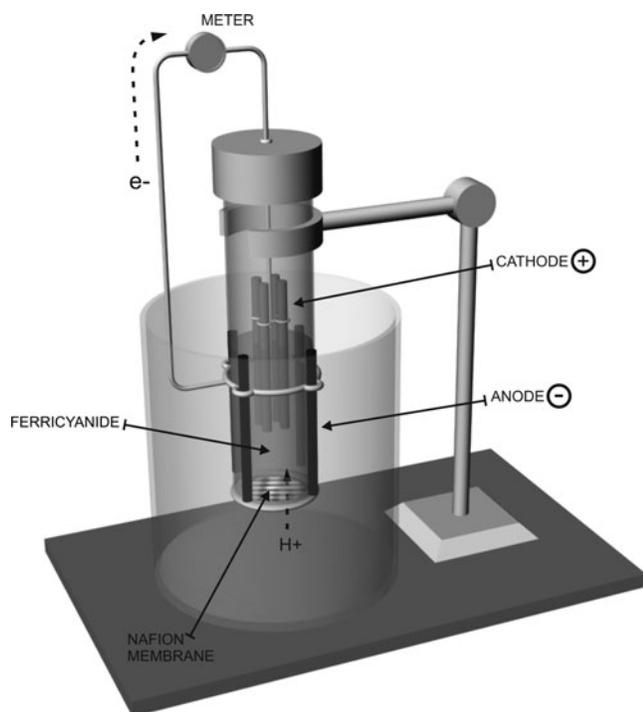


FIG. 1. Schematic representation of submersible MFC used to perform the experiments.

the control experiments, were replaced with sterilized culture or soil.

The cathode and anode were made of Toray carbon paper (TGP-H-120, density, 0.45 g cm^{-3} , porosity 78%, Toray Industries (America), Inc., NY, USA) for microbial culture experiments and graphite reinforced with carbon (0.7 HB pencil lead, Faber Castell, Brazil) for soil experiments. Both electrodes were connected to insulated copper wire with silver epoxy and covered thoroughly with insulating epoxy to avoid spurious currents that might arise from copper oxidation. The total area of the electrodes was 5.5 cm^2 for carbon paper and 3.76 cm^2 for graphite reinforced with carbon (anode and cathode areas are identical). The area of the Nafion membrane was 0.38 cm^2 .

To study the MFC behavior, the potential (E , volts) was measured at open circuit by using a Fluke 289 digital tester (Fluke Inc., Everett, WA) and different resistances ranging from 98Ω to $100 \text{ k}\Omega$ as an external load. The electrical current (I , amperes) was calculated according to Ohm's law

$$E = IR$$

and power (P , watts) was calculated according to

$$P = IE$$

where R is the resistance (Ω , ohms). Power and electrical current were normalized by the electrode area and were expressed as power and current density, (p) in W cm^{-2} and (j) in A cm^{-2} .

2.2. Microbial culture experiments

We considered two experimental groups: sterilized microorganism cultures (autoclaved at 121°C , 45 min) and

nonsterilized microorganism cultures. Both were placed in the anolyte compartment. To test the capacity of MFCs to detect life, we used two different microorganisms, as follows.

Saccharomyces cerevisiae (Baker's yeast) is a facultative anaerobic unicellular fungus which belongs to the domain Eukarya. Industrial *S. cerevisiae* (dry active yeast, from Calsa S.A., Argentina) was prepared by adding 3 g of the dry powder to 60 mL of phosphate buffer 100 mM, and adjusting pH to 7. Glucose 5 g L^{-1} was added as a carbon source, and aliquots of 60 mL for sterilized and nonsterilized groups were placed as anolytes and incubated in a thermostatic bath at $37 \pm 1^\circ\text{C}$ for the measurements.

Natrialba magadii is a prokaryotic microorganism that belongs to the domain Archaea and is a heterotrophic aerobic member of the family Halobacteriaceae. *N. magadii* was originally isolated from Magadii Lake in Kenya, Africa (Tindall *et al.*, 1984). It is an extremophilic haloalkaliphilic microorganism that lives in 3.5–4.0 M NaCl and pH values between 9 and 11 (optimum range). *N. magadii* (ATCC 43099) was grown aerobically at 37°C . Growth medium contained (g L^{-1}): yeast extract (5); NaCl (200); Na_2CO_3 (18.5); sodium citrate (3); KCl (2); $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1); $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (3.6×10^{-4}); $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (5×10^{-3}); pH was adjusted to 10.

Natrialba magadii liquid culture (at optical density around 0.5, exponential phase) was divided in aliquots of 36 mL. The aliquots for sterilized and nonsterilized groups were centrifuged 5 min at 45 rpm. The supernatant was discarded, and the pellet was resuspended in 36 mL of fresh medium, collected in a flask, diluted to reach 60 mL, and used as anolytes.

A resistor of 4600Ω was coupled to the circuit. After 12 h, this resistor was disconnected and measurements were taken 2 h later. The experiments were carried out at $37 \pm 1^\circ\text{C}$ in a thermostatic bath.

The catholyte was composed of ferricyanide (8.4 g L^{-1}) dissolved in a phosphate buffer solution (100 mM, pH = 7) for *S. cerevisiae* experiments and of saline solution [NaCl (200); Na_2CO_3 (18.5); sodium citrate (3); KCl (2); $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1); $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (3.6×10^{-4}); $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (5×10^{-3}), pH = 10] for *N. magadii*.

2.3. Soil experiments

To test the MFC capacity to detect life in soil samples, we used humus-rich (about 5%) topsoil, which is commercially available. The sample was divided into two subsamples of 70 g, sterile (control) and nonsterile, both in duplicate. For control, sterilized soil was obtained by autoclaving at 121°C in two cycles of 1 h 45 min and 45 min, separated by 24 h. Then, 40 mL of distilled water was added to each sample, and voltage was measured at the beginning of the experiment and every 24 h during a 144 h period. Each day, an external resistor of 4600Ω was coupled to the circuit. The resistance was disconnected 2 h before each measurement was done. Catholyte was phosphate buffer (100 mM, pH = 7) containing ferricyanide. The experiments were carried out at room temperature ($27 \pm 1^\circ\text{C}$).

3. Results

3.1. Culture experiments

In Figs. 2 and 3, we show the results obtained for the *S. cerevisiae* and *N. magadii* cultures, respectively, which are

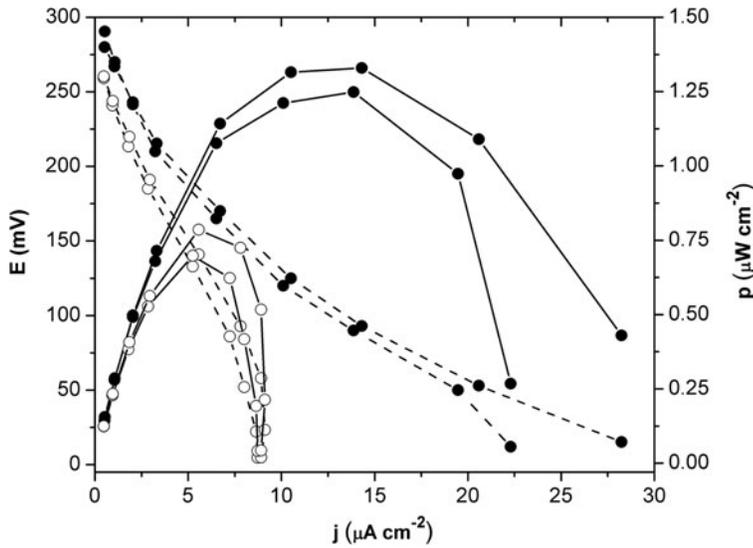


FIG. 2. MFC power density (solid lines, right scale) and potential (dashed lines, left scale) as a function of current density for *S. cerevisiae*. Experiments were performed in duplicate. Open circles: sterilized culture. Solid circles: non-sterilized culture.

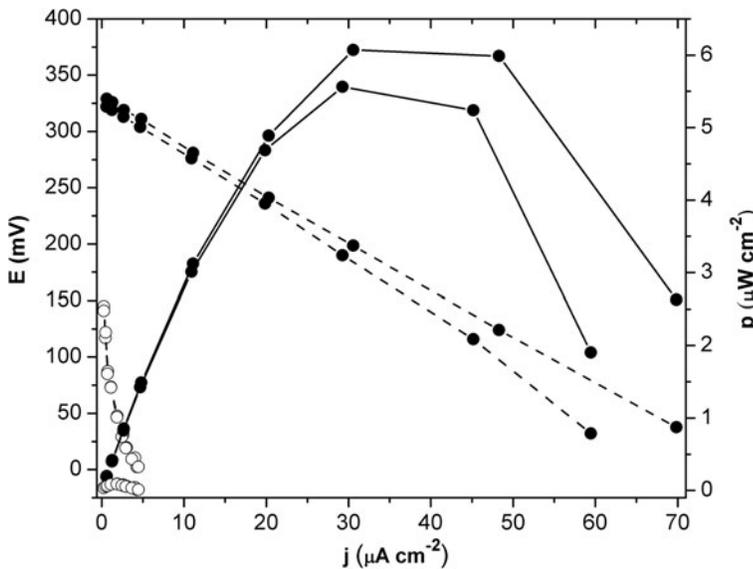


FIG. 3. MFC power density (solid lines, right scale) and potential (dashed lines, left scale) as a function of current density for *N. magadii*. Experiments performed in duplicate are shown. Open circles: sterilized culture. Solid circles: nonsterilized culture.

TABLE 1. SUMMARY OF DIFFERENT VALUES OBTAINED FROM CULTURE AND SOIL EXPERIMENTS

Sample/Experiment	Maximum power density ($\mu\text{W cm}^{-2}$)	Current density at maximum power density ($\mu\text{A cm}^{-2}$)	Potential at open circuit (mV)
Pure culture experiments			
<i>S. cerevisiae</i> (sterile)	0.74 ± 0.04	5.41 ± 0.16	277.0 ± 4.8
<i>S. cerevisiae</i> (nonsterile)	1.28 ± 0.03	14.00 ± 0.22	305.5 ± 0.5
<i>N. magadii</i> (sterile)	0.090 ± 0.002	1.860 ± 0.021	256.5 ± 2.5
<i>N. magadii</i> (nonsterile)	5.82 ± 0.25	30.00 ± 0.66	333.5 ± 8.5
Soil experiment			
Sterile soil ($t = 114$ h)	0.024 ± 0.085	0.36 ± 0.07	11.60 ± 1.40
Nonsterile soil ($t = 114$ h)	3.21 ± 0.29	8.50 ± 0.38	187.0 ± 5.01

In all cases, we list the mean value of the duplicate experiments \pm half the difference between them.

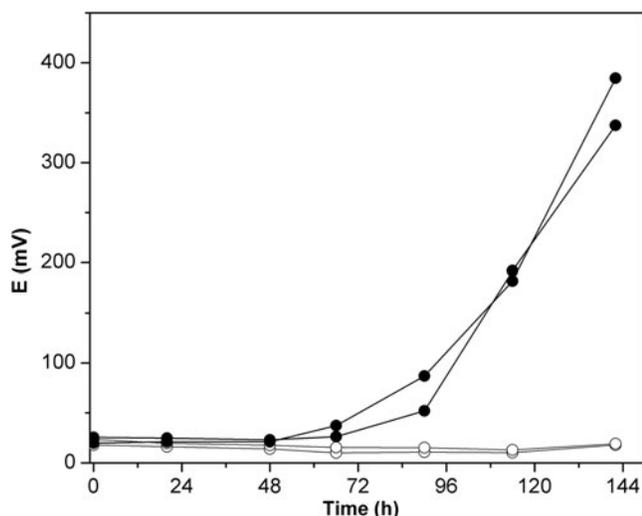


FIG. 4. MFC potential as a function of time, $R = 4600 \Omega$ for or soil experiments. Experiments in duplicate for sterile (open circles) and nonsterile samples (solid circles) are plotted.

also summarized in Table 1. In the figures, we show potential and power density as a function of current density. It can be seen that in both experiments power densities were larger when microorganisms were present in the anode than in the corresponding sterilized samples. On the other hand, in the experiments with microorganisms the maximum power densities occur at higher current densities than in the control experiments (Table 1).

3.2. Soil experiments

In Fig. 4, we plot the evolution of potential with time, with the system loaded with a fixed resistor (4600Ω). It can be seen that after around 48 h the potential in the nonsterile samples increased as a signal of the presence of microorganisms. On the other hand, the sterile samples showed no increment of the potential. In Fig. 5, we show the potentials, power, and current densities obtained for the soil experiments, which are also

summarized in Table 1. Here, we show potential and power density as a function of current density, measured 114 h after the beginning of the experiments. Also in this case, the power densities in the experiments with microorganisms are much larger, and the maximum occurs at higher current density than in the sterile soil.

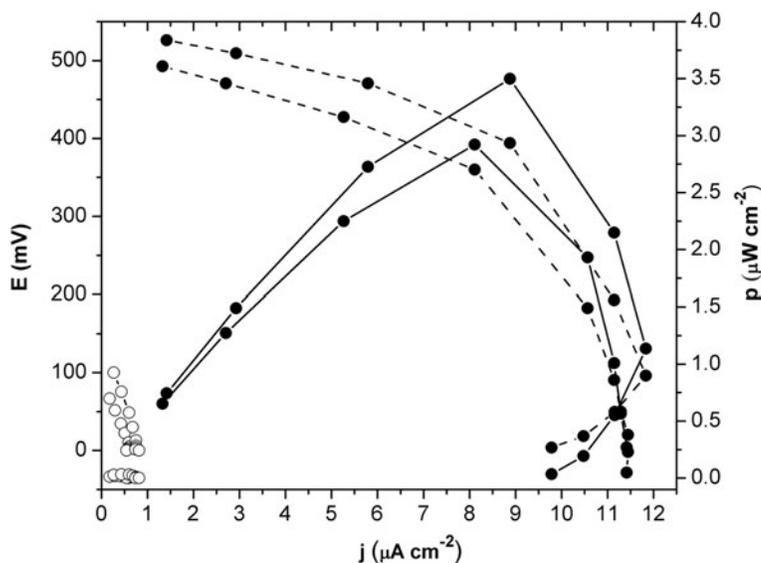
4. Discussion

Our experiment results show that power and current densities are significantly larger when microorganisms are present in the samples. This is due to the coupling of the microorganism's metabolism to the electrical circuit. Therefore, our MFC results demonstrate that this method can detect the presence of microbial life, which satisfies the requisites mentioned in the Introduction, and could be used for the *in situ* search for extraterrestrial life.

In principle, there are no impediments to the application of this method on other rocky planets, and it can be applied to liquid samples or in soil experiments. In this case, a rapid scan of a large spatial region could be performed by taking different soil samples and performing several experiments at the same time. Several studies have shown that MFCs can be employed in different experimental conditions from acid to alkaline (Raghavulu *et al.*, 2009; Liu *et al.*, 2010). Temperature working values were found in a range from 5°C to 60°C (Mathis *et al.*, 2008; Scott *et al.*, 2008). Up to now, salt concentrations used in MFCs were in the range of $0.1\text{--}0.4\text{M}$ NaCl (Liu *et al.*, 2005; Huang *et al.*, 2010). In the present work, we tested a concentration 10 times larger than previously documented. Therefore, we have demonstrated that the range of ionic strength in which MFCs can operate could be expanded to work in hypersaline environments and for the detection of extreme halophiles.

Additionally, it is important to note that, for the first time, we used MFCs with archaea, which constitute the vast majority of extremophiles, and we found that the values obtained in this case were larger than the values for the other microorganisms. In particular, *N. magadii* is a halophilic archaeon, and halophiles are usually proposed as "exophiles" due to their capacity to survive in extreme physicochemical conditions: not only high salt concentrations but also high UV or

FIG. 5. MFC power density (solid lines, right scale) and potential (dashed lines, left scale) as a function of current density in soil experiments performed in duplicate. Open circles: sterilized soil. Solid circles: nonsterilized soil.



ionizing radiation doses, low oxygen levels, and extreme temperatures and pH values (DasSarma, 2006).

Our approach in this work has been similar to that used to test, on the ground before launch, the Viking mission's biological experiments. In particular, the Labeled Release experiments were performed on pure cultures of microorganisms and soil samples (Levin and Straat, 1976), as in our study of the ability of MFCs to detect *in situ* microbiological life. Furthermore, our experiments involved different kinds of microorganisms—representatives of the three domains of life, Archaea, Bacteria and Eukarya—which are taxonomically and metabolically diverse, and in all cases the results were positive. Therefore, the method is able to detect different kinds of metabolisms and allows for the detection of life independently of its form, which is part of the main requirements for these kinds of tests.

However, in contrast to the Viking experiments, our methodology does not require the existence of carbon-based life; it is, therefore, a more general approach to the detection of life independently from its composition.

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Abbreviation

MFCs, microbial fuel cells.

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Address correspondence to:
Ximena C. Abrevaya
Pabellón IAFE
Universidad de Buenos Aires
Ciudad Universitaria
CC 67 Suc. 28
1428 Buenos Aires
Argentina

E-mail: abrevaya@iafe.uba.ar

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