Research Article

In Situ Search for Extraterrestrial Life:
A Microbial Fuel Cell–Based Sensor
for the Detection of Photosynthetic Metabolism

Federico Figueredo,[1] Eduardo Cortón,[1] and Ximena C. Abrevaya[2]

Abstract

Microbial fuel cells (MFCs) are bioelectrochemical systems (BES) capable of harvesting electrons from redox reactions involved in metabolism. In a previous work, we used chemoorganoheterotrophic microorganisms from the three domains of life—Bacteria, Archaea, and Eukarya—to demonstrate that these BES could be applied to the *in situ* detection of extraterrestrial life. Since metabolism can be considered a common signature of life ''as we know it,'' we extended in this study the ability to use MFCs as sensors for photolithoautotrophic metabolisms. To achieve this goal, two different photosynthetic microorganisms were used: the microalgae *Parachlorella kessleri* and the cyanobacterium *Nostoc* sp. MFCs were loaded with nonsterilized samples, sterilized samples, or sterilized culture medium of both microorganisms. Electric potential measurements were recorded for each group in single experiments or in continuum during light-dark cycles, and power and current densities were calculated. Our results indicate that the highest power and current density values were achieved when metabolically active microorganisms were present in the anode of the MFC. Moreover, when continuous measurements were performed during light-dark cycles, it was possible to see a positive response to light. Therefore, these BES could be used not only to detect chemoorganoheterotrophic metabolisms but also photolithoautotrophic metabolisms, in particular those involving oxygenic photosynthesis. Additionally, the positive response to light when using these BES could be employed to distinguish photosynthetic from non-photosynthetic microorganisms in a sample. Key Words: Astrobiology—Photosynthesis—Redox reactions—Life detection—Bioelectrochemical systems. Astrobiology 15, xxx–xxx.

1. Introduction

SPACE EXPLORATION that involves the search for life on other planetary bodies has been a major challenge for the discipline of astrobiology and remains, to date, an active avenue of research (Des Marais *et al.*, 2008).

Different efforts in astrobiology have focused on the *in situ* search for biosignatures of extinct or extant life in the Solar System. In particular, Mars has been the preferred target not only because of its physical proximity to Earth but more importantly due to its supposed Earth-like conditions in the past that suggest the planet may have been habitable at this time and potentially at the present (McCollom, 2006).

The development of new technology and instrumentation has been essential for the advancement of planetary exploration. The development of new chemical, biological, and physical techniques with which to study Earth life-forms in the terrestrial context has also provided new tools for the detection of life on other planetary bodies.

To date, two different missions have flown with the intent to detect, *in situ*, direct evidence of life on other planetary bodies: the Viking lander biology experiments and the Beagle 2 experiments that were part of the Mars Express mission (Young, 1976; Sims *et al.*, 1999). Both of these studies assumed carbon-based life-forms such as those found on Earth. Because the Beagle 2 mission was lost shortly after deployment, the Viking lander was the first, and only, mission to attempt detection of evidence of biological activity on the surface of a planetary body (Levin, 2014).

The results obtained by the Viking lander experiments (Klein *et al.*, 1976; Klein, 1977), however, were met with controversy (see, *e.g.*, Klein, 1999; Navarro-González *et al.*,

[1]Laboratorio de Biosensores y Bioanálisis (LABB), Departamento de Química Biológica e IQUIBICEN-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (UBA). Pabellón 2, Ciudad Universitaria, Ciudad Autónoma de Buenos Aires, Argentina.
[2]Instituto de Astronomía y Física del Espacio (IAFE, CONICET-UBA). Pabellón IAFE, Ciudad Universitaria, Ciudad Autónoma de Buenos Aires, Argentina.

2006; Biemann, 2007; Houtkooper and Schulze-Makuch, 2007; Levin, 2014), but in subsequent years several techniques have been proposed for the search and detection of extinct or extant life (*e.g.*, Kounaves *et al.*, 2002; Sims *et al.*, 2005; Hoehn *et al.*, 2007; Kiang *et al.*, 2007a, 2007b; Parro *et al.*, 2011).

Even at the present time there is no "universal" definition of life (Conrad and Nealson, 2001); however, there is general agreement as to how life-detection strategies should be approached such that unequivocal results are the end goal (*e.g.*, false positives, false negatives). There are indispensable requisites that life-detection experiments must accomplish. A major objective is that biotic and abiotic processes must be clearly distinguished from each other. Also, one of the most desirable features of a *bona fide* biosignature is that it should represent a ubiquitous characteristic of life on Earth. At the same time, search strategies should not be entirely Earth-centric such that the detection of "life as we don't know it" (Nealson *et al.*, 2002).

Possible biosignatures to be considered are those that result from reduction-oxidation (redox) processes and are involved in metabolism. Different kinds of metabolisms evolved on Earth to exchange matter and energy with the environment, all of which involve redox reactions that occur in cells as part of this process, which can be generally described as electron transfers between electron donors and acceptors (Nealson and Popa, 2005). This is a widespread characteristic for all life-forms on Earth.

In particular, most redox couples available on Earth have been employed by life as fuel. As a consequence, it is possible to see that life has vast redox versatility (Nealson *et al.*, 2002).

If we try to extend the argument discussed above beyond Earth, it is expected that the same principle can be applied. Biological processes should follow the same physical laws that rule the Universe, and living organisms can be considered as open systems that exchange matter and energy with their environment (von Bertalanffy, 1950). The diverse redox reactions involved in metabolism are a fundamental part of biological processes (Nealson and Popa, 2005).

Moreover, these biological redox reactions can be distinguished from those involved in abiotic processes. The capability of cells to catalyze these reactions at rates that are faster than those predicted for abiotic processes is an unequivocal characteristic of life. Therefore, this fact can be employed to distinguish biotic and abiotic processes, and as a consequence, the presence or absence of living organisms could be determined (Nealson and Popa, 2005).

Considering that redox processes involved in cellular metabolism can be employed as biosignatures for life detection, we explored in a previous work the feasibility of detecting them through a bioelectrochemical approach. In particular, we proposed and demonstrated for the first time that microbial fuel cells (MFCs) could be used as *in situ* life-detection devices (Abrevaya *et al.*, 2010). MFCs are bioelectrochemical systems (BES) similar to batteries and were originally designed for production of electricity from microorganisms or enzymes. However, many applications were later derived from these BES (Abrevaya *et al.*, 2015a, 2015b). In a typical MFC, redox compounds are oxidized by microorganisms at the anode where electrons are released. The electrons travel through an external circuit and are then released at the cathode to reduce an oxidant such as oxygen. This process generates an electric potential that can be measured.

Particularly, we previously showed that MFCs could allow the detection of microbial life based on chemoorganoheterotrophic metabolisms (from the domains Bacteria, Archaea, and Eukarya) (Abrevaya *et al.*, 2010).

The aim of the present study was to demonstrate the capability of a MFC to detect photolithoautotrophic metabolisms, in particular those involving oxygenic photosynthesis.

2. Material and Methods

2.1. Single-strain culture samples

Two microorganisms with photolithoautotrophic metabolisms, from the domains Bacteria and Eukarya, were employed for all the experiments.

We employed the microalgae *Parachlorella kessleri* (strain BAFC CA10, Juárez *et al.*, 2011) as a representative of the domain Eukarya. *Parachlorella kessleri* is an aerobic photosynthetic mixotrophic unicellular algae isolated from Laguna Verde, a volcanic mesothermal, sulfurous, and extremely acidic pond (pH values between 2.1 and 2.8), located in the Copahue-Caviahue Provincial Park (34°42′58″S, 71°03′14″W) in northern Patagonia, Argentina. This microorganism is the predominant species in the pond, being acid-tolerant and able to grow at pH values as low as 2.5–3.0 (Juárez *et al.*, 2011).

We selected a cyanophyte, *Nostoc* sp., as a representative of the domain Bacteria; it was kindly provided by Rizobacter Argentina S.A. *Nostoc* is a genus of colony-forming filamentous cyanobacteria, commonly found in aquatic and terrestrial environments including tropical and polar habitats (Dodds *et al.*, 1995).

Parachlorella kessleri and *Nostoc* sp. were grown autotrophically in liquid Bold's Basal Medium (Bischoff and Bold, 1963) and in liquid BG-11 medium (Rippka *et al.*, 1979), respectively, at $26°C \pm 1°C$, pH 6.6, under continuous agitation and illumination with photoperiod (80 μmol photons m^{-2} s^{-1}, 16:8 h light:dark cycle). These conditions were maintained for all the experiments. Three experimental groups were considered: microorganism cultures (nonsterilized samples), autoclaved microorganism cultures (121°C, 45 min) (sterilized samples), and sterilized culture medium devoid of cells (sterilized culture medium).

2.2. Microbial fuel cell architecture and operation

Microbial fuel cells were made of transparent poly(methyl methacrylate) with an anodic and a cathodic compartment (volume 50 mL and 7 mL, respectively) (Fig. 1). Both chambers were connected through a proton-exchange Nafion membrane (0.38 cm^2; DuPont, Wilmington, DE, USA).

The anodic compartment was designed to contain the sample to be tested and an electrode (anode) made of carbon paper (1 cm^2; Spectracarb 2050-L, Engineered Fibers Technology, LLC, Shelton, CT, USA).

The cathodic compartment was filled with sterilized culture medium devoid of cells. A cathode made of woven carbon cloth with a Pt load of 0.5 mg cm^{-2} (1 cm^2; GDE LT 120EW, E-TEK Division, PEMEAS Fuel Cell Technologies, Somerset, NJ, USA) was placed inside the cathodic compartment, which was continuously aerated (air-bubbled).
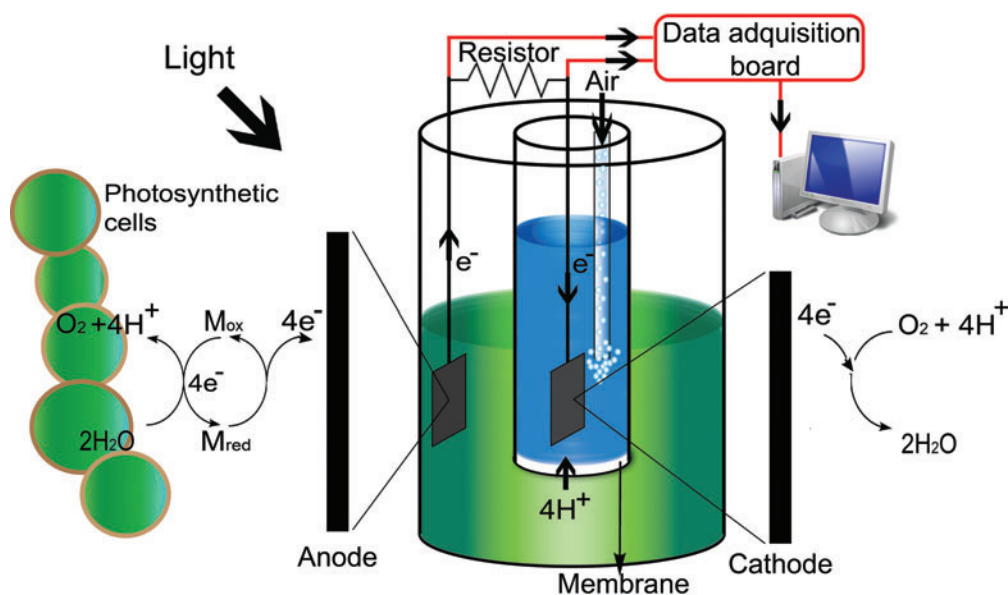
FIG. 1. Architecture of the MFC employed in the experiments. Electrochemical reactions occurring at the anode and cathode are also detailed. The electron transfer mechanism proposed in the anode, which involves chemical mediator redox reactions, is hypothetical (M_{red} = reduced mediator; M_{ox} = oxidized mediator). (Color graphics available at www.liebertonline.com/ast)

In the MFC that was designed for this work, the electrons that arise from the photosynthetic metabolism were harvested from the biophotolysis of water mediated by photosystem II. These electrons were released and then captured by the anode, which acts as an electron acceptor. The electrons were then transferred to the cathode, reducing oxygen in the presence of protons (passing through the Nafion membrane) to form water together with oxygen provided by air bubbling in this compartment (Pisciotta *et al.*, 2011). The latter reaction was catalyzed by the presence of Pt at the surface of the electrode. A schematic overview of these reactions is shown in Fig. 1.

The electric potential (E, volts) of the MFC was measured with a UNI-T digital multimeter (model UT71B, Uni-Trend Technology Limited, Guangdong Province, China). The electric current (I, ampere) was obtained according to Ohm's law and electric power (P, watt) conforming to the equation $P = IE$. The normalization of these values with the electrode area (anode) gave the current density (j, A cm^{-2}) and power density (p, W cm^{-2}). Polarization curves (E vs. j and p vs. j) were obtained when measurements were performed with different resistors connected to the circuit as an external load, ranging from 4.7 MΩ to 120 Ω.

Before performing the experiments, the MFCs were sterilized with H_2O_2 (30 vol) for 30 min and then washed thoroughly with sterile double-distilled water.

2.3. Detection of photosynthetic metabolic activity

The experiments for the detection of photosynthetic metabolic activity were performed by placing nonsterilized samples, sterilized samples, and sterilized culture medium of *P. kessleri* and *Nostoc* sp. (1.5 mg/mL dry weight) in the anodic compartment of the MFC.

Microbial fuel cells were left 24 h at open circuit (OC, no external load connected) for stabilization. The polarization curves were obtained at 24, 72, and 96 h for *P. kessleri* and

at 24, 48, and 96 h for *Nostoc* sp. The experiments were performed in duplicate, and the mean for each value, with the corresponding standard deviation, was plotted.

2.4. Light-dependent response

In another set of experiments, nonsterilized samples, sterilized samples, and sterilized culture medium of *P. kessleri* and *Nostoc* sp. (1.5 mg/mL dry weight) were placed in the anodic compartment of the MFCs. After 96 h at OC, the MFCs were connected to a 217 kΩ resistor, and electric potential measurements were recorded with a data acquisition board in continuum over the course of 5 days, at a sample rate of 10 Hz (National Instruments, Austin, TX, USA). Experiments were performed in duplicate.

Then the electric potential values were taken at the middle of the light period for the 2nd, 3rd, and 4th day from the beginning of the experiment. Current density values were calculated, and a mean for each experimental group with their corresponding standard deviations was obtained. An ANOVA analysis was performed ($p < 0.01$) and allowed for determination of whether there were any significant differences between the means of the groups considered. The current density values were used for each experimental group in duplicate (nonsterilized samples, sterilized samples, and sterilized culture medium). The means were compared by using Tukey's test.

2.5. Verification of the biological response

An experiment was performed to verify whether the metabolic reactions responsible for the increment on the electric potential had a biological origin.

To this end, the MFCs that contained nonsterile samples of the previous experiment (Section 2.4) were left 5 days at OC. Then, MFC polarization curves were obtained, both for *P. kessleri* and for *Nostoc* sp., through measurements of the

electric potentials of the anode using a series of resistors as an external load (ranging from $4.7\,M\Omega$ to $120\,\Omega$). These electric potentials were measured with respect to an Ag/AgCl reference electrode. The electric potential of the cathode was then obtained for each external load from the following equation (1), where the electric potential of the MFC (E_{MFC}) is equal to the electric potential obtained for the cathode (E_C) minus the electric potential of the anode (E_A).

$$E_{MFC} = E_C - E_A \qquad (1)$$

2.6. Limit of detection

To determine the limit of detection (LOD), different dry weights (from 0.1 to 1 mg/mL) were inoculated at the anode of MFCs for both *P. kessleri* and *Nostoc* sp. An estimation of the number of cells per milliliter was obtained by cell counting in a Neubauer chamber. After 24 h at OC, the MFCs were connected to $217\,k\Omega$ resistor, and electric potential measurements were performed for 2 days. Current density values were calculated at the middle of the light period (see Section 2.4).

To corroborate the results obtained for the LOD, an ANOVA analysis ($p < 0.01$) was performed that compared different values, one above and another below the LOD for each microorganism with the respective control groups.

3. Results

3.1. Detection of photosynthetic metabolic activity

The electric potential and power density as a function of current density were analyzed to assess the capability of MFCs to detect photosynthetic metabolisms driven by a process of biological origin. The results presented in Figs. 2 and 3 show that the electric potential and power densities of nonsterilized samples were higher than those obtained with sterilized samples or sterilized culture medium (inactive metabolism or absence of organisms and therefore metabolism, respectively).

The same results were obtained regardless of the time of measurement, although the differences seem to be more noticeable at 96 h, in particular because of the decrease in the electric potential and power density values observed for sterilized samples and sterilized culture medium. Despite this fact, the differences are always remarkable between samples that contain an active photosynthetic metabolism and those that contain inactive metabolisms or are devoid of microorganisms, which shows that the reactions are exclusively of biological origin.

3.2. Performance in continuum and positive light response

To determine the influence of light on the performance of the MFCs and their capacity to detect the presence of the photosynthetic metabolism, the electric potential of the MFC was recorded in continuum. In Figs. 4 and 5, it is clearly seen, as expected, that the electric potential obtained for nonsterilized samples (active metabolism) is higher than that for sterilized samples or for the sterilized culture medium (inactive metabolism or absence of metabolism,

respectively). A further analysis for *P. kessleri* showed that the means of the current density values during the light phase obtained for each experimental group were (in mA m^{-2}) 0.970 ± 0.154 (nonsterilized samples), 0.267 ± 0.094 (sterilized samples), and 0.088 ± 0.031 (sterilized culture medium). For the same analysis when using *Nostoc* sp., the means of the current density values obtained for each experimental group were (in mA m^{-2}) 1.789 ± 0.154 (nonsterilized samples), 0.181 ± 0.041 (sterilized samples), 0.0279 ± 0.006 (sterilized culture medium).

To analyze these results, a one-way statistical analysis of variance (ANOVA) was performed. The results show that the differences observed between the values for sterilized samples and sterilized medium were not statistically significant for *Nostoc* sp. or for *P. kessleri* ($p < 0.01$). Moreover, significant differences were observed when comparing the current density values for these groups with the current density values for nonsterilized samples ($p < 0.01$) (Fig. 6).

In addition, at the beginning of each light cycle it was always possible to see a rapid increase in the electric potential. This could be observed for both microorganisms and revealed a positive response to light. On the contrary, in the absence of light at the beginning of the dark cycle there was an abrupt drop in the electric potential.

Furthermore, this positive response to light appeared only when an active photosynthetic metabolism was present (nonsterilized samples). It was also possible to see a very slight response to light for sterilized samples (oscillation of 2 mV); however, the increment in the electric potential did not represent, in magnitude, those obtained for nonsterilized samples. For sterilized culture samples, the positive response to light was not observed.

3.3. Verification of the biological response

To confirm that the source of electrons was provided by the microorganisms in the anodic compartment, the electric potentials for anode and MFC were measured by using an Ag/AgCl reference electrode with different resistors as an external load. Figures 7 and 8 display the electric potential values as a function of current densities for different external loads, for both *P. kessleri* and *Nostoc* sp., respectively. As can be seen for both microorganisms, the electric potential of the MFC was governed by the reactions in the anode compartment. As was expected, the electric potential at the cathode was constant where abiotic reduction reactions are produced. This result indicates that the changes in the electric potential of the MFC were due to the redox reactions at the anode, where metabolically active microorganisms were present and releasing electrons.

3.4. Limit of detection

Taking into account that there are not significant differences between sterilized samples and sterilized culture medium (see results in Section 3.2), we considered the use of sterilized culture medium for the LOD calculation as a control group. The determination of the lineal range when the current density (*j*) is proportional to dry weight for *Nostoc* sp. and *P. kessleri* was 0.6–1.0 and 0.15–0.30 mg/mL, respectively (Fig. S1; supplementary material is available online at www.liebertonline.com/ast). Then, the LOD for *Nostoc* sp. and *P. kessleri* was 0.54 and 0.2 mg/mL,
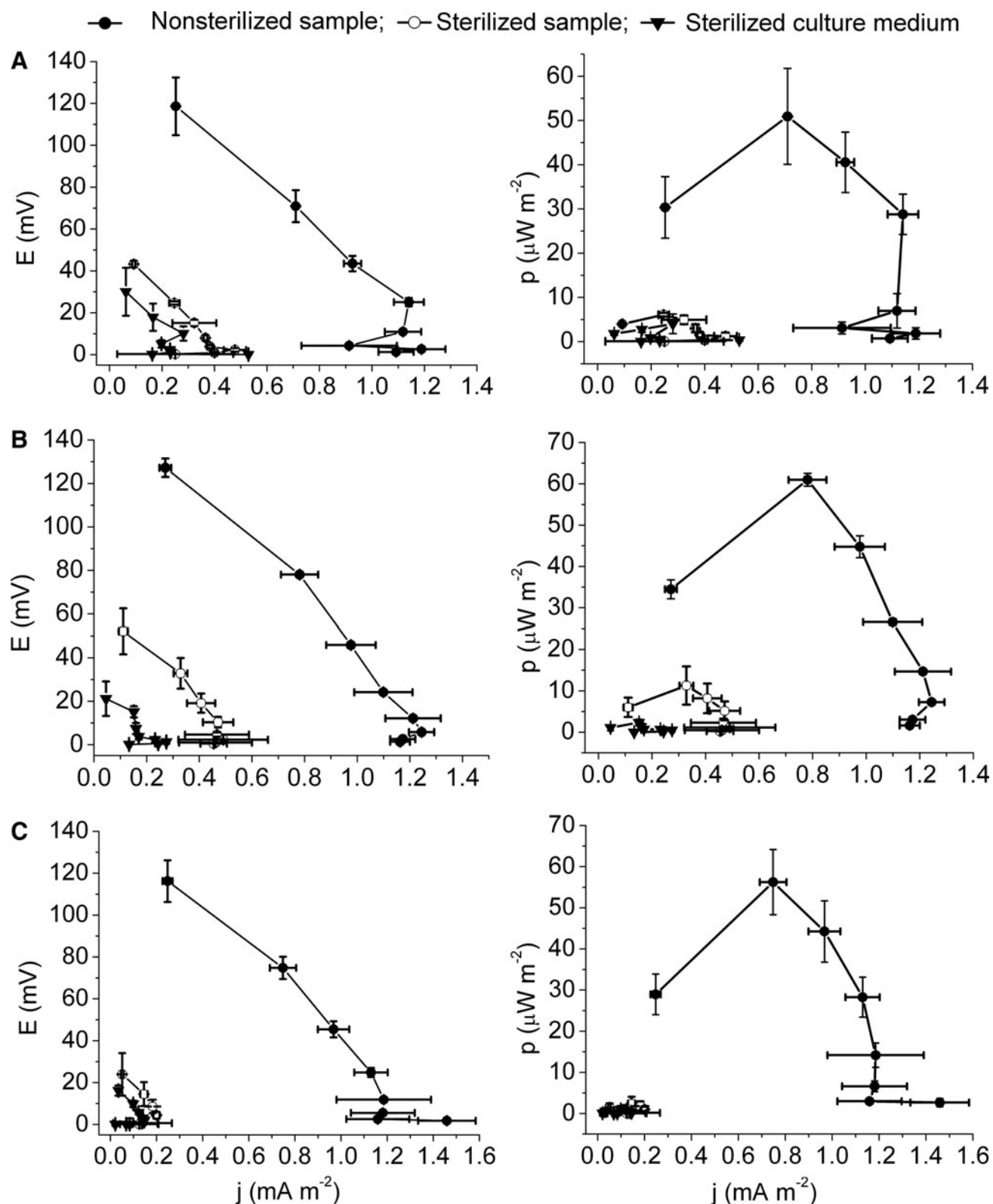
FIG. 2. Potential as a function of current density (left) and power density (right) for *P. kessleri*. Nonsterilized samples, sterilized samples, and sterilized culture medium in the anodic compartment at different times and in duplicate are shown. (**A**) 24 h, (**B**) 72 h, and (**C**) 96 h. Error bars represent the standard deviation of the mean based on duplicate experiments.
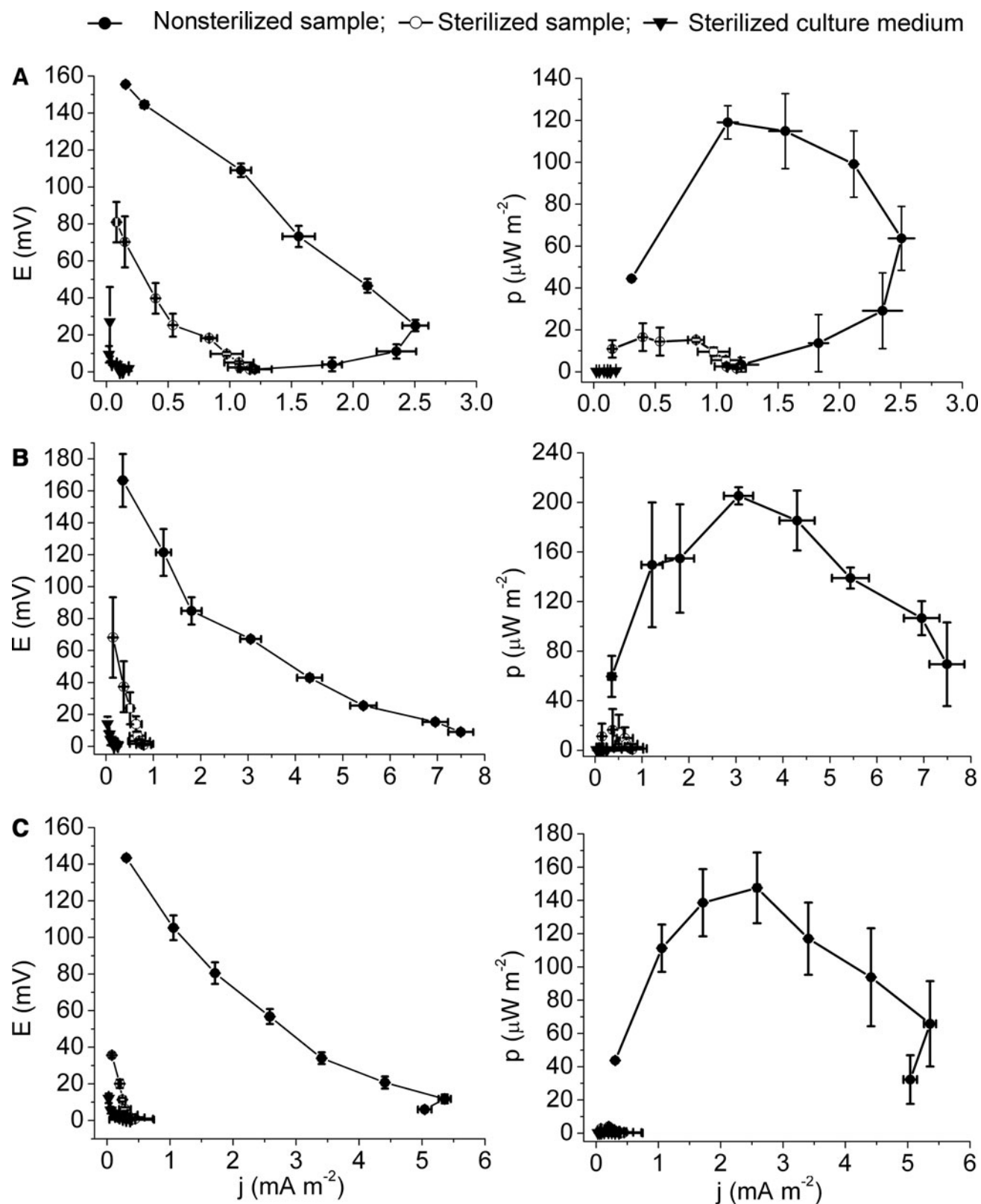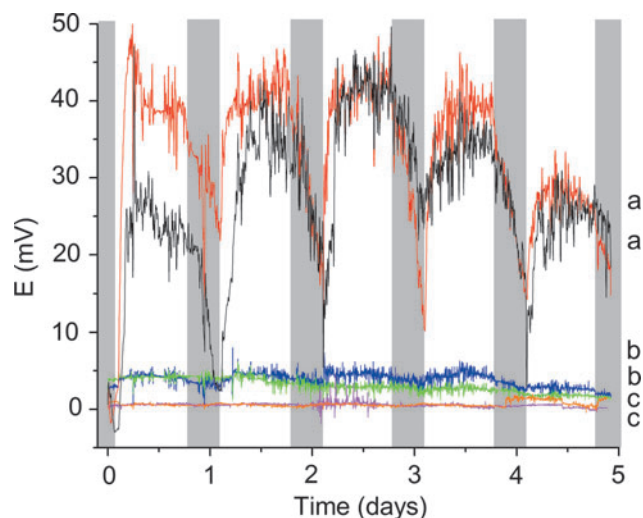
FIG. 3. Polarization curves (current density, left; power density, right) obtained for *Nostoc* sp. for nonsterilized samples, sterilized samples, and sterilized culture medium in the anodic compartment at different times and in duplicate. (**A**) 24 h, (**B**) 48 h, and (**C**) 96 h. Error bars represent the standard deviation of the mean based on duplicate experiments.
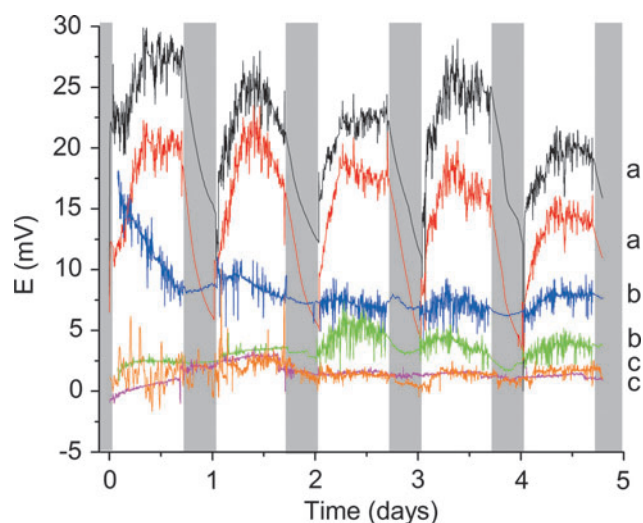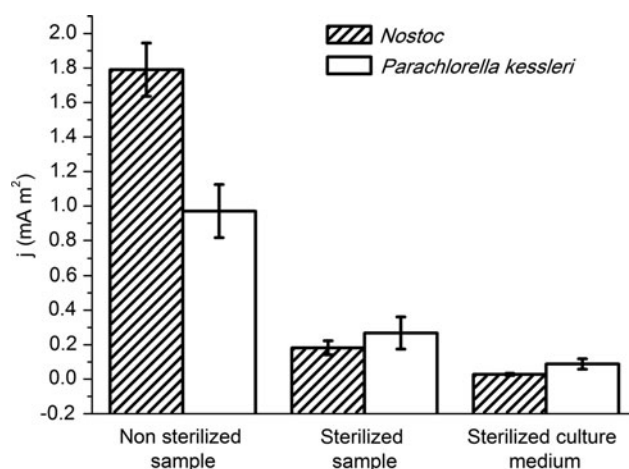
FIG. 4. Measurements in continuum showing potential (E) as function of time in MFCs containing *P. kessleri* non-sterilized samples (a, black and red lines), sterilized samples (b, blue and green lines), and sterilized culture medium (c, magenta and orange lines), in duplicate. An external resistor of $217\,k\Omega$ was connected to the circuit along the experiment. The measurements were done for 5 days (16:8 h light:dark cycles). Dark phases are indicated by gray bars. (Color graphics available at www.liebertonline.com/ast)

FIG. 6. Current density values obtained for *Nostoc* sp. and *P. kessleri* considering measurements of MFC potentials in continuum between days 2 and 4.

corresponding to 1×10^{6} and 1×10^{5} cells/mL, respectively (signal-to-noise ratio equal to 10). To confirm this result, a statistical analysis was performed (ANOVA), which showed that there are only significant differences between non-sterilized samples and sterilized culture medium ($p < 0.01$) for dry-weight values above the LOD.

FIG. 5. Measurements in continuum showing potential (E) as function of time in MFCs containing *Nostoc* sp. non-sterilized samples (a, black and red lines), sterilized samples (b, blue and green lines), and sterilized culture medium (c, magenta and orange lines), in duplicate. An external resistor of $217\,k\Omega$ was connected to the circuit along the experiment. The measurements were done for 5 days (16:8 h light:dark cycles). Dark phases are indicated by gray bars. (Color graphics available at www.liebertonline.com/ast)

4. Discussion

Microorganisms that display photolithoautotrophic metabolisms have been found in extreme environments on Earth (Rothschild and Giver, 2002; Warren-Rhodes *et al.*, 2007), and it may be possible to find them on other planetary bodies such as Mars (Bishop *et al.*, 2006; Marschall *et al.*, 2012; de Vera *et al.*, 2014).

In this work, we propose an *in situ* life-detection method that is based on photolithoautotrophic metabolisms. This represents a nontrivial extension of a previous work in which we showed the capability of MFCs as BES to be applied for the detection of chemoorganoheterotrophic metabolisms in extraterrestrial environments (Abrevaya *et al.*, 2010).

In particular, two different, though important, results were obtained by using these BES as *in situ* life sensors. The first outcome was that the electric potential and current density values were higher when nonsterilized samples were placed in the anode compartment, with respect to those obtained for sterilized samples or sterilized culture medium. This result is similar to that obtained with prokaryotic (cyanobacteria) and eukaryotic (microalgae) microorganisms (Figs. 2 and 3). Similar conclusions were obtained for measurements performed in continuum (Figs. 4 and 5). These findings demonstrate that this method is capable of distinguishing metabolic reactions from those produced as a consequence of abiotic reactions, and support and expand our previous studies with chemoorganotrophic metabolisms (Abrevaya *et al.*, 2010). The second relevant result in this work is that the method allows for the detection of a positive response to light when metabolically active microorganisms (nonsterilized samples) are in the anode compartment. This was shown by a light-dependent change in the electric potential (or current density) values (Figs. 4 and 5). This positive response to light is a general characteristic of photolithoautotrophic metabolisms and could be used as an indication of the presence of photolithoautotrophic life-forms. In fact, this phenomenon has also been reported for a diverse group of cyanobacteria and for different species of microalgae (*e.g.*, Pisciotta *et al.*, 2010; De Caprariis *et al.*,

2014). Therefore, this increment in electric potential, as a consequence of illumination, is part of a general mechanism that is not an exclusive feature of the microorganisms employed in this work. Moreover, this photosynthetic response to light could be used as another feature of the method to discriminate between samples containing photosynthetic microorganisms and nonphotosynthetic microorganisms. Pisciotta *et al.* (2010) demonstrated that MFCs that contain a chemoorganoheterotrophic bacterium such as *E. coli* do not respond to light as an increment in electric potential values. Therefore, employing the same BES used in this work, one could expect to distinguish between photosynthetic and nonphotosynthetic metabolisms in a sample to be analyzed.

In the case of photosynthetic MFCs, it is possible to see a positive response to light because of the electron flow produced by light-driven reactions in the metabolism. These electrons are donated to the anode by the photosynthetic electron transfer chain. Previous works have explored possible mechanisms of electron transfer from the photosynthetic microorganisms to the anode. Using photosynthetic MFCs as devices for electricity generation by employing cyanobacteria and including photosynthetic inhibitors has demonstrated that electrons originated from the photolysis of water molecules through photosystem II (Yagishita *et al.*, 1993; Zou *et al.*, 2009; Pisciotta *et al.*, 2010). Regardless of the source of electrons, two different mechanisms, in general, have been proposed for the electron transfer between the microorganisms and the anode. One implies a direct electron transfer (DET), in which the electrons are transferred through the anode via physical contact of the cell membrane with the electrode (including the possibility of the presence of previously described structures known as "nanowires"). The other mechanism involves an electron-mediated mechanism through metabolites (MET), which can act as redox molecules, commonly known as "mediators."
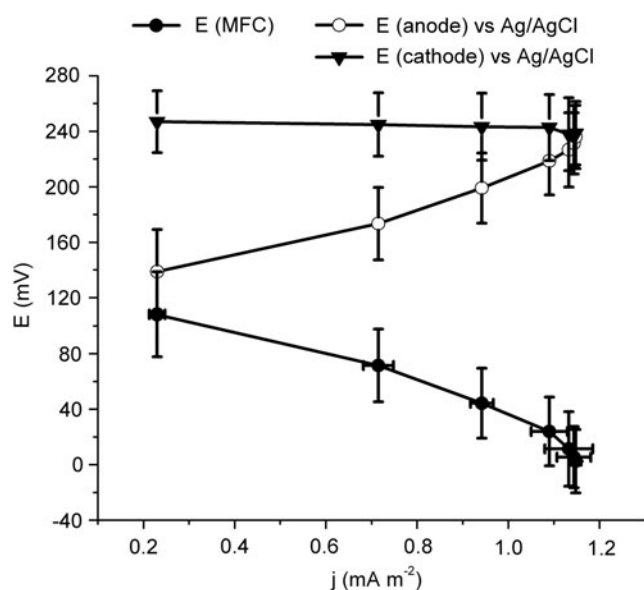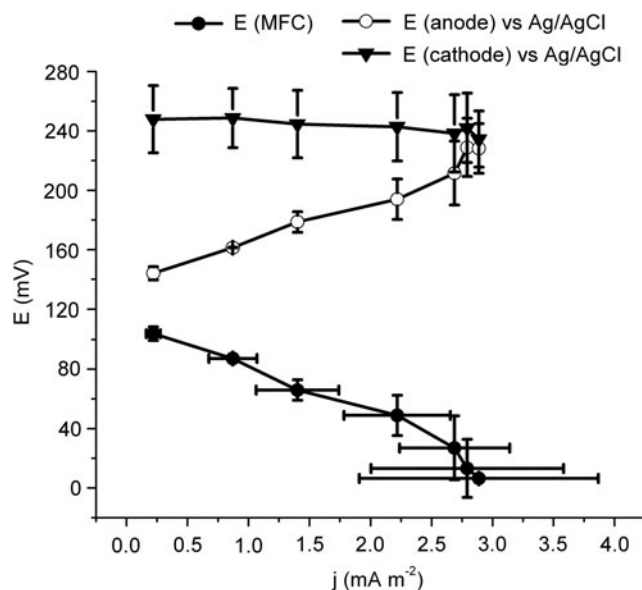
FIG. 8. Anode and cathode MFC potentials as function of current density for *Nostoc* sp. nonsterilized samples, measured after 96 h at OC.

The MET mechanism is exemplified in Fig. 1 (for further details on both mechanisms, see Schröder, 2007). In the case of cyanobacteria, DET has been suggested for *Synechocystis* sp. PCC6803 passing through nanowires (Gorby *et al.*, 2006). However, several cyanobacterial genera (including *Nostoc*) have shown that the electron transfer to the extracellular environment could be performed by plastoquinone and bd quinol oxidase acting as electron carriers (Pisciotta *et al.*, 2011). Nevertheless, it still remains unclear which mechanisms can operate for different genera of cyanobacteria and microalgae.

As part of our experiments, MFCs that contained sterilized samples of microorganisms or sterilized culture medium without microorganisms seemed to be very slightly responsive or unresponsive to light, respectively (Figs. 4 and 5). This is partially supported by other works employing culture medium devoid of cells as control groups for photosynthetic MFCs (Pisciotta *et al.*, 2010; De Caprariis *et al.*, 2014). Moreover, the very slight increment in the electric potential due to illumination for sterilized samples of microorganisms when compared with sterilized culture medium could be explained. As several works have demonstrated, when using isolated photosystems in bio-photosensor devices, it is possible to see a light-dependent electric current (Maly *et al.*, 2005; Teresaki *et al.*, 2007). However, isolated photosystems seem to be susceptible to degradation through photodamage (Maly *et al.*, 2005). In our case, we employed, for the first time, sterilized samples of microorganisms in photosynthetic MFCs. This is an indispensable control group for the proposed method. Considering the previously mentioned evidence and the composition of the sample (an autoclaved sample of microorganisms), it is possible to conjecture that the sterilizing process itself could not completely destroy the photosynthetic apparatus, and probably some remnants of photosystems or photosensitive molecules were still present in the sterilized samples. However, this does not represent a drawback of the method, as we have

FIG. 7. Anode and cathode MFC potentials as function of current density for *P. kessleri* nonsterilized samples, measured after 96 h at OC.

demonstrated through statistical analysis (ANOVA). Experiments performed during light phases showed not only nonsignificant differences between sterilized samples and sterilized culture medium but significant differences between these groups and nonsterilized samples (Fig. 6). Therefore, because the method that we propose is based on the use of control groups and the result is obtained through a comparison with them, these pseudo-positive responses to light do not affect the possibility of making a distinction between processes of biotic and abiotic origin.

In recent years, other life-detection methods proposed for the *in situ* detection of life have employed electrochemical sensors as part of the detection device such as the Life Detection Array (Kounaves *et al.*, 2002) and, as a subsequent development, the Microbial Detection Array (Hoehn *et al.*, 2007). Although these methods pointed to the presence of metabolism through the detection of gases or chemical disequilibrium in enclosed chambers, the proposed methodology depended on the growth of microorganisms. In the case of the BES developed in the present study, an increase in the number of cells as a requisite for life detection is not required. This feature one of this method's principal advantages.

It is also possible to compare MFCs as BES to detect metabolism in extraterrestrial environments with previous developments, in particular, those related to the biological experiments performed during the Viking mission, widely known as the tripartite biological experiment (Klein *et al.*, 1976; Klein, 1977). In comparison, our method has an advantage in that it does not depend on the chemical composition of the life-form—having the capacity to detect, for example, non-carbon-based life—as we already previously demonstrated in Abrevaya *et al.* (2010).

Our method also presents advantages over recently proposed methods, in that it does not employ the use of complex reagents or potentially labile molecules as antibodies (*e.g.*, Sims *et al.*, 2005; Parro *et al.*, 2011), which can be problematic (Kodadek, 2001). For example, there is the potential for degradation in extraterrestrial environments and during extended periods of transportation. Additionally, our method is not dependent on the presence of biosignatures, which are vulnerable to destruction by UV radiation at the surface of planets such as Mars (Dartnell and Patel, 2014).

Although the main goal of this study was to demonstrate the capability of MFCs as *in situ* life-detection sensors, in a future work it will be necessary to include these experiments in the context of a real mission to Mars or another planetary body. As a rough estimate, we consider that the total weight of the MFC—life-detection system, including an acquisition board—would be 1.5 kg, and the peak energy consumption would be 50 W considering MFCs and 800 W for periods of at least 5 min when a vapor-based sterilization system is in use as, for example, was described by Atwater *et al.* (1997).

5. Conclusions

We have demonstrated the potential for MFCs as BES to be used as sensors for *in situ* life detection, and their capability to detect life by comparison with a control group. Significant differences observed for nonsterilized and sterilized samples would be indicative of the presence of an active metabolism and therefore life. Measurements can be performed singly or in continuum. As an additional feature, the presence or absence of a positive light response could, in principle, be used distinguish between photosynthetic and nonphotosynthetic life-forms.

In future work, we intend to improve the application of these BES with the use of environmental samples and to design a new bioelectrochemical system to increase the capacity to detect smaller amounts of cells and identify metabolisms with different electron sources.

In conclusion, our results show that our method can not only detect photosynthetic metabolisms, but it also could allow for distinguishing photosynthetic from nonphotosynthetic life-forms, which would extend the application of MFCs as extraterrestrial life sensors.

References

Abrevaya, X.C., Mauas, P.J.D., and Cortón, E. (2010) Microbial fuel cells applied to the metabolically based detection of extraterrestrial life. *Astrobiology* 10:965–971.

Abrevaya, X.C., Sacco, N.J., Bonetto, M.C., Hilding-Ohlsson, A., and Cortón, E. (2015a) Analytical applications of microbial fuel cells. Part I: biochemical oxygen demand. *Biosens Bioelectron* 63:580–590.

Abrevaya, X.C., Sacco, N.J., Bonetto, M.C., Hilding-Ohlsson, A., and Cortón, E. (2015b) Analytical applications of microbial fuel cells. Part II: toxicity, microbial activity and quantification, single analyte detection and other uses. *Biosens Bioelectron* 63:591–601.

Atwater, J.E., Dahl, R.W., Garmon, F.C., Lunsford, T.D., Michalek, W.F., Wheeler, R.R., Jr., and Sauer, R.L. (1997) Miniature microwave powered steam sterilization chamber. *Rev Sci Instrum* 68:3924–3925.

Biemann, K. (2007) On the ability of the Viking gas chromatograph–mass spectrometer to detect organic matter. *Proc Natl Acad Sci USA* 104:10310–10313.

Bischoff, H.W. and Bold, H.C. (1963) *Phycological Studies. IV. Some Soil Algae from Enchanted Rock and Related Algal Species*, University of Texas Publication No. 6318, the University of Texas, Austin.

Bishop, J.L., Louris, S.K., Rogoff, D.A., and Rothschild, L.J. (2006) Nanophase iron oxides as a key ultraviolet sunscreen for ancient photosynthetic microbes. *International Journal of Astrobiology* 5:1–12.

Conrad, P.G. and Nealson, K.H. (2001) A non-Earthcentric approach to life detection. *Astrobiology* 1:15–24.

Dartnell, L.R. and Patel, M. (2014) Degradation of microbial fluorescence biosignatures by solar ultraviolet radiation on Mars. *International Journal of Astrobiology* 13:112–123.

De Caprariis, B., De Filippis, P., Di Battista, A., Di Palma, L., and Scarsella, M. (2014) Exoelectrogenic activity of a green microalgae, *Chlorella vulgaris*, in a bio-photovoltaic cells (BPVs). *Chemical Engineering Transactions* 38:523–528.

Des Marais, D.J., Nuth, J.A., III, Allamandola, L.J., Boss, A.P., Farmer, J.D., Hoehler, T.M., Jakosky, B.M., Meadows, V.S., Pohorille, A., Runnegar, B., and Spormann, A.M. (2008) The NASA Astrobiology Roadmap. *Astrobiology* 8:715–730.

de Vera, J.P., Dulai, S., Kereszturi, A., Koncz, L., Lorek, A., Mohlmann, D., Marschall, M., and Pocs, T. (2014) Results on the survival of cryptobiotic cyanobacteria samples after exposure to Mars-like environmental conditions. *International Journal of Astrobiology* 13:35–44.

Dodds, W.K., Gudder, D.A., and Mollenhauer, D. (1995) The ecology of *Nostoc*. *J Phycol* 31:2–18.

Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., Beveridge, T.J., Chang, I.S., Kim, B.H., Kim, K.S., Culley, D.E., Reed, S.B., Romine, M.F., Saffarini, D.A., Hill, E.A., Shi, L., Elias, D.A., Kennedy, D.W., Pinchuk, G., Watanabe, K., Ishii, S., Logan, B., Nealson, K.H., and Fredrickson, J.K. (2006) Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proc Natl Acad Sci USA* 103:11358–11363.

Hoehn, A., Brown, I., Clawson, J., Freeman, J.B., Kapit, J., Lynch, K.L., Young, S.M.M., and Kounaves, S.P. (2007) *Microbial Detection Array (MDA), a Novel Instrument for Unambiguous Detection of Microbial Metabolic Activity in Astrobiology Applications*, SAE Paper 2007-01-3190, SAE International, Warrendale, PA.

Houtkooper, J.M. and Schulze-Makuch, D. (2007) A possible biogenic origin for hydrogen peroxide on Mars: the Viking results reinterpreted. *International Journal of Astrobiology* 6:147–152.

Juárez, A.B., Vélez, C.G., Iniguez, A.R., Martinez, D.E., Rodriguez, M.C., Vigna, M.S., and Rios de Molina, M.C. (2011) A *Parachlorella kessleri* (Trebouxiophyceae, Chlorophyta) strain from an extremely acidic geothermal pond in Argentina. *Phycologia* 50:413–421.

Kiang, N.Y., Siefert, J., Govindjee, and Blankenship, R.E. (2007a) Spectral signatures of photosynthesis I. Review of Earth organisms. *Astrobiology* 7:222–251.

Kiang, N.Y., Segura, A., Tinetti, G., Govindjee, Blankenship, R.E., Cohen, M.J., Siefert, J., Crisp, D., and Meadows, V.S. (2007b) Spectral signatures of photosynthesis. II. Coevolution with other stars and the atmosphere on extrasolar worlds. *Astrobiology* 7:252–274.

Klein, H.P. (1977) The Viking biological investigation: general aspects. *J Geophys Res* 82:4677–4680.

Klein, H.P. (1999) Did Viking discover life on Mars? *Orig Life Evol Biosph* 29:625–631.

Klein, H.P., Horowitz, N.H., Levin, G.V., Oyama, V.I., Lederberg, J., Rich, A., Hubbard, J.S., Hobby, G.L., Straat, P.A., Berdahl, B.J., Carle, G.C., Brown, F.S., and Johnson, R.D. (1976) The Viking biological investigation: preliminary results. *Science* 194:99–105.

Kodadek, T. (2001) Protein microarrays: prospects and problems. *Chem Biol* 8:105–115.

Kounaves, S.P., Noll, R.A., Buehler, M.G., Hecht, M.H., Lankford, K., and West, S.J. (2002) Microbial life detection with minimal assumptions. *Proc SPIE* 4495:137–144.

Levin, G.V. (2014) The curiousness of Curiosity. *Astrobiology* 14:1–3.

Maly, J., Krejci, J., Ilie, M., Jakubka, L., Masojidek, J., Pilloton, R., Sameh, K., Steffan, P., Stryhal, Z., and Sugiura, M. (2005) Monolayers of photosystem II on gold electrodes with enhanced sensor response—effect of porosity and protein layer arrangement. *Anal Bioanal Chem* 381:1558–1567.

Marschall, M., Dulai, S., and Kereszturi, A. (2012) Migrating and UV screening subsurface zone on Mars as target for the analysis of photosynthetic life and astrobiology. *Planet Space Sci* 71:146–153.

McCollom, T.M. (2006). The habitability of Mars: past and present. In *Solar System Update*, edited by P. Blondel and J.W. Mason, Springer Praxis Books, Berlin/Chichester, pp 159–175.

Navarro-González, R., Navarro, K.F., de la Rosa, J., Iñiguez, E., Molina, P., Miranda, L.D., Morales, P., Cienfuegos, E., Coll, P., Raulin, F., Amils, R., and McKay, C.P. (2006) The limitations on organic detection in Mars-like soils by thermal volatilization–gas chromatography–MS and their implications for the Viking results. *Proc Natl Acad Sci USA* 103:16089–16094.

Nealson, K.H. and Popa, R. (2005) Metabolic diversity in the microbial world: relevance to exobiology. In *Micro-Organisms and Earth Systems, Advances in Geomicrobiology*, edited by G.M. Gadd, K.T. Temple, and H.M. Lapin-Scott, Cambridge University Press, Cambridge, UK, pp 151–171.

Nealson, K.H., Tsapin, A., and Storrie-Lombardi, M. (2002) Searching for life in the Universe: unconventional methods for an unconventional problem. *Int Microbiol* 5:223–230.

Parro, V., de Diego-Castilla, G., Rodriguez-Manfredi, J.A., Rivas, L.A., Blanco-López, Y., Sebastián, E., Romeral, J., Compostizo, C., Herrero, P.L., García-Marín, A., Moreno-Paz, M., García-Villadangos, M., Cruz-Gil, P., Painado, V., Martín-Soler, J., Pérez-Mercader, J., and Gómez-Elvira, J. (2011) SOLID3: a multiplex antibody microarray-based optical sensor instrument for *in situ* life detection in planetary exploration. *Astrobiology* 11:15–28.

Pisciotta, J.M., Zou, Y., and Baskakov, I.V. (2010) Light-dependent electrogenic activity of cyanobacteria. *PLoS One* 5, doi:10.1371/journal.pone.0010821.

Pisciotta, J.M., Zou, Y., and Baskakov, I.V. (2011) Role of the photosynthetic electron transfer chain in electrogenic activity of cyanobacteria. *Appl Microbiol Biotechnol* 91:377–385.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., and Stanier, R.Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol* 111:1–61.

Rothschild, L.J. and Giver, L.J. (2002) Photosynthesis below the surface in a cryptic microbial mat. *International Journal of Astrobiology* 1:295–304.

Schröder, U. (2007) Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency. *Phys Chem Chem Phys* 9:2619–2629.

Sims, M.R., Pillinger, C.T., Wright, I.P., Dowson, J., Whitehead, S., Wells, A., Spragg, J.E., Fraser, G., Richter, L., Hamacher, H., Johnstone, A., Meredith, N.P., de la Nougerede, C., Hancock, B., Turner, R., Peskett, S., Brack, A., Hobbs, J., Newns, M., Senior, A., Humphries, M., Keller, H.U., Thomas, N., Lingard, J.S., and Ng, T.C. (1999) Beagle 2: a proposed exobiology lander for ESA's 2003 Mars Express Mission. *Adv Space Res* 23:1925–1928.

Sims, M.R., Cullen, D.C., Bannister, N.P., Grant, W.D., Henry, O., Jones, R., McKnight, D., Thompson, D.P., and Wilson, P.K. (2005) The Specific Molecular Identification of Life Experiment (SMILE). *Planet Space Sci* 53:781–791.

Teresaki, N., Yamamoto, N., Tamada, K., Hattori, M., Hiraga, T., Tohri, A., Sato, I., Iwai, M., Iwai, M, Taguchi, S., Enami, I., Inoue, Y., Yamanoi, Y., Yonezawa, T., Mizuno, K., Murata, M., Nishihara, H., Yoneyama, S., Minakata, M., Ohmori, T.,

Sakai, M., and Fujii, M. (2007) Bio-photosensor: cyano-bacterial photosystem I coupled with transistor via molecular wire. *Biochim Biophys Acta* 1767:653–659.

von Bertalanffy, L. (1950) The theory of open systems in physics and biology. *Science* 111:23–29.

Warren-Rhodes, K.A., Rhones, K.L., Liu, S., Zhou, P., and McKay, C.P. (2007) Nanoclimate environment of cyano-bacterial communities in China's hot and cold hyperarid deserts. *J Geophys Res: Biogeosciences* 112, doi:10.1029/2006JG000260.

Yagishita, T., Horigome, T., and Tanaka, K. (1993) Effects of light, CO_2 and inhibitors on the current output of biofuel cells containing the photosynthetic organism *Synechococcus* sp. *J Chem Technol Biotechnol* 56:393–399.

Young, R.S. (1976) The origin and evolution of the Viking mission to Mars. *Orig Life* 7:271–272.

Zou, Y., Pisciotta, J., Billmyre, R.B., and Baskakov, I.V. (2009) Photosynthetic microbial fuel cells with positive light response. *Biotechnol Bioeng* 104:939–946.

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

E-mail: abrevaya@iafe.uba.ar

Abbreviations Used

BES = bioelectrochemical systems
DET = direct electron transfer
LOD = limit of detection
MET = electron-mediated mechanism through metabolites
MFCs = microbial fuel cells
OC = open circuit