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Inoculation procedures and characterization of membrane electrode assemblies for microbial fuel cells

G.K. Surya Prakash[a,*], Federico A. Viva[a], Orianna Bretschger[b], Bo Yang[a], Moh El-Naggar[b], Kenneth Nealson[b]

[a] Loker Hydrocarbon Research Institute and Department of Chemistry, University of Southern California, Los Angeles, CA 90089, USA
[b] Department of Earth Sciences, University of Southern California, Los Angeles, CA 90089, USA

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ABSTRACT

Membrane electrode assemblies were prepared following procedures adopted in the fabrication of polymer electrolyte membrane (PEM) fuel fells and used in microbial fuel cells (MFCs) with *Shewanella oneidensis* MR-1 as a single culture and sodium lactate as the electron donor. Improved inoculation procedures were developed and fuel cell performance with the biofilm density of microbes over the anode is discussed. A novel procedure to condition the membrane is also presented. Polarization measurements were carried out and power density plots were generated. Power density values of 300 mW m^{-2} are typically obtained while a maximum value of 600 mW m^{-2} is demonstrated indicating good performance for a single cell culture.

1. Introduction

In recent years, interest in microbial fuel cells (MFCs) has grown considerably not only because they provide a way to generate electricity but also because they can be coupled with wastewater treatment [1–5] given the ability of the bacteria used in MFCs to metabolize several carbon sources [6]. Among the bacteria used in MFCs are *Aeromonas* [7], *Clostridium* [8], *Geobacter* [9], *Enterococcus* [10,11] and *Shewanella* [2,10,12–15]. *Shewanella* is an interesting bacterium because it attaches to the electrode and transfers electrons without the need of a mediator [13], hence it is characterized as an electrochemically active bacteria (EAB). Recently the presence of pili, also called nanowires, was confirmed and they were hypothesized to play a significant role in the transport of electrons [16]. Recently, a comprehensive review on microbial fuel cells has been published by Logan [17].

The choice of electrode material, membrane and cell design in a MFC influence the overall power output. The plethora of MFC varieties found in the literature [3,4,18–27] shows that an optimal design has not been found. Some of the MFCs use both single chamber as well as dual chamber cell designs in which the anode and cathode are either free-standing in solution or separated by a proton-permeable membrane [26,28–30]. Acclimation of the microorganism on the fuel cell chamber is another aspect that has been recently investigated [31].

In polymer electrolyte membrane fuel cells (PEM FCs) with H_2 as a fuel, or in direct methanol fuel cell (DMFC) with methanol as a fuel, there is consensus that the way to obtain the maximum power is by constructing a membrane electrode assembly (MEA). The MEA is a thin array in which a polymeric membrane, which serves as the ionic conductor, is placed between two gas diffusion electrodes (GDE), the anode and the cathode. The catalysts for the anodic and cathodic reactions are located on the inner face of the GDE, in direct contact with the membrane. One of the MEA preparation processes involves hot pressing, in order to obtain a close contact between the GDE, the catalyst and the membrane.

Another issue in MFC design is the use of water in the cathode compartment. The low solubility of O_2 results in mass transport limitation at the cathode resulting in low power output. The use of Nafion® for MEA fabrication also presents problems. Nafion® is the most used membrane in PEM FC and has also been used as a membrane for MFC [28–30,32]. It has been pointed out that the use of Nafion® in MFCs is problematic due to the high concentration of ions like Na^+, a common cation in the buffers used in MFC and present at concentrations, which are nonexistent in PEMFC. Moreover, Nafion® has more affinity to anions like Na^+ and K^+ than H^+ [33] exacerbating the aforementioned problem. The price of Nafion® has also been indicated as a drawback for MFC [26]. The MFC cost has been related to the price of Nafion®, particularly because electrodes with big cross-sections would have to be used

* Corresponding author. Tel.: +1 213 740 5984; fax: +1 213 740 6679.
E-mail address: gprakash@usc.edu (G.K.S. Prakash).

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in order to obtain significant power, which would require a significant quantity of the membrane. Also the acidity of Nafion® has been identified as a problem for the growth of the bacteria. However, membranes like the interpenetrating network of polyvinylidene fluoride and polystyrene sulfonic acid (IPN PVDF–PSSA) [34] are inexpensive and have shown improvement with respect to Nafion® in DMFCs [35]. In terms of MFC cost, we believe that as in PEM FCs, it is the Pt used on the cathode for the oxygen reduction that actually drives the cost.

In previous publications [36–38], investigators have used *Shewanella oneidensis* MR-1 in MFC to study biochemical aspects of the microbe as well as the electrochemistry of the fuel cell. Although these devices proved useful, they were not intended to maximize the power output, total power obtained in these cells was in the order of 20 μW. Impressive power densities from 2000 mW m^{-2} [32,39] up to 4000 mW m^{-2} [11,40] have been reported, but these results were obtained with different fuel cell designs using consortia of microbes and mediators and not single cell cultures. In the present work, a fuel cell hardware used for DMFCs, was adapted for MFCs, i.e. two chambers separated by an MEA, one of the chambers being a dry cathode. MEAs using Nafion® and PVDF–PSSA IPN membranes were prepared and tested with *S. oneidensis* MR-1 as a single culture microorganism in the anode and lactate as electron donor fuel to maximize the power density.

2. Experimental

2.1. Microorganism preparation

S. oneidensis MR-1 was grown aerobically in batch cultures using a defined medium: 18 mM sodium lactate (Sigma–Aldrich) as the sole carbon source, 50 mM PIPES buffer ($C_8H_{18}N_2O_6S_2$, Sigma–Aldrich), 7.5 mM NaOH (Sigma–Aldrich), 28 mM NH_4Cl (Sigma–Aldrich), 1.3 mM KCl (EMD Chemicals Inc.), 4.3 mM $NaH_2PO_4{\cdot}H_2O$ (EMD Chemicals Inc.), 100 mM NaCl (EMD Chemicals Inc.), 10 mL L^{-1} of vitamin solution [41], 10 mL L^{-1} amino acid solution and trace mineral stock solutions [36]. All solutions were prepared using DI water from a NANOPure Infinity model D8961 by Barnstead. The final optical density at 600 nm (OD_{600}) of the culture was measured using a spectrophotometer (Beckman DU 530), and used to calculate an experimental dilution of OD_{600} = 0.4 in the MFC anode compartment. The appropriate volume of cells was then injected into each experimental setup such that approximately 5×10^9 cells mL^{-1} were present for each evaluation.

2.2. Membrane electrode assembly, MFC housing and electrochemical measurements

For the MFC housing, a column flow-type graphite current collector with an area of 25 cm^2 for DMFC (purchased from Electrochem) was adapted by machining an anode chamber in house from a solid block of graphite (d = 1.2 g cm^{-3}, conductivity 0.22 mΩ cm^{-1}) (purchased from www.thegraphitestore.com). The anode chamber had a volume of 70 cm^3, and a glass window to allow visual control of the liquid level. The MEAs were prepared by cutting two 5 cm × 5 cm pieces of carbon paper. Toray, TGP H-120 non-wet-proof carbon paper was used as the anode material and TGP H-60 10 wt% wet-proof for the cathode, both provided by E-tek. Pt black (HiSPEC 1000, Alfa Aesar) was used as a cathode catalyst and was applied on one face of the electrode by a direct painting method. A suspension was prepared by mixing the catalyst with both deionized water and Nafion® ionomer (5% in alcohol, Aldrich) in a 1:3:1 ratio. The cathode catalyst loading obtained was ca. 5 mg cm^{-2}. For the anode, the same amount of Nafion® ionomer as used in the cathode was diluted with a few drops of DI water and also applied on one face of the electrode. The membranes used were Nafion® 117 (175 μm, Ion Power) and PVDF-PSSA. Prior to MEA fabrication, Nafion® membranes were pretreated by boiling them in a solution of 3% H_2O_2 (50% solution, EMD Chemicals Inc.) for 1 h, boiling in deionized water for another hour, boiling in 3% H_2SO_4 (ACS grade, EMD Chemicals Inc.) for 1 h and finally washing several times with deionized water. Preparation of PVDF–PSSA membrane (thickness ca. 250 μm) has been previously reported [35]. The membrane was placed between the two electrodes and hot pressed at 900 kg force at 140 °C for 50 min. The obtained MEAs were rehydrated by immersing them in deionized water at 60 °C for 6 h. MilliQ deionized water was obtained from a Millipore Direct-Q3 system. Finally, the cells were assembled by inserting the MEA between the graphite current collectors and using thin Teflon films (50–150 μm) as gaskets. A uniform torque of 36 N m^{-1} was applied to each bolt used to assemble the cell. Fig. 1(A) and (B) shows a schematic representation of the MEA and the assembled cell. After assembling, the FC with MEA inside was autoclaved (AMSCO Scientific, model SG-

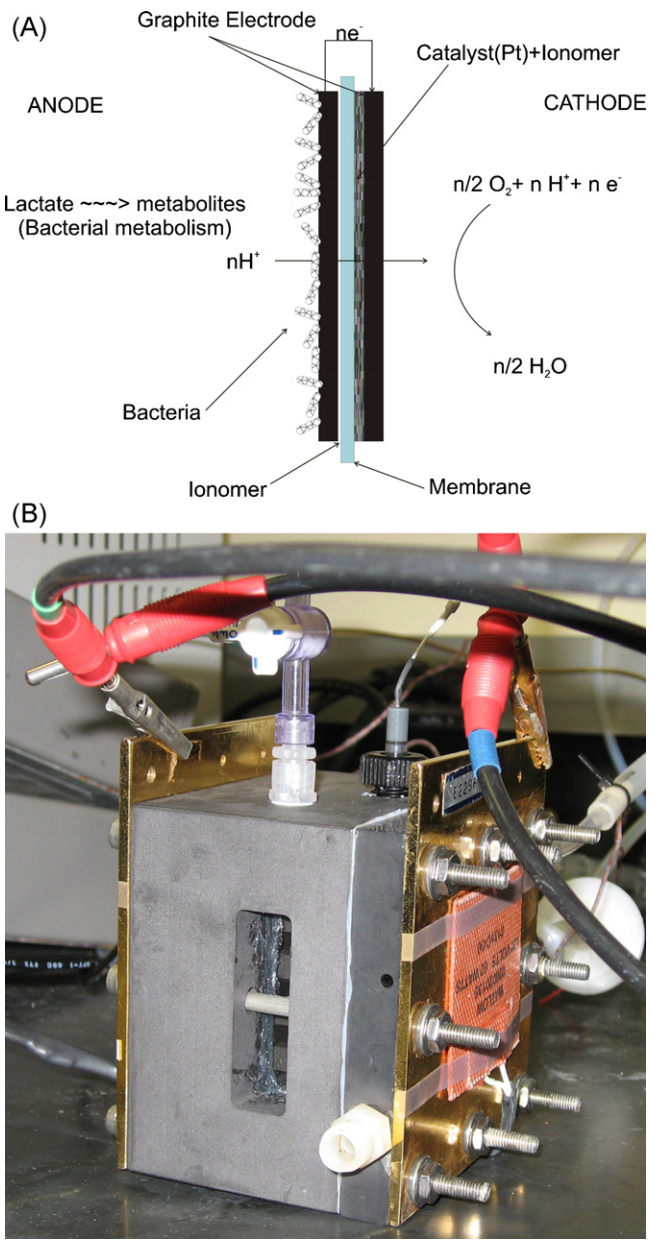

Fig. 1. (A) Schematic representation of the MEA. (B) Photograph of assembled fuel cell.

120) prior to microbial inoculation. A PIPES buffer (50 mM PIPES, 7.5 mM NaOH, pH 7.0) was used in the anode compartment as the diluting solution for the bacteria. Anaerobic conditions were achieved at the anode by sparging the compartment with sterile filtered Argon (ultra high pure grade Gilmore) for 30 min. Inoculation of the microorganism was carried out using two different procedures discussed in Section 2.3. Sodium lactate (100 mM) was used as the sole carbon source. After inoculation, the MFC was placed in such a way that the MEA was always in a horizontal position (with the anode at the top) throughout the experiment, allowing the bacteria to settle on the anode. O_2 (compressed, Gilmore) was circulated through the dry cathode at a flow of 7 mL min^{-1} unless stated otherwise. Additional lactate injections were administered thereafter, when the cell voltage dropped to baseline levels. MFCs were operated for a period of 20–30 days with the addition of sodium lactate when the potential dropped significantly. The temperature of the MFC was maintained at 28 °C using heating pads. Electrochemical measurements were performed using a Solartron SI 1287 potentiostat. Polarization measurements were performed galvanodynamically, sweeping the current at 0.1 mA s^{-1} from the open circuit voltage (OCV) until the cell voltage reached 0.01 V. Power densities (P) were obtained from the polarization plot by multiplying the voltage by the current density (j) using the electrode area of 25 cm^2 for the calculation.

2.3. Inoculation procedures

The two different inoculation procedures referred to as inoculation procedure 1 (IP1) and inoculation procedure 2 (IP2) are described. In IP1, after autoclaving the MFC with the MEA inside, the anode was filled with Pipes buffer. The necessary amount of the cultivated microbes was added in order to obtain the OD stated in Section 2.1. The microbes were fed with a solution of sodium lactate for a final concentration of 100 mM on the anode and deaerated with Ar for 30 min while oxygen was circulated through the cathode. The MFC was then connected to the potentiostat while the OCV was monitored. For IP2, after the MFC was autoclaved, the anode was filled with Pipes buffer and sodium lactate for a 100 mM concentration on the anode. The cell was then allowed to rest for 24 h. On the next day, the anode was emptied and the microbes with the desired OD in the buffer previously deaerated were added followed by sodium lactate addition. A 1 kΩ resistance was connected between anode and cathode and maintained at all times. The MFC was not handled for another 24 h, while oxygen was circulated through the cathode. Next, the anode was emptied again and the previous day's procedure was repeated. All these steps were performed in a consistent way for each MFC prepared. Once the second inoculation was completed, the MFC was connected to the potentiostat and the cell voltage across the resistance monitored. In both procedures the voltages (OCV or cell voltage) were continuously monitored, i.e. without interruption, for the period of operation and polarization measurements conducted on a continuous basis.

2.4. Scanning Electron Microscopy (SEM) images

For the SEM images, the MEAs with attached biofilms were removed from the fuel cells right after the period used (Section 2.2) and immediately fixed in glutaraldehyde (2.5% in DI water, Electron Microscopy Sciences). Samples were then subjected to a serial dehydration protocol using increasing concentrations of ethanol (200 proof, Pharmco-Aaper). After three final changes in ethanol, the samples were then dried using hexamethyldisilazane (Electron Microscopy Sciences). The desiccated samples were coated with evaporated carbon and viewed with using a Zeiss-LEO 1550 VP FESEM using an in-lens secondary electron detector.

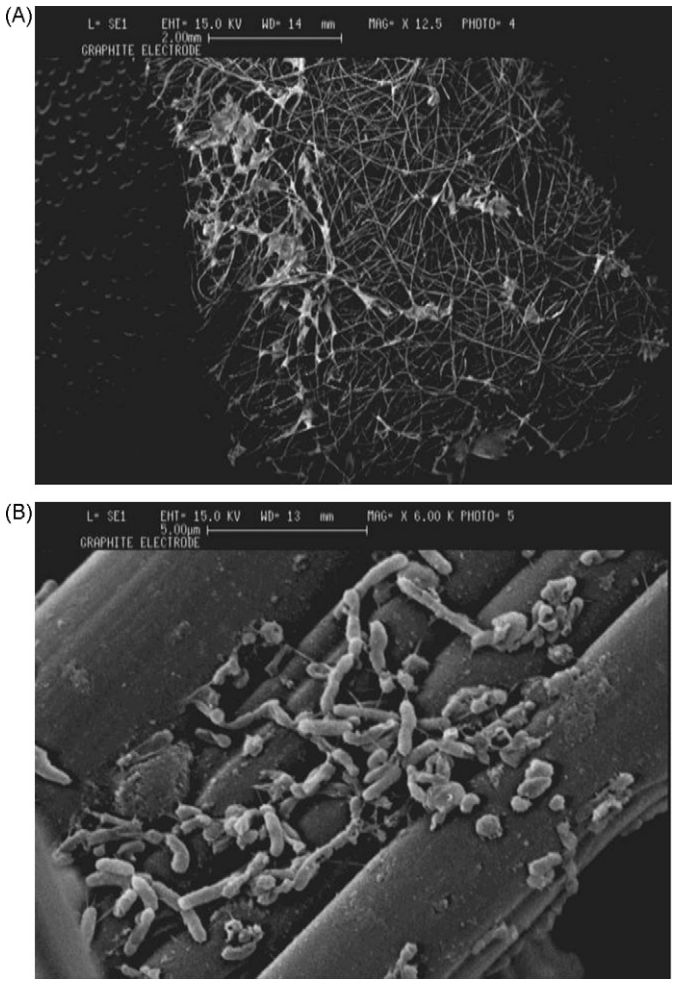

Fig. 2. SEM images for an MEA used in a FC inoculated using IP1. (A) 12.5× magnification. (B) 6000× magnification.

3. Results and discussion

The microorganism inoculation procedure was optimized after testing of the several assembled MFCs. This change from the most direct IP1 procedure to IP2 came with the goal of obtaining a dense biofilm over the electrode and ultimately improving the overall power output of the MFC. Initially, after testing a number of MFCs using the IP1 protocol, direct observation of the electrodes as well as SEM images of the anode obtained once the FC were open showed low amounts of microbes and poor biofilms as can be seen in Fig. 2(A) and (B). These results were consistent under IP1 protocol. This fact together with erratic values obtained for OCV and polarization plots prompted a change to the inoculation procedure essentially in three ways. The first change consisted of filling the cell with buffer and lactate immediately after autoclaving. This step was a way to prevent contamination since sometimes the microbial culture did not reach the required optical density (OD) for inoculation on time. Also adding the lactate solution was considered necessary to keep the cell and membrane humid until inoculation, even though the membrane of the MEA does not dry out during autoclaving. Another relevant change consisted of the two subsequent microbial inoculations with the resting period in between, which was expected to increase the number of microbes forming the biofilm. The final change consisted of the connection of a 1 kΩ resistance from the first inoculation, between the anode and the cathode, in order to have the microbes in an active metabolism under anaerobic conditions from the moment they were intro-

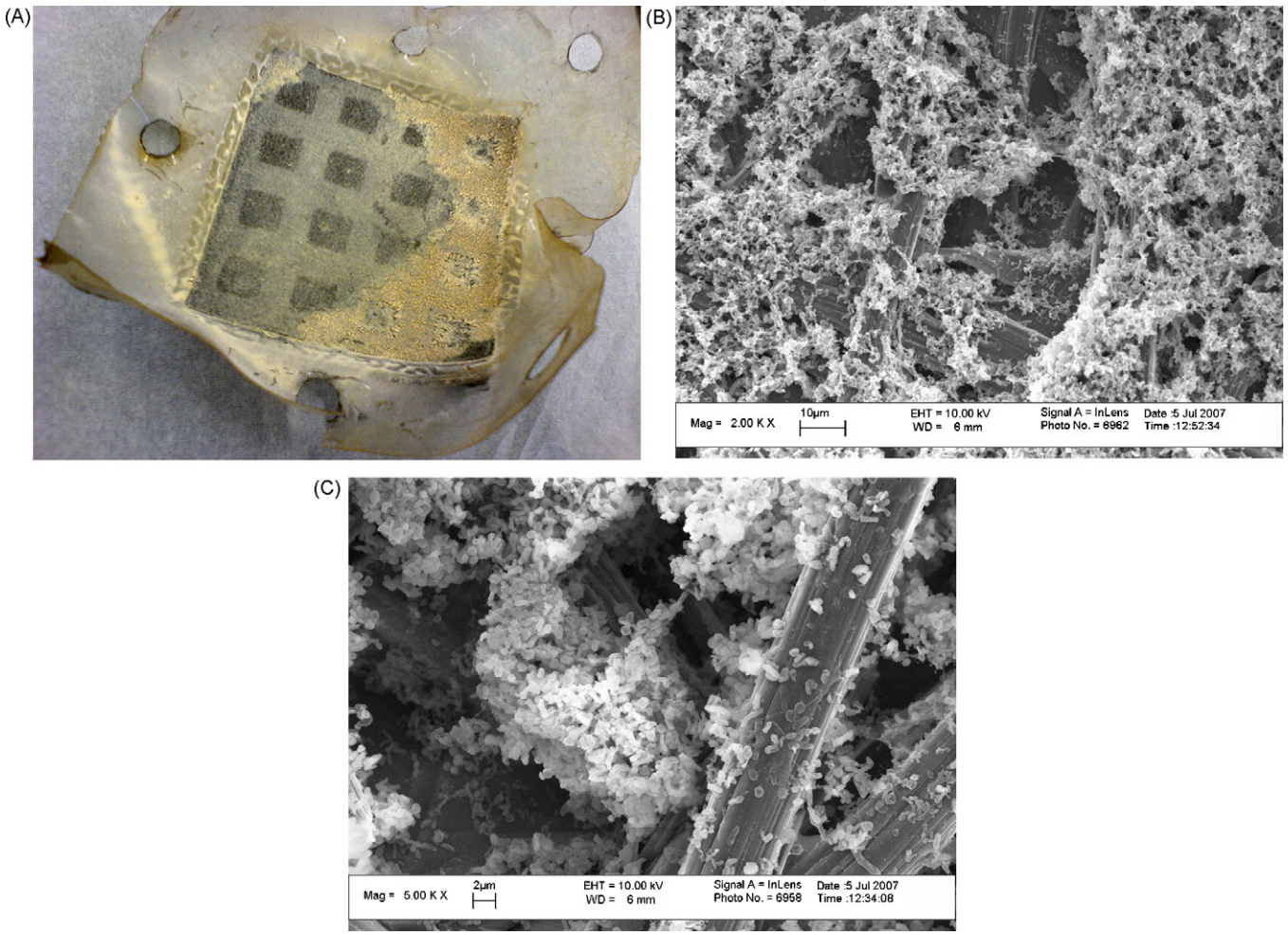

Fig. 3. (A) Digital photo taken of an MEA used in a FC inoculated using IP2. (B) SEM image at 2000× magnification. (C) SEM image at 5000× magnification.

duced into the anode compartment. Otherwise, in case of open circuit conditions, i.e. no current flowing, the bacteria will be in a kind of dormant state. These changes, i.e. IP2 protocol, proved to be much more efficient in obtaining a dense biofilm, as seen in Fig. 3(A) and (B). By comparing the SEM images in Figs. 2 and 3, it can be observed that after fixation of the biofilm, a magnification of 12.5× was necessary to view the biofilm in Fig. 2(A) using IP1 while in Fig. 3(A) using IP2 showed considerable biofilm coverage without magnification. In Fig. 2(B), which corresponds to an SEM image obtained with 6000× magnification, very few microorganisms appear to be present in the biofilm. This low biofilm density for MFC using the IP1 protocol was observed on the entire electrode surface. All anodes prepared for MFC testing using the IP1 protocol always presented lower electrode coverage that accounted for only 30% of the whole area. On the other hand, with IP2 protocol, as seen in Fig. 3(B) obtained with 2000× magnification, and Fig. 3(C)

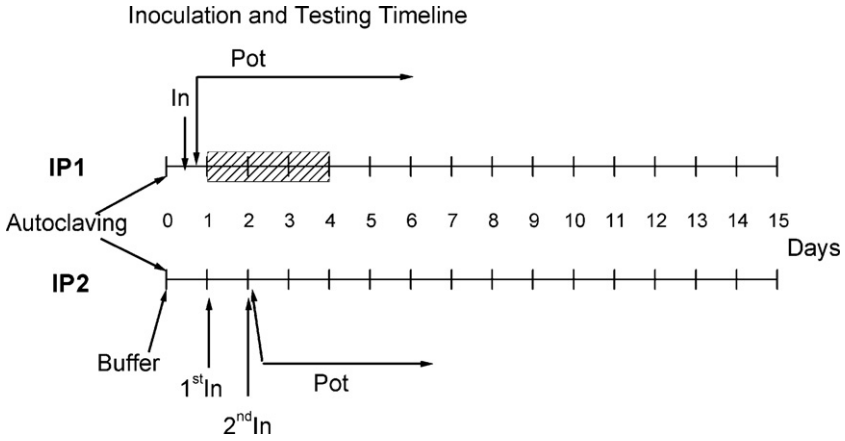

Fig. 4. MFC timeline for inoculation procedures 1 and 2 after autoclaving the cell assembly. Inoculation is showed by the "In" label while "Pot" represents connection to the potentiostat. The shaded box represents the unusable days for IP1.

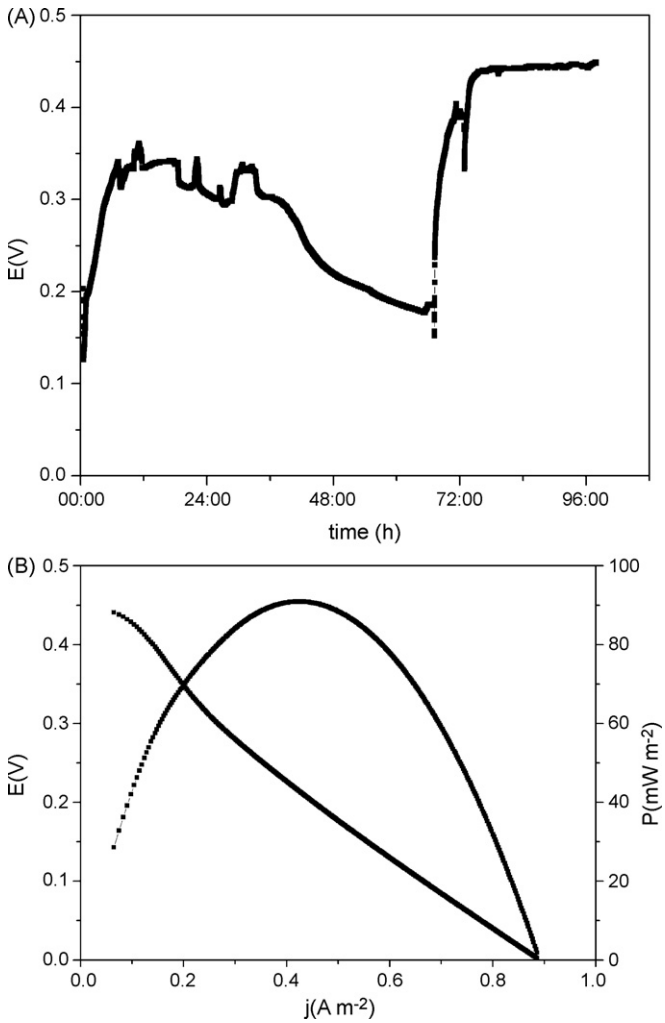

Fig. 5. (A) OCV and (B) polarization and power density plots for an MFC inoculated with IP1 Temperature 28 °C and O_2 flow of 7 mL min^{-1} at the cathode.

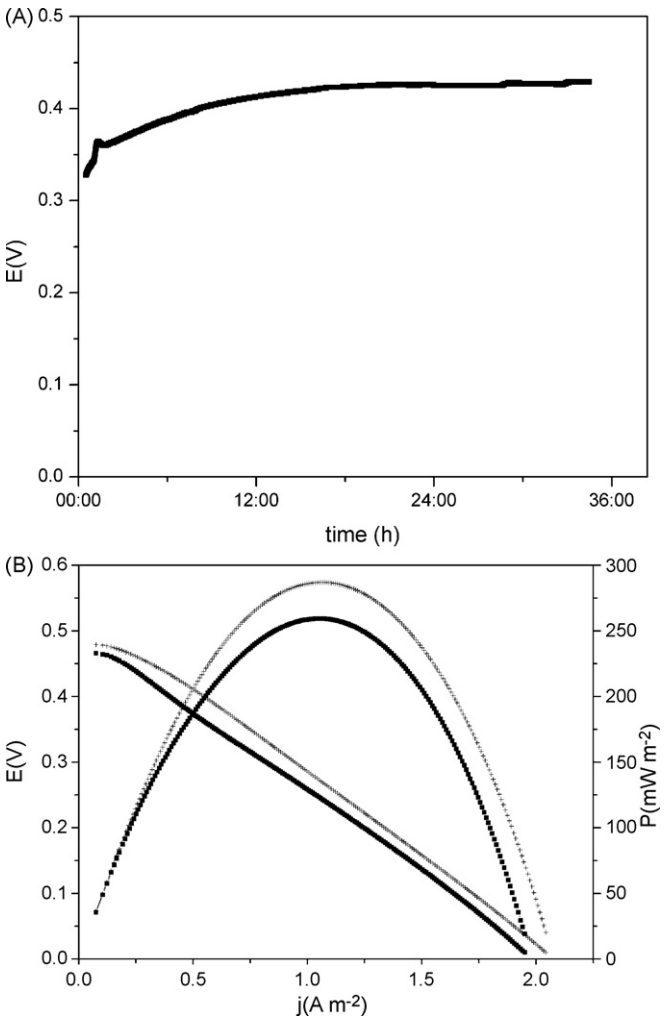

Fig. 6. (A) Cell voltage across a 1 kΩ resistance and (B) polarization and power density plots for an MFC inoculated with IP2 temperature 28 °C and O_2 flow of 7 mL min^{-1} at the cathode.

obtained with 5000× magnification, the images give a clear idea not only of the very densely populated biofilm, but also the better overall coverage, up to 60–70% of the electrode area. These images also show that acidic Nafion® and related proton exchange membranes do not present any hindrance in the bacterial growth over the carbon paper electrode.

The rationale for changing the inoculation procedure from IP1 to IP2 was to obtain a better biofilm. Apart from the SEM images, the other indication that a better biofilm was formed was by the results from cell voltage and polarization. Fig. 4 presents a timeline for the FC test for the two inoculation procedures, where the inoculation and connection to the potentiostat are in reference to the autoclaving of the MFC housing. In the early experiments, using IP1, the OCV was monitored immediately after inoculation, and polarization measurements were performed once a constant OCV was obtained. This usually occurred after a period of 2–3 days after the inoculation. Periods of 1–2 days were reported by other authors [30]. Referring to Fig. 4, a steady OCV for MFC using IP1 was obtained on day 5. Once this steady potential was obtained, polarization measurements were conducted on the MFCs. Fig. 5(A) and (B) shows the OCV vs. time and polarization and power plot for a MFC inoculated under IP1 protocol. As can be seen in Fig. 5A, a steady OCV could be obtained only after a period of 3 days after inoculation (as indicated in timeline shown in Fig. 4). After the voltage drops to a minimum, sodium lactate was added. At that point, after such addition, the voltage rises and maintains a constant value, which allowed polarization measurements to be performed. The OCV becomes steady only after 4 days of inoculation (day 5 in Fig. 4). Polarization was therefore obtained starting from day 5. The maximum power density (P_{max}), i.e. the top of the parabola, and the limiting current density (j_{lim}), i.e. the last current value in the polarization, obtained in Fig. 5(B) as well as for the rest of the MFC prepared using IP1 protocol were found more than satisfactory when compared to previous results [36–38]. However the best MFC prepared, using the IP1 protocol, yielded a power density of 120 mW m^{-2} and 1.1 A m^{-2} as the j_{lim}, which is subsequently shown to be rather low. All of the MFCs inoculated with IP1 protocol yielded the best results in term of polarization and power plots from day 5 to days 6–7 (Fig. 4). After that period, performance decreased steadily. Again, the unsteady OCV presented during the initial days of the fuel cell testing was an indication that the microbes needed some period of adaptation inside the cell. When IP2 protocol was used, the cell voltage rather than the OCV was monitored from the second inoculation, with the 1 kΩ resistance connected between the anode and the cathode, i.e. day 3 in Fig. 4. Fig. 6(A) and (B) shows the voltage vs. time across the resistance and polarization and power plots for a MFC using the IP2 method. It can be seen that from the moment the cell is connected to the potentiostat, on Day 3 in Fig. 4, the voltage indicates a high value. That value was maintained for a period of 2–3 days, days 4–5 in Fig. 4, and as the figures show, voltage across the resistance was

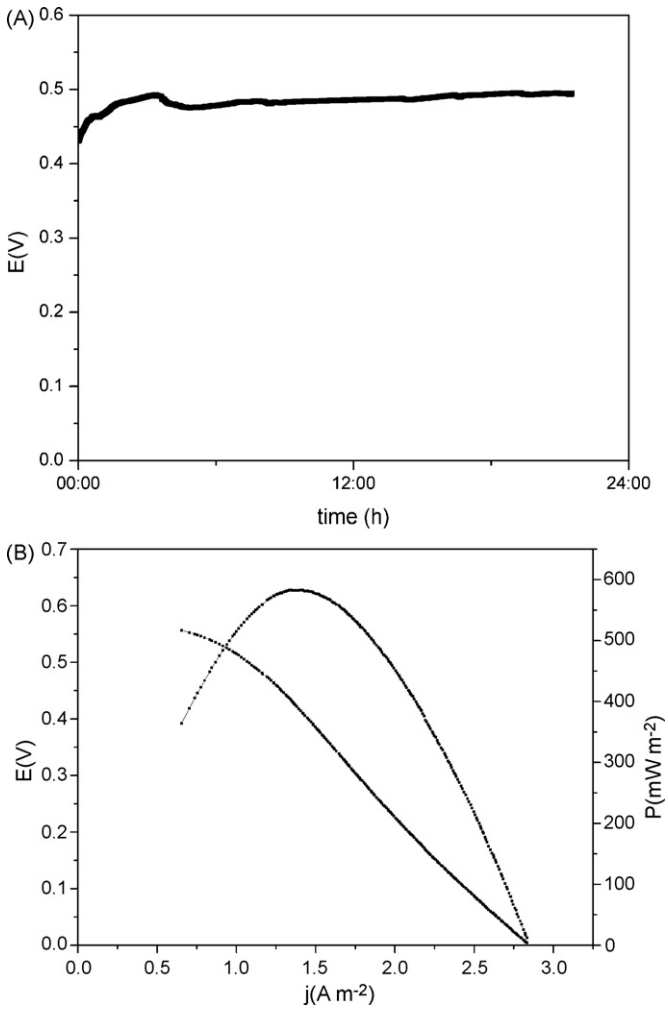

Fig. 7. (A) Cell voltage across a 1 kΩ resistance and (B) polarization and power density plots for a MFC prepared with Nafion® 117, inoculated by IP2 and conditioned once the FC was assembled as described in Section 3. Temperature 28 °C and O_2 flow rate of 7 mL min^{-1} at the cathode.

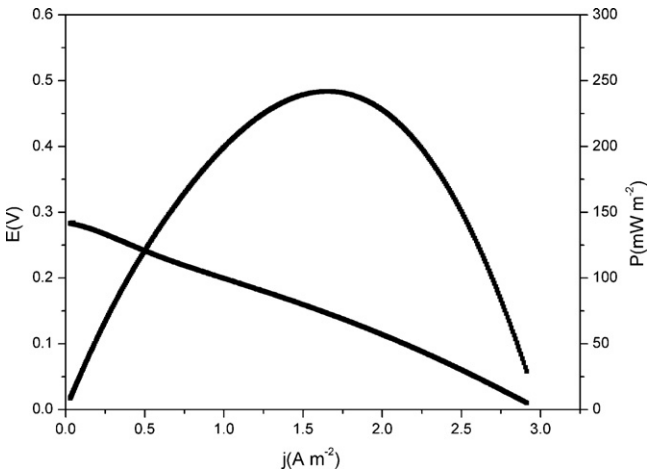

Fig. 8. Polarization and power density plots for a MFC with a PVDF-PSSA MEA inoculated by IP2. Temperature 28 °C and O_2 flow rate of 7 mL min^{-1} at the cathode.

higher than the OCVs values for MFCs using IP1 protocol, indicating that MFC with IP2 produced more power than with IP1. This behavior was shown consistently in all the MFCs assembled with the IP2 protocol. In Fig. 6(B), it can be seen on how the cell voltage (with the resistance), which was ca. 400 mV, increased to an OCV of 470 mV, as measured by the potentiostat at the beginning of the polarization measurement. In terms of P_{max} and j_{lim}, the polarization and power plots presented big improvements compared to MFCs under IP1 protocol. The P_{max} went from average values of 120 mW m^{-2} for IP1 to values always above 200 mW m^{-2} for IP2 as can be seen in Fig. 6(B). The j_{lim}, also showed an increase by a factor of two in Fig. 6(B) compared to Fig. 5(B). These results can be directly related to the biofilm density of the microorganism and show that the steps taken under protocol IP2 in order to improve the inoculation procedure were beneficial. Moreover in Fig. 6(B), a value of 260 mW m^{-2} represented by (■) can be seen, which was obtained 1 day after the second inoculation, while the plot (+) which yielded a P_{max} value of 290 mW m^{-2} corresponds to measurements performed 15 days after the second inoculation, i.e. days 18–19 in Fig. 4. With the MFC inoculated using IP1 protocol, measurements performed on subsequent days never yielded better results than measurements performed during the initial days, as was mentioned earlier. This lack of improvement can be explained by the fact that the biofilm obtained under IP1 protocol is poor compared to that obtained using IP2, again related to the inoculation procedure. As the same amount of bacteria was used for each procedure, this result indicates that for a more direct inoculation like IP1 the microorganism stay mostly in a planktonic state instead of forming a uniform biofilm. In addition, with the continued supply of O_2 to the cathode for the entire period of testing, similar power densities were realized during the 15 days of testing indicating that the permeability of O_2 through Nafion® and subsequent dissolution in the anode solution is not a problem in our cells.

An important step in DMFC and related fuel cells is the conditioning of the membrane of the assembled fuel cell, which is accomplished by forcing protons to move from anode to cathode through the membrane by drawing current in a non-steady state manner. This procedure opens up “channels” in the membrane, which allows protons to move easily later on through the membrane during the operation of the fuel cell and polarization measurements. In other words, it reduces the internal resistance by reducing the opposition to proton movement. This is observed by a decrease in the MEA resistance before and after this procedure. In MFC, since the microbes are the “catalysts” on the anode side, this method would be impractical. In order to force protons from the anode to the cathode, water was electrochemically split to hydrogen and oxygen in the MFC after the cell was assembled and before it was autoclaved. The anode was filled with a 0.1 M H_2SO_4 solution. Since the anode lacks a catalyst, a Pt wire electrode was introduced and used as anode for the oxidation of water to oxygen, while on the MEA cathode, protons get reduced to molecular hydrogen. The redox reaction for water was only possible because the protons moved through the membrane to reach the cathode. The electrolysis of water was performed for 6 h at a current, wherein the production of oxygen at the anode was noticeable and steady. After this membrane conditioning, the MFC was autoclaved and inoculated via the IP2 protocol. Fig. 7 shows the result for the assembled MFC. Fig. 7(A) shows the cell voltage after the second inoculation with the 1 kΩ resistance until it was stopped for testing, while Fig. 7(B) shows the polarization and power plot. For this MFC, the cell voltage across the resistance was measured to increase from a value of 500 mV, to an OCV of 570 mV. Both the j_{lim} and the P_{max} increased when compared to MFCs using IP2 prior to membrane conditioning, as presented in Fig. 6(A) and (B). The P_{max} showed an increase in the maximum power to 600 mW m^{-2}. These results demonstrate that this kind of MEA conditioning is not only useful, but also shows that considerable power is lost in the MFC by the internal resistance of the membrane.

MEAs were also prepared with PVDF–PSSA, conditioned as mentioned above and inoculated using IP2 protocol. Polarization and power results are shown in Fig. 8. Firstly, there is a noticeably low OCV (ca. 300 mV). This low OCV could be a result of the membrane thickness, which is a little bit higher than the Nafion® 117, and to the fact that this membrane was prepared to withstand higher currents, or as a result of higher oxygen permeability through this membrane. However, even with a low OCV, the P and j_{lim} are comparable with those of Nafion® MEAs presenting higher OCVs. The P_{max} of 245 mW m^{-2} is comparable with the P_{max} for the MFC shown in Fig. 6(B), while the j_{lim} is slightly above the j_{lim} of the MEA in Fig. 7(B). That value of j_{lim} represents a total of 7 mA for the MFC, which is again among the highest value obtained presently in our laboratory for single cell culture. This kind of membrane is promising because if the OCV could be increased (e.g. by reduction of the thickness), a much higher power density is expected.

4. Conclusion

If MFCs based on single culture cell organism such as *Shewanella* MR-1 are to be practical, a simple and useful design is needed. Construction of MEA, using the same procedure as used for PEMFC, for MFC was carried out. A novel way of conditioning the membranes was also found. The MEA presents the best contact between electrodes and membrane, thereby reducing the internal resistance, and also the use of a dry cathode moves away from the complication of having air or oxygen bubbled through into the cathode solution. The results presented here for the MFC built in house not only increased the power output compared to the traditional simple fuel cell designs [36–38] by 2–3 orders of magnitude but these results also show improvements in power density from early to later tests performed in this study. The results also demonstrated the importance of microbial inoculation or microbial accommodation times in the anode chamber for the formation of adequate biofilm and their impact on the overall power output. Furthermore, the results indicate that although a limit on the power output exists for MEAs using Nafion® due to its affinity for the Na^+ ion, which decreases proton conductivity, this limit has not yet been reached at the realized power densities. A more economical option than Nafion® was employed using PVDF–PSSA membrane. The results obtained however did not show any tangible progress. On the other hand, we believe that there is much room for improvement since this particular PVDF–PSSA membrane was initially designed for DMFC.

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