Sustainable Hypersaline Microbial Fuel Cells: Inexpensive Recyclable Polymer Supports for Carbon Nanotube Conductive Paint Anodes

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Microbial fuel cells are an emerging technology for wastewater treatment, but to be commercially viable and sustainable, the electrode materials must be inexpensive, recyclable, and reliable. In this study, recyclable polymeric supports were explored for the development of anode electrodes to be applied in single-chamber microbial fuel cells operated in field under hypersaline conditions. The support was covered with a carbon nanotube (CNT) based conductive paint, and biofilms were able to colonize the electrodes. The single-chamber microbial fuel cells with Pt-free cathodes delivered a reproducible power output after 15 days of operation to achieve 12 ± 1 mW m^{-2} at a current density of 69 ± 7 mA m^{-2}. The decrease of the performance in long-term experiments was mostly related to inorganic precipitates on the cathode electrode and did not affect the performance of the anode, as shown by experiments in which the cathode was replaced and the fuel cell performance was regenerated. The results of these studies show the feasibility of polymeric supports coated with CNT-based paint for microbial fuel cell applications.

Keywords: carbon nanotubes · conductive paints · hypersalinity · microbial fuel cells · wastewater

Introduction

A microbial fuel cell (MFC) is a bioelectrochemical system that converts chemical energy from civil and industrial wastewaters into electrical energy.[1] The core of this technology is the ability of some microorganisms to catalyze the oxidation of the organic matter in wastewaters and reduce electron acceptors.[2] These microorganisms, that can communicate with both the anode and the cathode of a MFC, are classified as electrogenic, or electrochemically active bacteria, because of their ability to perform extracellular electron transfer.[3] The interest in MFCs is due to the high theoretical efficiency of energy generation ($\geq$80%) that has been reported. However, the overall energy efficiency of the devices is less than the theoretical value because the majority of the organisms cannot use complex substrates, thus, they rely on low-molecular-weight organic acids that are provided by fermenting bacteria.[4] In the last 15 years, this technology has seen important advancements and improvements,[5] and different prototypes have been reported and applied in field.[6] Although MFCs are not yet commercially competitive for large-scale power generation, some interesting applications could be implemented at the current technology stage. MFCs can be utilized to power remote sensors for environmental monitoring[6a,7] and applied directly as biosensing tools.[8] Further research is needed to achieve the commercial application of MFCs. In particular, more effort should be focused on the understanding of the fundamental bioelectrochemical processes of the technology, such as extracellular electron transfer and electron transport networks inside biofilms,[9] substrate oxidation,[10] and the oxygen consumption and redox processes at the cathode.[11] As the surface concentration of redox-active compounds in electroactive biofilms is of a higher interest than the bulk concentration, chemical and enzymatic microsensors have been developed specifically for application in biofilms and complex matrices.[12]

With the goal of the commercial application of MFCs, another critical aspect that needs to be improved is the material selection for the construction of the devices. The majority of previous studies report electrode materials that would be too expensive (e.g., Pt) or fragile for real industrial applications (e.g., carbon cloth).[13] For commercial applications, different stainless steels have been investigated both as the anode and cathode supports.[14] The results show that, under specific conditions, no corrosion caused by bacterial activity was obtained on AISI 304.[14b] Copper was also tested as anode support, and electrochemically active biofilms were able to colonize it, with no antimicrobial activity in the tested medium and negligible corrosion.[14c] However, under hypersaline conditions, the electrochemical behavior of metal electrodes can strongly affect the

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stability and viability. The high concentration of chlorides increases the corrosion rate and pitting, which would cause the early failure of the electrode. The application of MFCs under hypersaline conditions is of primary interest, as wastewaters with a high salinity cannot be treated in normal biodegradation plants because of osmotic stress and plasmolysis on bacterial cells.[15] Recently, it has been demonstrated that halotolerant bacteria can be applied in MFCs to perform the remediation of contaminated water.[16] We have demonstrated that halotolerant bacteria obtained from the Great Salt Lake can be used as the inoculum in single-chamber MFCs and act as bioelectrocatalysts both for the anodic and the cathodic reactions.[17] In our previous study, carbon cloth was used as the electrode material for the anode of the MFCs.

The goal of this work is to develop an electrode material for in field applications having the following characteristics: sustainable, cost effective, good mechanical properties, resistant to hypersaline solutions, suitable for biofilm development, and recyclable to decrease waste generation after the replacement of the electrode. Accordingly, a recyclable polymeric substrate was investigated as the support for an anode. The polymeric support was coated with a conductive paint based on carbon nanotubes (CNTs). The price of the electrodes depends on the thickness of the coating and the CNT content and is $ 10–50 m^{-2}. Conductive paints based on CNTs are characterized by a relatively high electrical conductivity and they adhere readily to polymeric supports. Carbon-based electrodes modified with CNTs have been reported previously. They show good bacteria colonization and facilitate extracellular electron transfer.[18] CNTs were also grown on conductive materials, such as a Cr/Ni catalyst layer[19] and nonconductive materials.[20] Unlike metal anodes, the electrodes used in this work do not corrode, which makes them easy to implement in MFCs operated in hypersaline environments such as industrial wastewaters and wastewaters in coastal cities.

Results and Discussion

Trends in potentials under load

The evolution of the difference of the potential between the anode and cathode of the MFCs with an external load of 2000 Ω is shown in Figure 1 (number of biological replicates $n=3$). The MFCs started to develop a difference of potential after 1 day from the start up and reached the maximum value of 23 ± 4 mV at 7 days of operation. After this peak, the difference of potential sharply decreased. Fresh acetate was added on day 8 (final concentration 9 g L^{-1}), but only an insignificant increase of the difference of potential was obtained, followed by a decrease to 1.4 ± 0.2 mV. A decrease of the performance was already observed in our previous work, in which the addition of acetate did not lead to the recovery of the difference of potential after 16 days of operation of the MFCs.[17] Notably, the MFCs are operated using lake solution from the Great Salt Lake (Utah), and the precipitation of nonconductive inorganic compounds on the electrode surface can inhibit or completely hinder the electron transfer by blocking the active sites on the

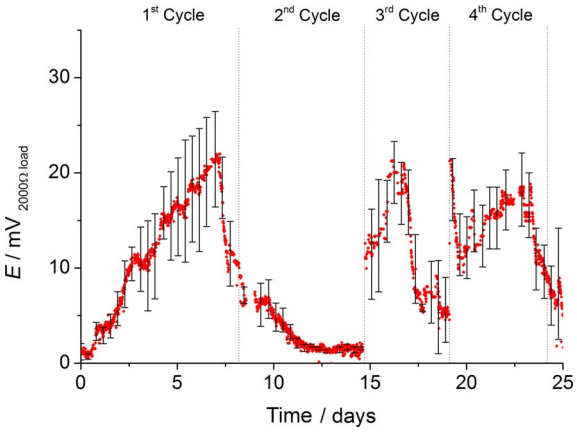

Figure 1. Difference of potential evolution between the anode and cathode of the MFCs with an external load of 2000 Ω. The cathodes were substituted on day 14. Vertical dashed lines indicate acetate addition and degradation cycles (from the first to the fourth cycles).

electrode surface as demonstrated in a study by Santini et al.[21] This aspect is further discussed in the SEM section. Here, the cathodes were substituted after 14 days, to explore the possible recovery of the MFCs, and new acetate was added (final concentration 9 g L^{-1}). An immediate increase of the difference of potential was obtained, which decreased again after 18 days and was recovered with a new addition of the substrate at day 19. The difference of potential decreased again at day 23, and the following additions of acetate did not lead to the recovery of the potential to previous levels. These results showed that although the activated carbon-based cathode was not stable over the time frame, the CNT anode supported on the polymer was stable, and under turnover conditions (in the presence of substrate at saturating concentrations), an average continuous current generation of 47 ± 6 mA m^{-2} was obtained (cycles 1, 3, and 4).

Control MFCs experiments were conducted with autoclaved bacteria growth and autoclaved lake solutions. The control experiments showed that no potential difference developed between the anode and cathode of any MFCs in the absence of active bacteria (Figure S1). This control response indicated that the difference of potential appeared from the metabolic activities of the viable microorganisms in the samples.

Open-circuit potential evolution

The evolution of the open-circuit potentials (OCPs) of the anodes and the cathodes for the MFCs is reported in Figure 2. The grey area indicates the evolution of the OCP for the overall MFCs ($OCP_{MFC}=OCP_{CATHODE}-OCP_{ANODE}$). After an initial shift of the cathode OCPs from 0.16 ± 0.02 to 0.064 ± 0.002 V, the values stabilized at approximately 0.13 ± 0.02 V throughout the experiments. However, the analysis of the anode OCPs is more interesting to this study. After the start-up of the MFCs, the anode OCP stabilized to negative values of approximately -0.17 ± 0.03 V for 6 days. The OCP changed to more positive values and the new addition of substrate at day 8 did not shift the OCP to lower values. Interestingly, if the cathodes were

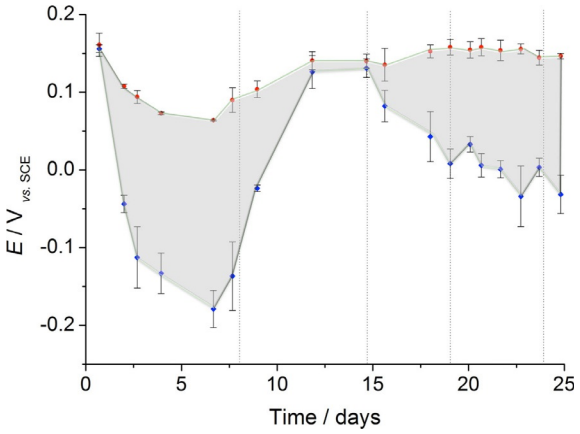

Figure 2. OCP evolution for anodes (blue) and cathodes (red) of the MFCs. Vertical dashed lines indicate acetate addition and degradation cycles. Grey area indicates the OCP evolution of the MFCs. Cathodes were substituted on day 14.

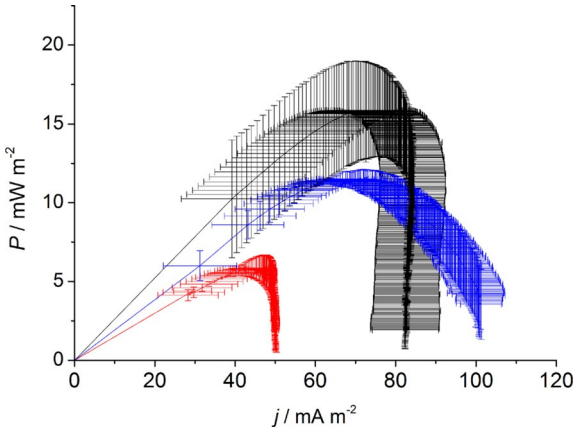

Figure 3. Power curves of the microbial fuel cell. Day 6 (black line), day 10 (red line), days 15, 19, and 22 (blue line). The cathodes were substituted on day 14.

replaced, we obtained a strong recovery of the anode OCPs, which stabilized at -0.04 ± 0.01 V, and the OCP of the MFCs was approximately 0.2 V. A mutual influence between the two electrodes was reported previously for single-chamber MFCs, so this response is expected.[14b,22] Throughout the evolution of the OCP for the overall MFCs, values of approximately 200 mV are obtained. These values are lower than those obtained in our previous work with carbon cloth anodes in hypersaline MFCs ($\approx$400 mV). In particular, the OCP of the anodes is more positive in the present work. It has to be considered that to be able to withdraw electrons from microorganisms, the potential of the anode electrode must be more positive than the potentials of the membrane proteins used by the microorganisms to exchange electrons in a direct electron-transfer process or more positive than the potential of the redox mediator in a mediated electron-transfer process. It is possible that the different surface forced the microorganisms to use different pathways to perform extracellular electron transfer (EET), which resulted in a more positive potential of the electrode. The study of EET mechanisms for the bacteria species of the anodic biofilm will be the objective of future investigations.

Power curves

Three separate MFCs were prepared and tested by using quasi-stationary polarization at 0.1 mV s^{-1}. The average power curves for the MFCs are reported in Figure 3 with the corresponding error bars. The maximum power output was obtained at day 6, which was 16 ± 3 mW m^{-2} at a current density of 74 ± 9 mA m^{-2}. This current density is 36% lower than that obtained with carbon cloth anodes,[17] but carbon cloth allows for biofilm formation on both sides of the electrode. Future work should explore the coating of both sides of the polymer support with the conductive CNT layer. The power output decreased drastically at day 10 to 5.8 ± 0.6 mW m^{-2} at a current density of 48 ± 4 mA m^{-2}. After the substitution of the cathodes, the power output of the MFCs stabilized at 12 ± 1 mW m^{-2} at a current density of 69 ± 7 mA m^{-2} for one week of operation. Therefore, the decrease in the performance of the MFCs obtained after 10 days can be associated mostly with the biofouling of the cathodes as their substitution led to a recovery of the power output for all the MFCs.

Quasi-stationary polarization of the anode and cathode

To investigate the behavior of the single electrodes, quasi-stationary polarization was performed on both the anode and cathode. Representative current–voltage (*i–V*) curves are shown in Figure 4. The polarizations performed on the cathodes at 7 days showed the lowest OCP and the lowest current response, compared to the polarizations performed at days 15, 18, and 22. In these days, the cathode OCP stabilized to a more positive potential and the electrodes showed similar current responses. Conversely, the anodic behavior is more complex. At day 7, a limiting current was achieved rapidly, which indicates diffusional limitations. In the following days, a limiting current was not achieved, and a higher current response was obtained at day 22. This behavior indicates that the diffusional limitation was overcome throughout the operation of the MFC. This result might be explained by an increased amount of self-secreted redox mediator by the bacterial cells that facilitate the electron-transfer process or the establishment of a better communication pathway between bacteria that constitute the anodic biofilm and electrode surface. Investigation of this aspect is complicated by the presence of a mixed species biofilm under our experimental conditions. However, based on the quasi-stationary polarization measurements performed at the anode, we can state that the current response of the electrode improves over time. These results indicate that the anode made of a polymeric substrate coated with the conductive CNT paint is less affected by aging problems than the cathode electrode. Accordingly, such an anode could be of great interest for application in MFCs that operate for long periods in hypersaline solutions.

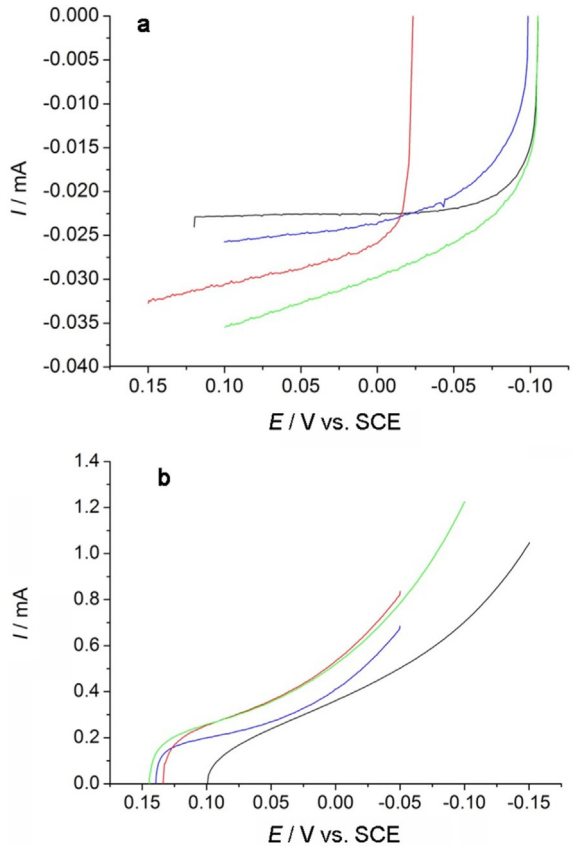

Figure 4. Quasistationary polarization for the a) anode and b) cathode of MFCs. Day 7 (black lines), day 15 (blue lines), day 18 (red lines), day 22 (green lines). The cathodes were substituted on day 14. Scan rate = 0.2 mV s^{-1}.

SEM analysis

The SEM images were obtained on the clean anode and on the anode after 20 days of operation (Figure 5 a, b, respectively). Clearly, biomass developed on top of the electrode, although the colonization of the electrode was not uniform. Conversely, a thick layer of deposits can be recognized on the waterside of the cathode electrode after 14 days of operation if the cathode was removed and substituted in the MFCs. The deposits can be attributed to the development of biofilm together with the deposition of different products because of the chemical composition of the lake solution. As introduced earlier during the discussion of the trend of potentials, the inorganic fouling of the cathodes was reported as a possible reason for the loss in the performance of the MFC in long-term operation by blocking the active sites on the electrode surface. In our previous work, a considerable amount of $MgCl_2$ was detected at the cathode surface.[17] In summary, the SEM images showed that the anode was colonized by biofilm but not uniformly. Future research will be devoted to facilitating and improving the colonization of the anode, and additional surface modification approaches will be explored, which would allow us to improve the power performance of the complete MFC system.

Conclusions

The results of this work showed that a polymeric support coated with a conductive carbon nanotube (CNT) paint can be used as an anode in a single-chamber microbial fuel cell with a comparable performance to the common anode material carbon cloth but with a greater physical stability and lower cost. These characteristics make this electrode more suitable than carbon cloth for real applications. Its chemical stability allows application in a broad range of pH values (pH 3.5–10), which is much larger than the typical pH range in which MFCs are operated.[22] This finding opens new research possibilities because it will be possible to develop new single-chamber MFC configurations using 3 D-printed plastic reactors modified with the conductive paint to obtain reliable and inexpensive MFC devices for application in water purification in hypersaline solutions. Further research is needed to maximize the bacteria colonization and communication with the plastic-CNTs anode. Moreover, the developed CNTs-modified polymeric support could be applied to different bioelectrochemical systems such as bio-fuel cells, in which enzymes are immobilized on the electrode surface,[23] or in biological photovoltaic devices for direct interaction with thylakoid[24] to obtain reliable and low-cost electrodes.

Experimental Section

Bacteria sampling

Bacteria samples were collected at White Rock Bay, Antelope Island State Park, Utah. The lake solution was filtered through 10 μm filters (Sigma–Aldrich, Chemrus disposable filter tunnels 40 mL) to eliminate suspended solids and stored at 4 °C until use for inoculation. The conductivity of the lake solution used in the experiments was 100.8 ± 0.9 mS cm^{-1}. The salt content was determined by evaporating 15 mL of lake solution and weighing the dry salts and was 140 ± 20 g L^{-1}.

Bacterial growth

Bacteria were grown by following a procedure reported elsewhere.[17] The final composition of the growth medium was (per liter of deionized water): 0.5 g KH_2PO_4 (Macron Chemicals), 1 g NH_4Cl (Macron Chemicals), 1 g Na_2SO_4 (Macron Chemicals), 1 g $CaCl_2$ (Sigma–Aldrich), 1.83 g $MgCl_2 \times 6H_2O$ (Fisher Scientific), 1 g yeast extract (Sigma–Aldrich), 0.1 g L-ascorbic acid (Sigma–Aldrich), 0.013 g sodium thioglycolate (Sigma–Aldrich), 6.38 g sodium citrate (Sigma–Aldrich), 0.5 g $FeSO_4 \cdot 7H_2O$ (Sigma–Aldrich), 1.75 g sodium lactate (Sigma–Aldrich), 2 g sodium acetate trihydrate (Macron Chemicals), and 35 g NaCl (VWR Analytical). The pH of the solution was adjusted to 7.5–7.8 using 2 M NaOH. Before inoculation, the growth medium was autoclaved at 121 °C for 15 min (Harvey SterileMax ST75925). The growth medium was then purged with Ar for at least 4 h to eliminate dissolved oxygen. Furthermore, all inoculations were performed in an anaerobic chamber under Ar flow to ensure anaerobic conditions. After inoculation, the solution was maintained at 35 °C. The MFCs were inoculated with bacterial cells in the early-stationary phase at 55 h of the bacterial growth.

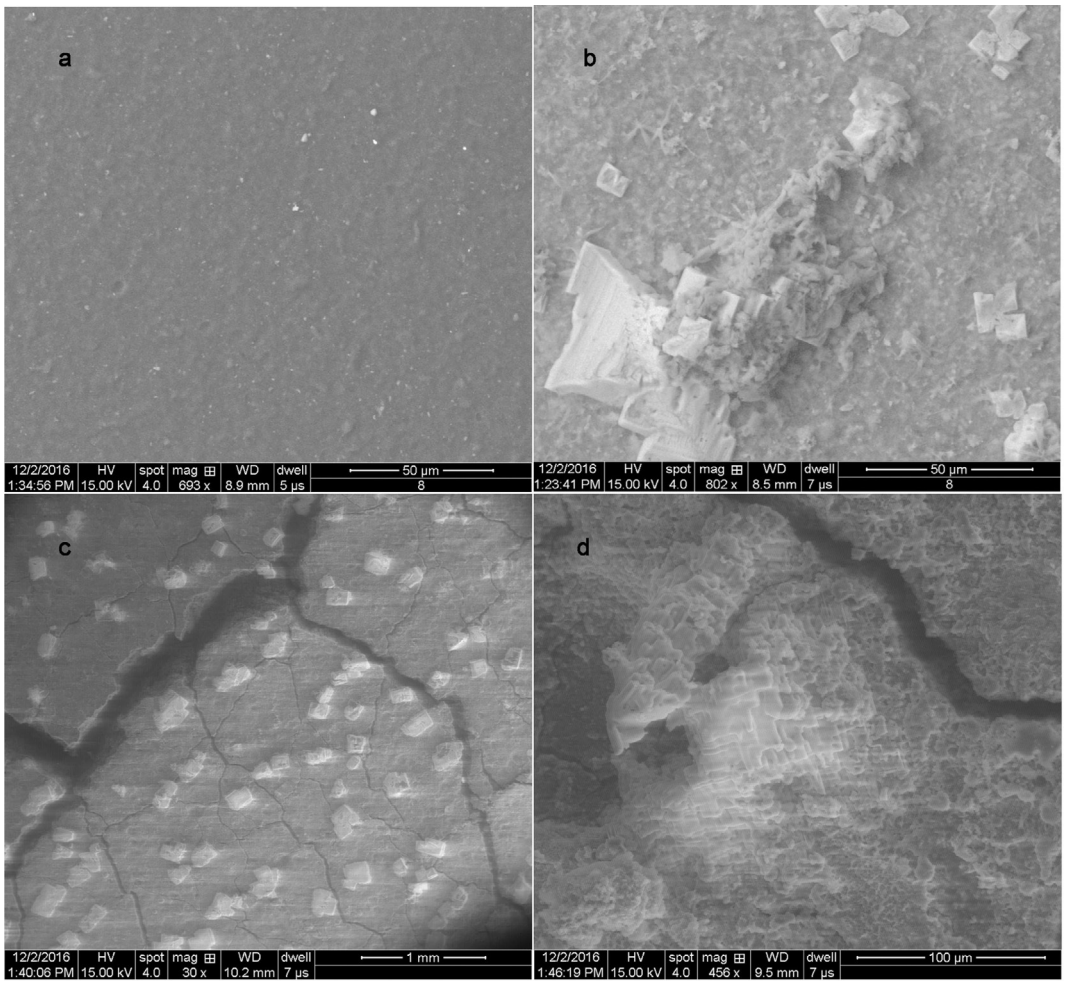

Figure 5. SEM analysis of the anode and cathode. a) Bare electrode surface before bacteria colonization in the MFC; b) biomass accumulation on the anode surface after 20 days of operation; c,d) water side of the cathode electrode after 14 days of operation, which shows a thick layer of precipitates that inhibits the electron-transfer process by blocking the active sites of the electrode surface.

MFC setup

The MFCs consisted of a single-chamber electrochemical cell with an opening on the side in which the cathode electrode was placed (Figure 6). The anodes were made of acrylonitrile butadiene styrene substrate (ABS) coated with polyurethane paint that contained 4–5% of multiwalled carbon nanotubes (Verniciature Bresciane s.r.l., Castegnato, Italy). The thickness of the coating was approximately 30 µm, and the electrical resistivity was 22 $\Omega\,sq^{-1}$. The

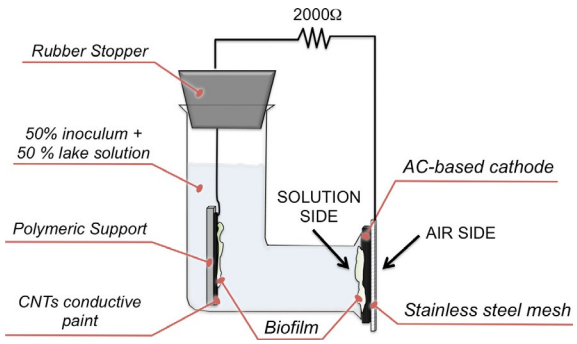

Figure 6. Schematic of the MFCs setup.

electrical connection was obtained utilizing a conductive silver epoxy paste (Electron Microscopy Sciences, Hatfield USA) to connect a copper wire to the polymeric support coated with the paint. The silver paste was cured by heating at 65 °C for 15 min. Following the curing of the silver paste, the electrical connection was insulated by at least three layers of nonconductive epoxy resin (ITW Devcon, Danvers USA). The cathode was made by mixing 80% commercial activated carbon (NORIT SX Ultra, Sigma–Aldrich) with 20% poly(tetrafluoroethylene) (PTFE, Sigma–Aldrich) and pressing the paste onto a stainless-steel mesh (McMaster-Carr, Robbinsville, NJ, USA) following the procedure of Santoro et al. and Merino-Jimenez et al.[25] The MFCs were set up in triplicate and filled with 29 mL of lake solution, 29 mL of bacteria culture media, and 2 mL of 2.9 M sodium acetate (final concentration 9 $g\,L^{-1}$) for a total volume of 60 mL. The substrate was added at the start of the experiment and again when the difference of potential between anode and cathode decreased to a stable value, which defined a new degradation cycle. The anode and cathode of all the MFCs were connected to an external load of 2000 Ω and maintained at 30 °C during operation. For the control experiments, the lake solution and the bacteria culture media were autoclaved (121 °C for 15 min) and utilized to set up MFCs operated under the conditions described.

MFC electrochemical characterization

To characterize the MFCs, the difference of potential between the anode and cathode with the external load of 2000 Ω was recorded every 10 min by using a multimeter (Measurement Computin, USB-1608G) connected to a portable computer. The electrochemical performances of the MFCs were then evaluated by plotting quasi-stationary polarization curves (Bio-Logic VSP) by using a three-electrode set up with the anode and cathode as working electrodes (WE) for anodic and cathodic polarizations, respectively, Pt mesh as a counter electrode (CE), and a saturated calomel electrode (SCE) as a reference electrode (RE) ($E = +0.241$ V vs. standard hydrogen electrode (SHE)) at a scan rate of 0.2 mV s^{-1}. Power curves were obtained from the quasi-stationary polarization curves (Bio-Logic VSP) performed at a scan rate of 0.1 mV s^{-1}, in which the cathode was used as the WE and the anode as CE and RE. Before any electrochemical experiments were performed (power curves or quasi-stationary polarizations), the MFCs were maintained under open-circuit condition for at least 30 min to allow the system to stabilize and to avoid erroneous evaluations. The power P was calculated as the potential (E) times the current density (j). The OCPs of the anode and cathode and the pH evolution of the MFC were also recorded. After 14 days, the cathodes were substituted, and after 20 days of operation one of the MFCs was stopped and the anode was removed. The electrodes were dried in sterilized petri dishes at RT (20 ± 2 °C) before we performed SEM (FEI Quanta 600 FEG).

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Conflict of interest

The authors declare no conflict of interest.

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