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# Single medium microbial fuel cell: Stainless steel and graphite electrode materials select bacterial communities resulting in opposite electrocatalytic activities

B. Erable [a,*], N. Byrne [b], L. Etcheverry [a], W. Achouak [b], A. Bergel [a]

[a] Laboratoire de Génie Chimique, Université de Toulouse, CNRS, INP, UPS, Toulouse, France
[b] Laboratoire d'Ecologie Microbienne de la Rhizosphère et d'Environnements extrêmes, CNRS-CEA-Université Aix-Marseille, Service de Biol. Végétale et Microbiol, Environnementales/IBEB/DSV, CEA Cadarache, 13108 Saint Paul Lez Durance, France

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ABSTRACT

A graphite electrode and a stainless steel electrode immersed in exactly the same medium and polarised at the same potential were colonised by different microbial biofilms. This difference in electroactive microbial population leads stainless steel and graphite to become a microbial cathode and a microbial anode respectively. The results demonstrated that the electrode material can drive the electrocatalytic property of the biofilm opening perspectives for designing single medium MFC.

This new discovery led to of the first demonstration of a "single medium MFC." Such a single medium MFC designed with a graphite anode connected to a stainless steel cathode, both buried in marine sediments, produced 280 mA $m^{-2}$ at a voltage of 0.3 V for more than 2 weeks.

## Introduction

All fuel cells obey the same basic principle: the oxidation of a fuel (electron donor) on the anode produces electrons that are driven through the external electrical circuit to the cathode, on which they are transferred to the final oxidising agent (electron acceptor). Fuel cell design must be engineered to avoid the fuel and the oxidising agent both reacting simultaneously on the same electrode instead of exchanging electrons via the external circuit. Separator-less (or membrane-less, because the separator is often a membrane) fuel cells can be designed (i) if the anode and the cathode are strictly selective for the fuel and the oxidising agent respectively; or (ii) if the cell design is built in a way in which not lot of fuel or oxidising agent can permeate and interfere inside the opposite compartment.

In microbial fuel cells (MFCs) [41], the electro-catalysis is ensured on the anode by a microbial biofilm that oxidises the

* *Corresponding author.*
E-mail address: benjamin.erable@ensiacet.fr (B. Erable).

fuel and transfers the electrons produced to the electrode material [46]. Many electroactive bacterial strains have been identified on anodes [22,29] and different electron transfer mechanisms have been identified: diffusible electrochemical mediators, conduction through outer-membrane cytochromes [16] or networking through extracellular cytochromes [26,43] or conductive nanowires [23,47]. Similarly, electroactive bacteria can also develop on cathodes [15] and catalyse the reduction of oxygen, sulphates, nitrates, or $CO_2$ but mechanisms of extracellular electrons exchange are less comprehensively understood. Several MFC designs have been implemented so far and all need two different phases or at least two different compartments physically separated to be put into contact (Fig. 1): one for the anode the other for the cathode. In two-chamber MFCs [2,28,48] the separation between the anode and cathode compartments is ensured by specific membranes (Fig. 1a). In benthic MFCs [4,27,32,36,39] the gravity ensures natural separation between solid sediments in which the anode is embedded, and liquid seawater, where the cathode is immersed (Fig. 1b). When abiotic air-cathodes are used, the cathode itself ensures the separation between the anode solution and the gas phase thanks to a hydrophobic porous layer [24,25]. (Fig. 1c). In all cases, ionic transfer at the interface between anodic and cathodic compartments (i.e. through the membrane or at the liquid/solid interface) is a key parameter often increasing MFC internal resistance [35]. The use of a common medium (i.e. homogeneous aqueous solution) for both the anodic and cathodic reactions appears as an evidence for (i) simplifying the design of MFCs and (ii) removing the limitations related to ionic transfer at the interface of compartments physically separated.

The implementation of microbial anodes and microbial cathodes in a unique electrolyte is completely feasible especially since the demonstration of several electronic exchange mechanisms between reversible electroactive biofilms and electrodes [3,6,44]. These "reversible electrodes" are mostly microbial anodes firstly formed in complex environments (wastewater, activated sludge, garden compost, etc) that under depletion of reducers (e.g. acetate) and in the presence of an alternative oxidant (oxygen for example) switch their initial electrocatalytic activity towards the electro reduction of the oxidant. In competition, that is to say in the presence of both a reducing and an oxidizing agent, the oxidation reaction seems to be the preferred reaction of the electroactive microorganisms. Why? Especially because the concentrations involved are usually very different. Indeed, concentrations of synthetic reducers (acetate, glucose, etc) used in the literature on the reversible microbial electrodes are of the order of 15–20 mM COD equivalent whereas in the case of oxygen as the oxidant for example, the maximum concentration is the maximum solubility in aqueous medium at pH 7.0 and 20 °C, it means 0.24 mM. The unbalance is very important, the ratio of concentrations is at least 60 in favor of the reducers. This actually means that if a reversible electrode is exposed to an environment where oxygen and excessive acetate coexist, traces of reduction current may persist but they are a minority even negligible in comparison to the oxidation current. Indeed, in the case of a reversible microbial electrode, considering an exchange current $J_0$ identical to the oxidation of the acetate or the reduction of oxygen, and no limitation of mass transport phenomena, the theoretical ratio of the maximal current is given by the ratio of the concentrations of species that can to be oxidized or reduced at the electrode. Here, the $J_{max}$ for the oxidation of acetate should be at least 60 times greater than the $J_{max}$ obtained for the reduction of oxygen.

Now, without exogenous addition of acetate (or other organic substrates), marine sediments naturally contain only acetate concentrations of about 1 μM to 1 mM depending on the location, the season and biogeochemical dynamics [18]. Potential biochemical electrons acceptors available in sediments are in soluble forms such as oxygen (sub-surface) or nitrates or sulfates or in complexes forms as Fe(III) or Mn(II). Anyway, their cumulative concentration never exceeds a hundred of μM. The ratio between organic reducing agents (acetate type electron donors) and microbial electrons acceptors is not far from the unit value in sediments, offering therefore, from a theoretical point of view, more opportunities for selectively directing the formation of biofilm capable of catalyzing a redox reaction over another.

The use of electrodes made of different materials is proposed to promote the specific settlement of bacteria with different/opposite electro-catalytic properties. The carbon based materials are basically used as the anode material in the MFC not supplemented in artificial fuel like benthic MFC. In

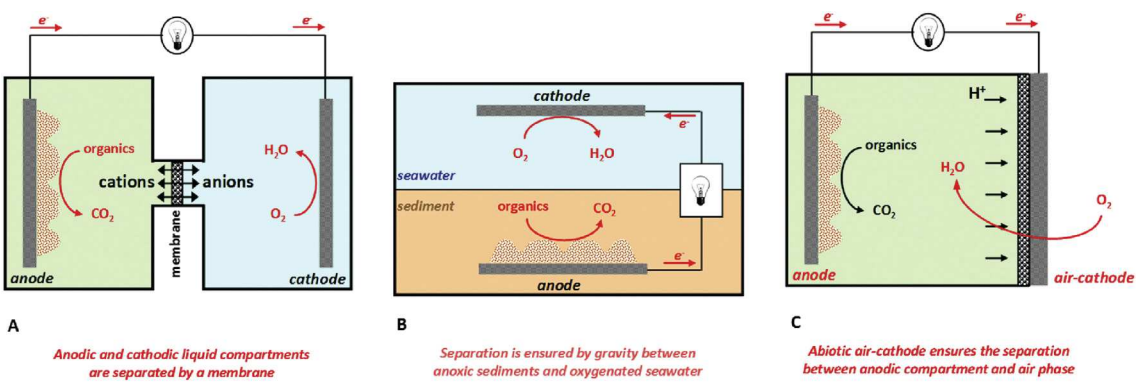


**Fig. 1 – Design of the most widely described MFCs. A: Dual chamber microbial fuel cell, B: Benthic microbial fuel cell, C: Air-cathode microbial fuel cell.**

addition, for unknown reasons, it was already clearly demonstrated in several studies with *Geobacter sulfurreducens* [45] or with marine multispecies biofilm [14], that stainless steel was a favorable material to the formation of microbial cathode capable of reducing respectively fumarate or oxygen.

In this study, we first designed a MFC with two electrodes made of different materials (stainless steel vs. graphite) buried in a common electrolyte consisting of marine sediments. To our knowledge, this was the first example of an MFC able to operate with the two electrodes immersed in a single medium. To go deeper in understanding the bioelectrochemical phenomena involved, several experiments were then conducted in electrochemical bioreactor with electrodes polarized at constant potential to (I) validate the opposite electrochemical behavior of the two electrode materials, (II) determine which of the electrode material, the applied potential or the bacterial composition of biofilms was at the origin of the observed phenomenon, (III) establish strong assumptions on the electrochemical reactions and kinetics occurring at each electrode.

## Material and methods

### *Sediments*

Marine sediments were collected in the port of La Tremblade (France). A uniform mixing containing solid marine sediments (3/4 v/v), seawater (1/4 v/v), acetate (1 mM) was created by 2 h of stirring.

### *Single medium (sediments) MFC*

A graphite electrode (20 $cm^2$) and a 254 SMO stainless steel (25 $cm^2$) electrode were totally buried in 500 mL of hydrated sediments and connected through an electrical resistance of 220 Ω (day 0 to day 13) and then 100 Ω (day 13 to day 21) using titanium wires as current collectors. Current and power values were calculated as a function of time from the cell voltage measured across the resistance. Power curves were recorded by varying the external resistance from 33 kΩ to 1 Ω on days 6, 12 and 21. The cell was equipped with a saturated calomel reference electrode (SCE, Radiometer Analytical, +0.241 V/SHE) to record the anode potential in parallel with the polarisation curves.

### *Electrochemical set-up for constant polarisation and pre-polarised MFC*

Experiments were performed in single compartment bioelectrochemical reactors (500 mL) equipped with a 3-electrode system composed of a graphite (20 $cm^2$) or a 254 SMO stainless steel (25 $cm^2$) working electrode, a saturated calomel reference electrode and an 15 $cm^2$ Pt grid as auxiliary electrode. The working electrode was located far (around 10 cm) from the auxiliary electrode but as close as possible (around 0.5 cm) to the reference electrode [38]. Each reactor contained 500 mL of hydrated sediments. The working electrodes were polarized at −0.1 V vs. SCE (chronoamperometry) using a multi-channel VSP potentiostat (Bio-Logic SA, software EC-Lab) and the current was recorded every 15 min.

For pre-polarised MFCs, electrodes were first individually polarised for 10 days at −0.1 V vs. SCE and then the two electrodes were coupled through a 220 Ω external resistance in a bioreactor containing hydrated sediments.

### *Microbial community analysis*

The biofilms covering the stainless steel and the graphite electrodes were collected in sterile synthetic seawater by scraping vigorously the electrode surfaces with a sterile glass spreader. DNA was extracted from cell suspensions and then used as a template for 16S rRNA gene amplification by PCR using primers P2 and P3 [33]. PCR products were analysed using the denaturing gradient gel electrophoresis (DGGE) fingerprinting technique. Predominant 16S rRNA gene bands were cut off from the DGGE gels and identified by sequencing. The sequences obtained were submitted to the BLAST program of the National Center for Biotechnology Information [1] and to the Sequence Match of the Ribosomal Database Project to identify the closest relatives.

## Results and discussion

### *Single medium MFC in marine sediments*

Our first attempt of MFC in a unique environment was conducted by immersing a graphite electrode and a 254SMO stainless steel electrode in marine sediments supplemented with 1 mM of acetate. The two electrodes were connected through an external resistance of 220 Ω. Quickly, the voltage between the two electrodes began to increase (Fig. 2) in the image of what had been observed by Reimers et al. in 2001 with the first benthic MFC demonstration. A quasi steady current of 0.61 mA (i.e. a current density of 240 $mA/m^2$) was measured for up to 4 days (day 8–day 12). During the increase of the current, two MFC power tests were conducted on day 6

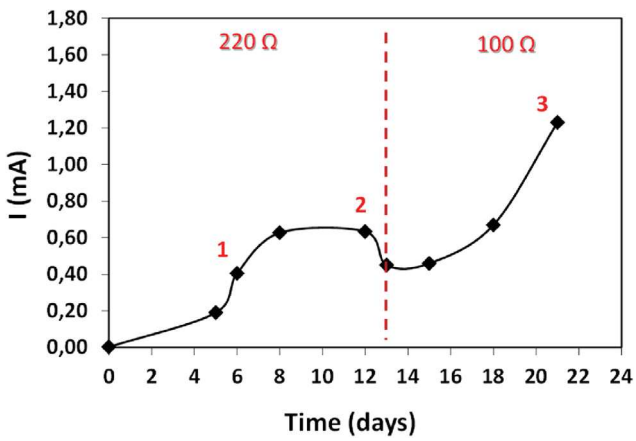


**Fig. 2 – Evolution of the current supplied by a single medium MFC implemented in marine sediments. The anode is graphite and the cathode is stainless steel.**

and day 12 (labeled 1 and 2 in Fig. 2). Between day 6 and day 12, the maximum current output the MFC has almost doubled from 0.58 mA to 1.06 mA (Fig. 3a). The maximum power density increased from 20 mW $m^{-2}$ to 47 mW $m^{-2}$. The current-potential curves (Fig. 3b) showed that the increase in MFC performance was related to the improvement of both the anodic and the cathodic kinetics (labels 1 and 2). The anode overpotential (about 400 mV) was always higher than that of the cathodic branch (barely higher than 100 mV), showing that the output power of the single medium MFC was limited by the bioelectrochemical kinetics of the anode.

To force the MFC to debit more, the electrical resistance between the anode and the cathode was reduced to 100 Ω. Basically, lower external resistance favors slightly higher current densities and enrichment of electrocatalytic biofilms in electroactive bacteria [19,20]. The external resistance change from 220 Ω to 100 Ω led to a sharp increase in the potential of the anode, while the potential of the cathode kept almost stable. Under the 100 Ω external load, the current supplied by the MFC increased again following an exponential allure between day 13 and day 21 (Fig. 2). This new exponential current increase was probably due to the additional growth of microbial electroactive species on the anode in response to the modification of the electrode potential. The polarization curve obtained at day 21 confirmed a visible improvement of anode kinetics (the slope of the current-potential curve multiplied by 2), while the cathode displayed always the same kinetics (Fig. 3b). The improvement of the anode kinetics while the cathode was not affected by the resistance change, is an element supporting the enrichment in electroactive species of the anode microbial community. Overall, the passage from 220 Ω to 100 Ω largely permitted to maximize the performance of the MFC. The maximum current doubled from 1.06 mA to 2.01 mA and the peak power density reached 118 mW $m^{-2}$. This performance was of the same order of magnitude as those already reported by optimised MFCs formed from natural marine environments ($J_{max}$ 150 mA $m^{-2}$, Pmax 100 mW $m^{-2}$) [11,37,46].

This experiment launched the concept of the single medium MFC using marine sediments as the common electrolyte, in which both anode and cathode were immersed. From a fundamental point of view, this realization raised questions to justify the behavior of the stainless steel material as a cathode and the graphite material as an anode:

- What is the nature of the electrode material or the electrode potential the key parameter for the establishment of an anode or a cathode behavior?
- In the common medium, was it the same biofilm with reversible electrocatalytic properties that developed on both electrode materials? or the biofilms were not similar and they were therefore composed of significantly different microbial species?

### *Chronoamperometries with stainless steel and graphite electrodes*

To distinguish between electrode material or electrode potential to tip the balance towards anode or cathode properties, two experiments were conducted with stainless steel and graphite electrodes polarized at the same potential, −0.1 V/SCE. This potential value was chosen because it ranged between the free potential of the cathode and the free potential of the anode of the single medium MFC let at open circuit (Fig. 3b). A lot of studies working with marine sediments have also reported this potential as an appropriate value to select for electroactive microbial species with graphite [12] or stainless steel electrodes [2,14].

A graphite electrode and a stainless steel electrode were buried in the same hydrated sediments constantly agitated with 1 mM acetate in a closed bioreactor. Both electrodes were polarised at −0.1 V/SCE. No significant current was observed during the first day (less than 0.02 A $m^{-2}$). The graphite electrode then showed a growing positive current (current of oxidation), which reached a plateau in the range of +0.50 to 0.65 A $m^{-2}$ from day 7 (Fig. 4a). Such a sigmoid current evolution characterises the gradual formation (Monod kinetics) of an electroactive microbial biofilm on the electrode surface. In parallel, the stainless steel electrode gave a reduction current that reached a plateau around −1.4 A $m^{-2}$ from day 4 (Fig. 4b). The research of microbial presence on the surface of graphite and stainless steel electrodes by fluorescence microscopy imaging [21] clearly revealed the presence of microbial biofilms on the two electrode materials. Just like what was observed in the single medium MFC in which electrodes worked at varying potential values, the electrodes polarised at the same potential in the same medium exhibited an anode and a cathode behaviour related to the development of

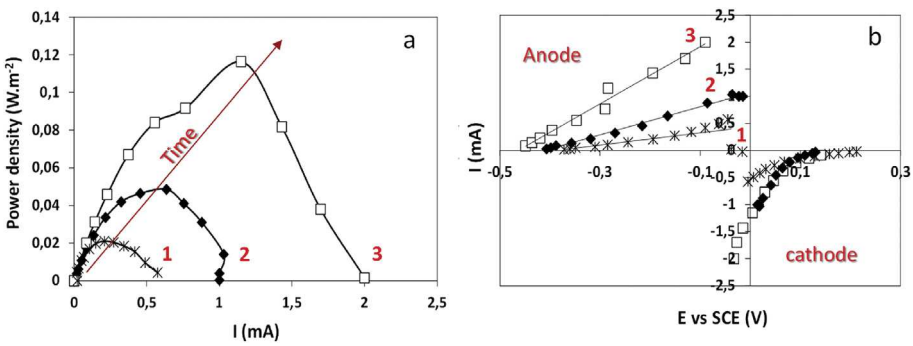


**Fig. 3 – Single medium MFC performance at different stages of electrocatalytic biofilms development. Power density/Current (a) and Current/Potential (b) curves measured at different times indicated on Fig. 2.**

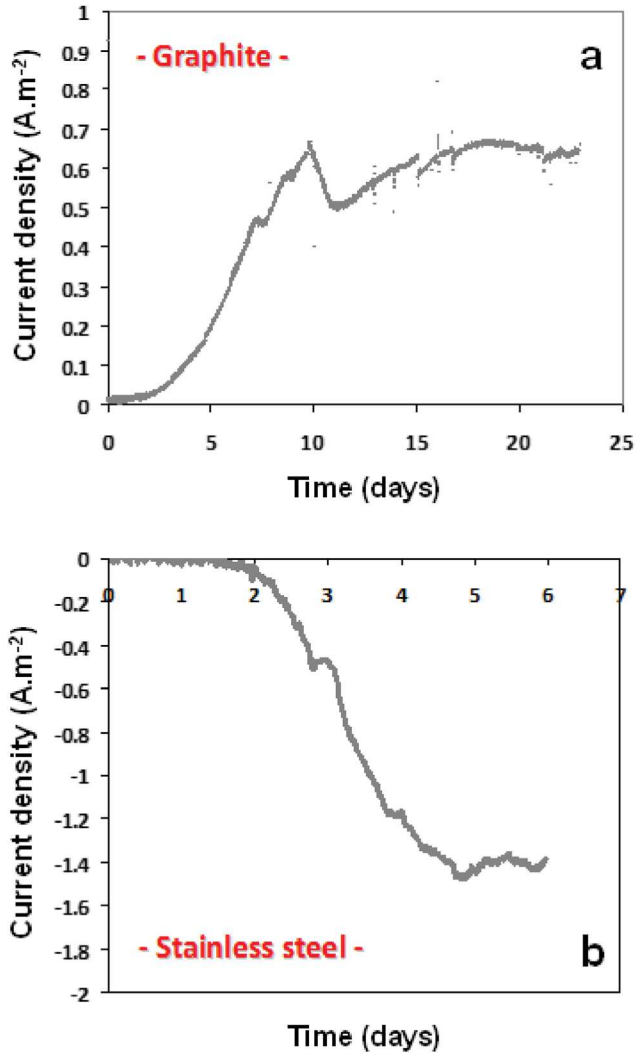


**Fig. 4 – Evolution of the current density on graphite (a) and stainless steel (b) electrodes polarised at −0.1 V/SCE in marine sediments.**

microbial biofilms on electrodes surface. In conclusion, the electrode material and not the electrode potential, was consequently at the origin of the anode or cathode formation.

Actually, the most curious was the possible catalysis of a cathodic reaction on stainless steel electrode in sediments. Usually at this electrode potential (−0.1 V/SCE), and especially in the presence of higher concentrations of acetate (10–20 mM) [12,13], anodic biofilms spontaneously develop on stainless steel. Here, the cathodic behaviour at −0.1 V/SCE was replicated 2 times with a stainless steel electrode, giving between −0.9 and −1.5 A $m^{-2}$ of cathodic current (Fig. 5).

The few examples of cathodic biofilm developed on stainless steel in this potential range were obtained without acetate for the catalysis of the oxygen reduction [2,14]. An attempt to enhance the oxygen supply by bubbling air into the solution did not success in increasing the overall electrocatalytic activity of the stainless steel biocathode (Fig. 5). In contrast, a loss of 85% of the current density was observed after aeration of the bioreactor for 10 min, enough to exclude oxygen as a major electron acceptor of the stainless steel

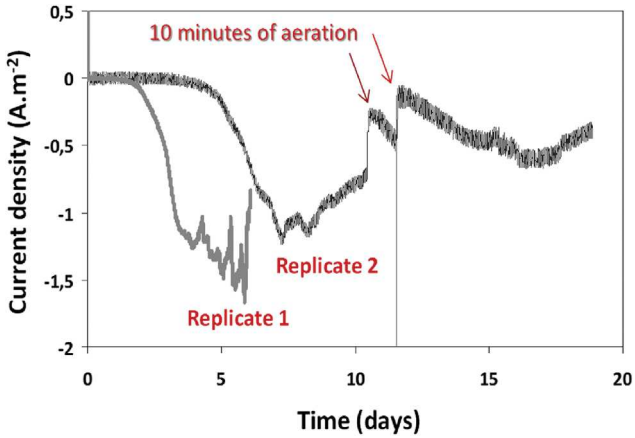


**Fig. 5 – Replication of the experiments performed with stainless steel electrodes embedded in the sediments. Evolution of the current density on stainless steel electrodes polarised at −0.1 V/SCE in marine sediments and effect of periodic sediment aerations for 10 min.**

biocathode. After the aeration period, the return to the steady state performance of the biocathode was gradual and required several days. This inhibitory or even irreversible effect of the aeration on the biocathode performance showed that the microbial communities involved in the reduction activity were probably mainly dominated by anaerobic species.

### *Microbial community within the electroactive biofilms*

The microbial diversity of biofilms formed on the stainless steel biocathode and on the graphite bioanode was assessed by denaturing gradient gel electrophoresis (DGGE) analysis

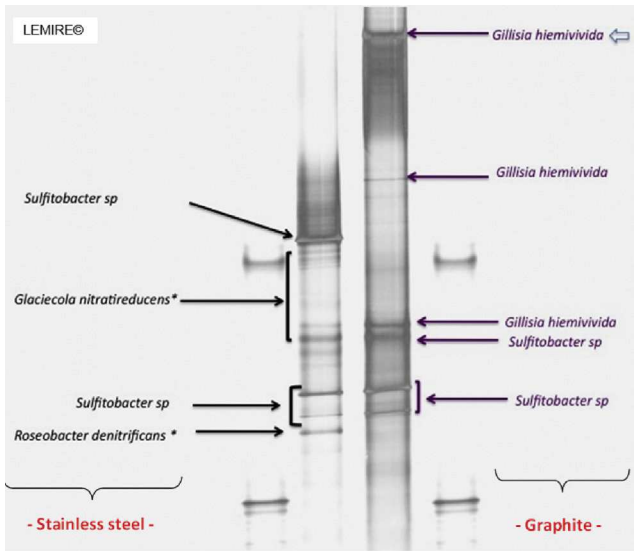


**Fig. 6 – Analysis of the bacterial composition of electroactive biofilms collected on stainless steel and graphite electrodes by denaturing gradient gel electrophoresis (DGGE). Asterisks indicate bacteria that are exclusive to the stainless steel cathode and the white arrow indicates bacteria that are exclusive to the graphite anode.**

(Fig. 6). Bacterial species related to *α-Proteobacteria* and *δ-Proteobacteria* were predominant in the biofilm collected from the stainless steel electrode, while *α-Proteobacteria* and *Bacteroidetes* were mainly present inside the biofilm from the graphite electrode. These groups of bacteria have been commonly observed in electroactive biofilms from sediments [13,31]. *Sulfitobacter* sp. were found in both anodic and cathodic biofilms. *Sulfitobacter* sp. (*α-Proteobacteria*) were described as heterotrophic bacteria abundant in coastal and open ocean environments especially when a constant source of inorganic sulphur is present. Concerning the presence of Sulfitobacter sp. in sediments microbial fuel cells, the genus *Sulfitobacter* has already been identified once in aerobic cathodic biofilms formed on stainless steel [40]. Some species of the genus *Sulfitobacter* have privileged interactions with algae and could therefore be serious candidates in association with photosynthetic algae to synergistically produce electricity using light microbial solar/fuel cells [17,30]. On the anode, *Sulfitobacter* sp. are suspected to oxidize organic compounds, sulfite and thiosulfate, while on the cathode, the only possible electron acceptor is oxygen since *Sulfitobacter* sp. has no specific mechanism of anaerobic respiration. In contrast, *Gillisia hiemivivida* and *Glaciecola nitratireducens,* which were detected for the first time as predominant bacteria in electroactive biofilms, were only present on graphite and stainless steel, respectively. Species of genus *Gillisia* (*Bacteroidetes*) have already been detected in several aquatic habitats (seawater sample, microbial mat, sponge, etc) [34]. Classically, *Gillisia hiemivivida* is capable of oxidizing a broad range of organic compounds using oxygen or nitrates as final electron acceptor (both aerobic and anaerobic respiration).

The genus *Glaciecola* like many other genera of *δ-proteobacteria* (*Alteromonas*, *Pseudoalteromonas*, *Idiomarina*, and *Colwellia*) is widely present in global oceans. *Glaciecola nitratireducens,* isolated from a surface seawater sample [42] has the particularity to reduce nitrate. In the same way, the marine *Roseobacter denitrificans* strain grows not only photoheterotrophically in the presence of oxygen and light but also anaerobically in the dark using nitrate as an electron acceptor [5]. So far, electroactive species of the genus *Roseobacter* have only been highlighted in aerobic cathode catalyzing the reduction of oxygen [9,14,40].

It should be noted that the analysis of the bacterial community in the crude sediment did not reveal the predominance of microbial genera that were enriched on the electrodes. Even stronger, species of the genus *Glaciecola* or *Gillisia* have not been detected in the crude sediment.

### *Electrodes polarization before starting a single medium MFC*

To start a MFC, the choice of the value of the external resistance is crucial. Opposite theories have been developed on this subject. For some authors, the formation of an electroactive biofilm must be initiated with a great resistance, which is then gradually reduced to acclimate the bacterial populations to exchange electrons with the electrode. For others, an MFC must start with low external resistance to quickly select the best performing electroactive species that will form the basis of the biofilm. One way to bypass this difficult choice is to pre-form electroactive biofilms on electrodes under controlled potential before using them as the anode or cathode in MFC [8,38]. Most of the time, the pre-formation of the electroactive biofilm can significantly shorten the overall startup time of the MFC [49]. In this objective, a graphite anode and a stainless steel cathode buried in hydrated sediments were prepared separately by 4 days' polarisation at −0.1 V/SCE. After individual polarizations, both electrodes were then placed in the same reactor and connected through a 220 Ω electrical resistance. The MFC directly provided about 280 mA $m^{-2}$ and was run for 2 weeks. The polarisation curve recorded after 2 days of electrodes connection showed a maximum power density (Pmax) of 70 mW $m^{-2}$ and a maximum current density ($J_{max}$) of 500 mA $m^{-2}$ when short-circuited (Fig. 7a). These performances were of the same order of magnitude as those registered after 12 days with the single medium MFC in the absence of pre-polarisation (Fig. 3 a, curve 2). The start-up time of the MFC is cut in half when microbial electrodes are first prepared individually under constant polarisation.

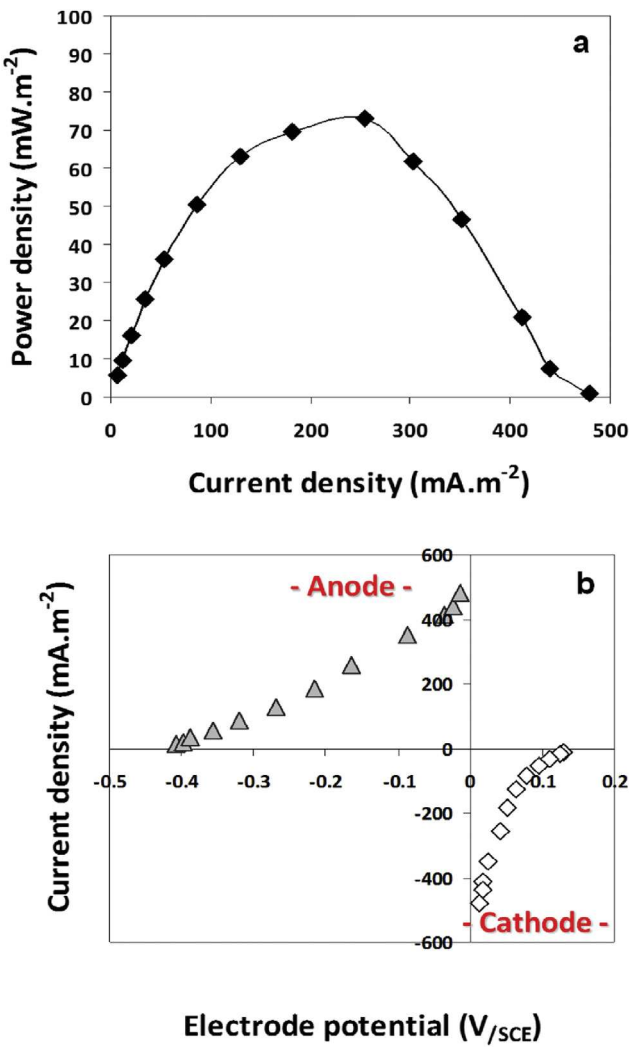


**Fig. 7 – Single medium microbial fuel cell performance after pre-polarisation of electrodes. Power density/Current density (a) and Current density/Potential (b) curves generated by a laboratory-scale sediments microbial fuel cell.**

*Possible electrodes reactions and kinetics*

As shown in Figs. 3b and 7b, the graphite microbial anode exhibited excellent kinetic characteristics, with a low open circuit potential around −0.16 V/SHE (−0.40 V/SCE), i.e. close to the equilibrium potential of acetate oxidation:

$$CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 8e^- + 7H^+$$

$$E^{0'}_{CO2\ /acetate} = -0.24\ \text{V/SHE at pH 7.0} \quad (1)$$

as already observed for MFCs fed with acetate [7,49]. The bioanode also revealed a high exchange current density ($J_0$) of about 60 mA $m^{-2}$ for comparison, $J_0$ related to hydrogen oxidation in $H_2SO_4$ 1 N on nickel electrodes is 70 mA $m^{-2}$ [50]. The stainless steel microbial cathode also showed good characteristics with $J_0$ of 20 mA $m^{-2}$. The reduction, which began at +0.38 V/SHE (+0.14 V/SCE), may correspond to the reduction of nitrate into nitrite catalysed by the nitrate reducing bacterial communities present in the biofilm:

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$$

$$E^{0'}_{NO3/NO2} = +0.43\ \text{V/SHE at pH 7.0} \quad (2)$$

*Glaciecola nitratireducens* and *Roseobacter denitrificans* are typically denitrifying strains and a majority of species from the genus *Sulfitobacter* can also respire nitrate. The attempts to determine nitrate and nitrite ion concentrations did not bring useful information Indeed, methods for the determination of the nitrate/nitrite couple are relatively robust in the clear waters but becomes more complicated in complex environments such as sediments. Too much interferences perturb the measurements (adsorption phenomena on the solid particles, the presence of ammonium, etc). Nevertheless, definitively, the possible oxygen reduction cannot be envisaged, because (i) the dissolved oxygen was rapidly consumed in the closed bioreactor by the aerobic bacteria contained in the upper layers of sediments and (ii) the aeration of the electrolyte had a negative influence on cathodic performance (Fig. 5).

It was proved here that the nature of electrode material gives a new key for developing microbial anode and cathode selective enough to oxidise a fuel and to reduce an oxidiser contained in the same medium. A new generation of MFCs that do no longer require two distinct phases to be separated can now be launched (Fig. 8).

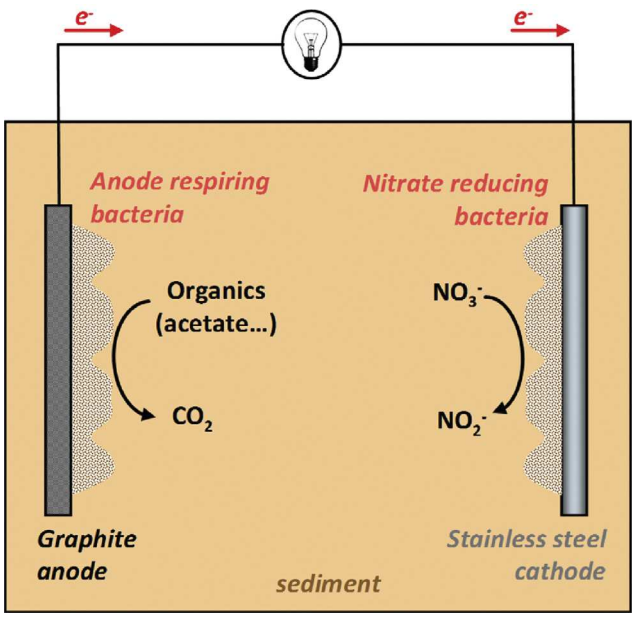


**Fig. 8 – Single medium (sediment) microbial fuel cell mechanism. Conceptual illustration of the biofilm-driven electrochemical reactions occurring on graphite anode and stainless steel cathode. On the anode, electrons extracted from acetate microbial oxidation are transmitted through the external circuit to the cathode, on which nitrate is used as electron acceptor by electroactive nitrate-reducing bacteria.**

## Conclusions

For the first time, a microbial cathode that operated in sediments was designed. The formation of a cathodic electroactive biofilm was closely related to the nature of the electrode material, stainless steel, since the use of graphite under identical operating conditions resulted in the formation of a more common acetate-reducing microbial anode. Based on this discovery, the new concept of "single medium MFC", which involves a graphite anode and a stainless steel cathode immersed in hydrated sediment, was validated. This first "single medium MFC" debited stable current density of 280 mA $m^{-2}$ over two weeks without any substantial limitation of the phenomenon.


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