



**HAL**  
open science

## Development of bioanodes rich in exoelectrogenic bacteria using iron-rich palaeomarine sediment inoculum

Fatima-Zahra Ait-Itto, James Behan, Mathieu Martinez, Frédéric Barrière

### ► To cite this version:

Fatima-Zahra Ait-Itto, James Behan, Mathieu Martinez, Frédéric Barrière. Development of bioanodes rich in exoelectrogenic bacteria using iron-rich palaeomarine sediment inoculum. *Bioelectrochemistry*, 2024, 156, pp.108618. 10.1016/j.bioelechem.2023.108618 . insu-04328268

**HAL Id: insu-04328268**

**<https://insu.hal.science/insu-04328268v1>**

Submitted on 12 Dec 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Development of bioanodes rich in exoelectrogenic bacteria using iron-  
2 rich palaeomarine sediment inoculum

3 Fatima-Zahra Ait-Itto <sup>a,b</sup>, James A. Behan <sup>a</sup>, Mathieu Martinez <sup>b</sup>, Frédéric Barrière <sup>a\*</sup>

4 <sup>a</sup> *Université de Rennes, CNRS, Institut des Sciences Chimiques de Rennes, UMR 6226, Rennes,*  
5 *France*

6 <sup>b</sup> *Université de Rennes, CNRS, Géosciences de Rennes – UMR 6118, Rennes, France*

7 \*Corresponding author: frederic.barriere@univ-rennes.fr

8

9

10 **Abstract**

11 Microbial Fuel Cells (MFC) convert energy stored in chemicals into electrical energy thanks to  
12 exoelectrogenic microorganisms who also play a crucial role in geochemical cycles in their  
13 natural environment, including that of iron. In this study, we investigated paleomarine  
14 sediments as inoculum for bioanode development in MFCs. These sediments were formed  
15 under anoxic conditions ca. 113 million years ago and are rich in clay minerals, organic matter,  
16 and iron. The marlstone inoculum was incubated in the anolyte of an MFC using acetate as  
17 the added electron donor and ferricyanide as the electron acceptor in the catholyte. After  
18 seven weeks of incubation, the current density increased to 0.15 mA.cm<sup>-2</sup> and a stable +700  
19 mV open circuit potential was reached. Community analysis revealed the presence of two  
20 exoelectrogenic bacterial genera, *Geovibrio* and *Geobacter*. Development of electroactive  
21 biofilms was correlated to bulk chemical transformations of the sediment inoculum with an  
22 increase in the Fe(II) to Fe<sub>total</sub> ratio. Comparisons to sediments sterilized prior to inoculation  
23 confirmed that bioanode development derives from the native microbiota of these  
24 paleomarine sediments. This study illustrates the feasibility of developing exoelectrogenic  
25 biofilms from iron-rich marlstone and has implications for the role of such bacteria in broader  
26 paleoenvironmental phenomena.

27

28 **Keywords:**

29 Exoelectrogenic bacteria, biofilms, bio-anode, paleomarine sediments, clay minerals

## 30 **Introduction**

31 The process of Fe (III) reduction by exoelectrogenic bacteria is crucial in the iron geochemistry  
32 cycle and plays a vital role in the degradation of natural and contaminant/waste organic  
33 compounds [1–3]. Exoelectrogenic bacteria have potential applications in the fields of  
34 bioremediation and biotechnology, as they can oxidize organic contaminants such as aromatic  
35 hydrocarbons anaerobically in anoxic petroleum-contaminated aquifers and simultaneously  
36 reduce Fe (III) [4]. For example, one study suggests that exoelectrogenic bacteria also play a  
37 fundamental role in Precambrian banded iron formations, as evidenced by the strong  
38 correlation between isotopically-light carbonates and iron minerals accumulations that  
39 occurred through a combination of organic matter mineralization and Fe (III) reduction [5].  
40 Thus, much research has been conducted to better understand the interaction between  
41 microorganisms and sediments in different environments, as this relationship plays a role in  
42 biogeochemical cycles where bacteria can alter the physical and chemical state of sediments  
43 [6]. Marine sediments are well-known as a source of abundant electrochemically active  
44 bacteria [7] and have been used as inoculum in sediment Microbial Fuel Cells (MFCs) for  
45 simultaneous energy production and bioremediation [8]. It is essential to highlight that the  
46 amount of energy obtained in the MFCs devices depends on several factors, including the  
47 quantity of organic matter and type of exoelectrogenic microorganisms present in the  
48 sediments [9,10]. Moreover, it can be argued more broadly that climatological factors and  
49 geological conditions play a role in MFC development, by influencing the microbiota  
50 composition of natural inoculum. In this context, the exploration of paleomarine sediments  
51 from a geological perspective offers a promising avenue for pinpointing actual and past  
52 environments conducive to the development of microorganisms, and selecting appropriate  
53 sediments for inoculation of MFCs [11].

54 In this study, we focus on the development of electroactive biofilms using paleomarine  
55 sediment inoculum formed in hemipelagic environment, characterized by a large deposition  
56 of organic-rich layers with accumulation of weathering products [12]. These ancient geological  
57 deposits are rich in clay minerals, including smectite, illite, kaolinite and chlorite, but also  
58 showcase high iron contents (primarily Fe(III)) as well as organic matter. These conditions may  
59 provide a favorable environment for the development of electroactive bacteria. Herein we  
60 demonstrate the development of acetate-metabolizing bioanodes in a microbial fuel cell  
61 system using these iron-rich paleomarine sediments as the inoculum. Physico-chemical

62 characterization of the sediments in the anolyte after continuous operation shows a distinct  
63 increase in the proportion of Fe(II) oxides to the total iron oxide content (Fe(II)/Fe<sub>total</sub>)  
64 consistent with the presence of exo-electrogens utilizing abundant Fe(III) in the sediments as  
65 the sole electron acceptor under anaerobic conditions. Using 16S rRNA amplicon sequencing  
66 we correlate these changes in sediment composition to the development of mixed biofilms  
67 containing *Pelobacter* genus and some well-known exo-electrogens species from the genera  
68 *Geovibrio* and *Geobacter*.

## 69 **2. Materials and methods**

### 70 **2.1 Sample collection**

71 The Paleomarine Sedimentary rock (PS) used in this study are marlstone (mixture between  
72 clay and calcite), with organic matter content around 1.3 %. The samples were collected from  
73 Col de Pré-Guittard section of the Vocontian basin in France, located approximately at 11 km  
74 north-northwest of Rémuzat, Drôme (Supporting Information Figure S1). The clay mineral  
75 assemblages of the Col de Pré-Guittard section consist mainly of smectite (averaging 63%),  
76 illite (averaging 19%), and kaolinite (averaging 11%), along with approximately 5% chlorite  
77 [13]. The selected marlstone sample was crushed into powder using an agate mortar to  
78 achieve an average particle size < 125 µm. X-ray diffractometry (XRD) was employed to  
79 identify the mineral phases present in the sediment, revealing the presence of quartz, calcite,  
80 gypsum, and clay minerals (Supporting Information Figure S2). The Scanning electron  
81 microscopy (SEM) image of the PS revealed a platy-shaped structure of clay mineral particles.  
82 The elemental composition of PS recorded the presence of high levels of alumino-silicate  
83 (approximately 20% of Si and 8% of Al) arising from the clay, as well as a significant quantity  
84 of Fe element, approximately 5.8% (Supporting Information Figure S2).

### 85 **2.2 Chemical reagents**

86 Potassium acetate (> 99%, anhydrous), glutaraldehyde (25% solution), potassium  
87 hexacyanoferrate III (K<sub>3</sub>Fe(CN)<sub>6</sub>), potassium phosphate dibasic (K<sub>2</sub>HPO<sub>4</sub>), and potassium  
88 phosphate monobasic (KH<sub>2</sub>PO<sub>4</sub>) were purchased from Sigma Aldrich and used without further  
89 purification.

### 90 **2.3 Bio-anode Setup & electrochemical measurements**

91 The marlstone samples were incubated under anoxic conditions that are favorable for the  
92 development of exoelectrogenic bacteria as previously reported [14] [15]. Briefly, an H-type  
93 reactor was used with 250 mL compartments separated by a Cation Exchange Membrane

94 (Fumasep® FKM). Graphite rods (Morgan AM&T) with a diameter of 0.5 cm and height of 20  
95 cm were used as electrodes in both compartments. For the anodic compartment, the  
96 electrode was cleaned using pure water in ultrasound for 10 min, then covered by parafilm to  
97 fix the surface at 4.9 cm<sup>2</sup>. In the anodic compartment, 8.05 g of marlstone samples was mixed  
98 with 250 mL of phosphate buffer (10 mM) prepared using ultrapure (18.2 MΩ.cm) water. The  
99 chosen concentration is consistent with the previous experiments using low ionic strength  
100 electrolytes which successfully developed bioanodes from mixed inoculum[16] The pH of the  
101 anodic compartment was measured and adjusted to pH 7 using 2M NaOH. Potassium acetate  
102 (10 mM) was regularly added as an electron donor from filtered anoxic stock solutions.  
103 The cathodic compartment was filled with 250 mL of phosphate buffer (10 mM) containing  
104 potassium hexacyanoferrate (50 mM) as an electron acceptor. The cathode showed a stable  
105 potential (*ca.* 0.2 V vs Ag/AgCl 3\_M\_KCl) which remained constant throughout the experiment.  
106 The two compartments were connected by an external resistor 1-4 kΩ to match the internal  
107 resistance of the MFC as determined via impedance spectroscopy measurements using a Multi  
108 Autolab / M204 potentiostat [15]. These pilots are designated as PS/MFC (PS/MFC 1 and  
109 PS/MFC 2).

110 A control cell (PS/Control) was also established and incubated under identical conditions  
111 without connection to an external cathode designated as (PS/Ctrl 1 and PS/Ctrl 2). To maintain  
112 the MFC under anaerobic conditions, the anodic compartment was purged with argon gas for  
113 30 minutes once every 5 days on average. MFCs were incubated in the dark at 30 °C in a  
114 thermostatic bath (Supporting Information Figure S3). The open circuit potential (OCP)  
115 between the electrodes was measured periodically using a digital multimeter or potentiostat.  
116 MFCs were left at open circuit for at least 30 minutes prior to taking the measurement. To  
117 monitor the development of exo-electrogenic bacteria from the marlstone samples, the bio-  
118 anode was regularly studied using cyclic voltammetry in the range -0.8 to +0.4 V vs Ag/AgCl 3  
119 M KCl at a scan rate of 2 mV/s unless otherwise specified. Electrochemical measurements  
120 were performed using a three-electrode set-up where the bioanode served as the working  
121 electrode, Ag/AgCl (3 M KCl) as the reference electrode placed in the anolyte, and the cathode  
122 (graphite rod) was used as a counter electrode. All experiments were conducted in duplicate  
123 under an inert atmosphere using Ar saturation. Samples of solution or sediments from the  
124 MFCs were taken under argon flux with sterile syringes to minimize disturbance to the  
125 bioanodes and preserve anaerobic conditions. Additional control experiments were

126 performed using sterilized inoculum: the paleomarine sediments were dispersed in a small  
127 volume of ultrapure water and autoclaved (Autoclave Steril Elite™, Fisher Scientific brand) for  
128 1 hour at 121°C and then incubated under identical conditions to the PS/MFC.

129

#### 130 **2.4. X-Ray Photoelectron Spectroscopy (XPS)**

131 In all cases, powder samples for XPS analysis were prepared by pressing the powder (0.1 g) on  
132 carbon tape mounted on the conductive sample holder. XPS data were obtained using a  
133 NEXSA G2 (ThermoFischer Scientific) spectrometer using an Al K $\alpha$  X-ray source working at  
134 1486.6 eV with a spot size of 200  $\mu\text{m}^2$ . Survey spectra (0-1000 eV) were acquired with an  
135 analyzer pass energy of 200 eV (1 eV/step); high-resolution spectra used a pass energy of 50  
136 eV (0.1 eV/step). Charge compensation using a flood gun was applied to all powder  
137 preparations and the binding energies corrected to the position of the C 1s peak assigned to  
138 adventitious carbon at 284.8 eV. Spectra were peak-fitted using commercial software  
139 (CasaXPS Version 2.3.18). Deconvolutions were performed using Shirley background  
140 subtraction in all cases. Peak areas used in quantitative analysis were normalized by relative  
141 sensitivity factors for atomic percentage determination (C 1s = 1, O 1s = 2.93, Si 2p = 0.817, Ca  
142 2p = 5.07, Fe 2p = 16.42, K 2p = 3.97, Mg 2s = 0.575, P 2p = 0.817, Na 1s = 8.52, Al 2p = 0.537).

#### 143 **2.5. X-Ray Diffraction (XRD)**

144 To determine the mineral phases, present in the marlstone samples, X-Ray Diffraction (XRD)  
145 was conducted. Dry powder samples were analyzed using a Bruker D8 Endeavour  
146 diffractometer equipped with CuK $\alpha$  radiations, a LynxEye\_XE\_T detector, and a Ni filter. The  
147 instrument was set at a voltage of 40 kV and an intensity of 25 mA. The goniometer scanning  
148 ranged from 2° to 65° for each analysis. The identification of mineral phases was accomplished  
149 by analyzing their primary diffraction peak (d001).

#### 150 **2.6. Scanning Electron Microscopy imaging (SEM) and Inductively Coupled Plasma Mass 151 Spectroscopy (ICP-MS)**

152 SEM samples were prepared following the protocol of reference [17]. First, the samples are  
153 fixed overnight with 2.5% glutaraldehyde in buffer phosphate solution (0.1 M, pH 7) at room  
154 temperature. Then, samples were washed using a phosphate buffer solution (pH 7) and  
155 immersed respectively in an aqueous solution containing increasing ethanol concentrations  
156 from 50%-100% as previously reported [15]. Finally, the samples were critical-point dried to

157 replace the remaining water and ethanol solvent with CO<sub>2</sub> and Au-coated. The analysis was  
158 performed using a JEOL JSM 7100 F EDS EBSD Oxford Scanning Electron Microscope.  
159 To estimate the overall concentration of oxides and total iron content, we used an ICP-MS  
160 (Inductively Coupled Plasma-Mass Spectroscopy) iCapQ ThermoFisher performed by the  
161 National Service of Rocks and Minerals Analysis (SARM: [http://helium.cprg.cnrs-  
162 nancy.fr/SARM/](http://helium.cprg.cnrs-nancy.fr/SARM/)) in Nancy. Prior to the analysis, it is necessary to prepare the samples through  
163 alkaline fusions using lithium metaborate (LiBO<sub>2</sub>). Fe(II) content was established using  
164 potentiometric titration for calculations of the ratio Fe(II)/Fe<sub>total</sub>.  
165

## 166 **2.7. DNA extraction from the biofilm**

167 After the incubation and development of the biofilm, DNA extraction was performed on all  
168 samples using the DNeasy PowerSoil DNA extraction kit (Qiagen®) according to the  
169 manufacturer's protocol. Prior to DNA extraction the biofilm developed on the surface of the  
170 electrode was gently scraped using a spatula sterilized with absolute ethanol. To investigate  
171 the distribution of bacterial communities throughout the entire anodic compartment, DNA  
172 was also extracted from the solution-sediment interface. The concentration of DNA was  
173 verified using a Nanodrop® and all samples reported had sufficient DNA to proceed with  
174 sequencing. The 16S rRNA gene sequencing was done by the GeT-Biopuces laboratory at INSA  
175 Toulouse. The variable region V4-V5 of the 16S rRNA was targeted using the primers 515F (5'-  
176 GTGCCAGCMGCCGCGGTAA-3') and 928R (5'-CCCCGYCAATTCMTTTRAGT-3'). The amplified  
177 products were then sequenced using an Illumina MiSeq instrument, and the results were  
178 obtained in the FastQ format [15].

## 179 **3. Results**

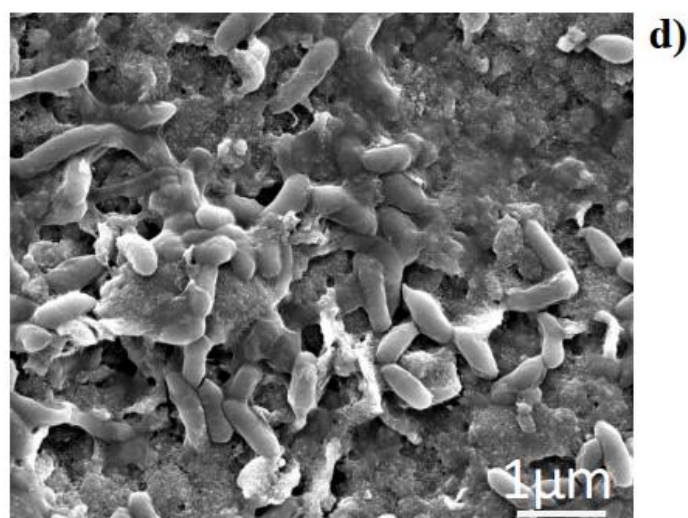
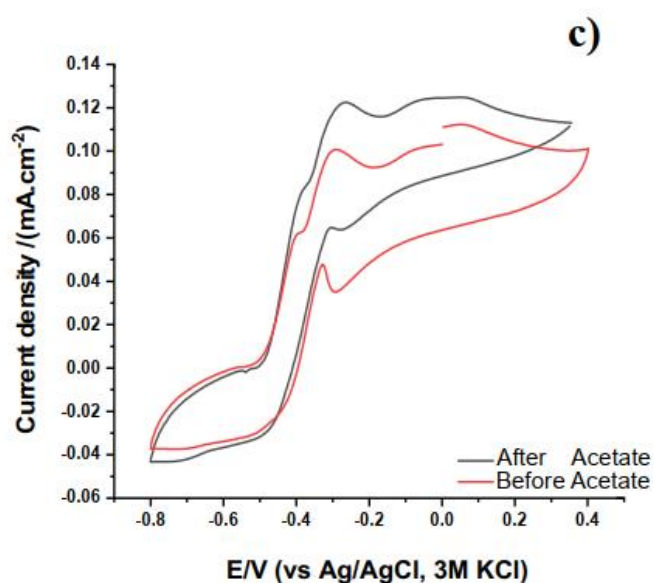
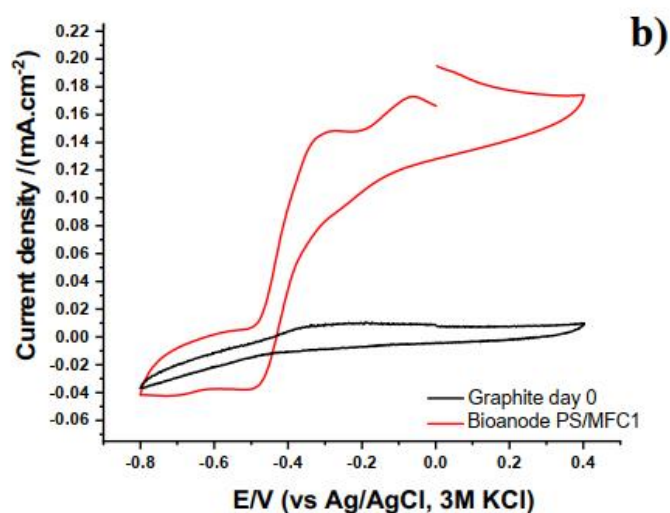
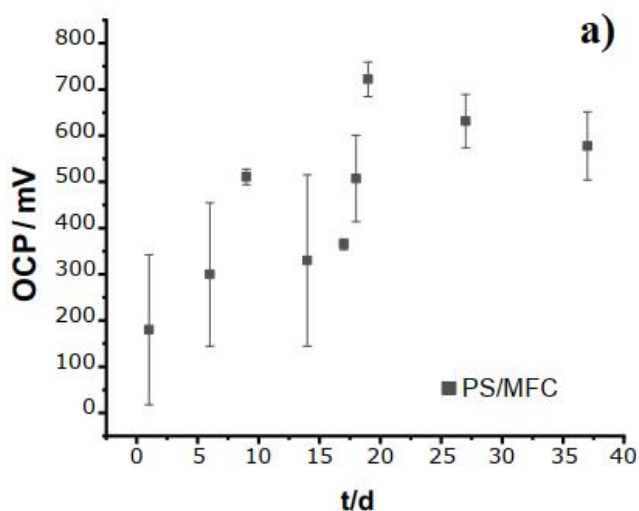
### 180 **3.1. Characterization of the bioanode developed at the surface of the electrode**

181 During the initial days of incubation, the Open Circuit Potential (OCP) was 200 mV (Figure 1a)  
182 versus the cathode which held a constant potential of 0.2 V vs Ag/AgCl (3 M KCl) throughout  
183 the experiment, maintained using 50mM of potassium hexacyanoferrate (III) as an electrolyte  
184 in the cathodic compartment [18]. Cyclic voltammograms showed a typical graphite  
185 background with only capacitive current (Figure 1b, black curve). After 14 days of incubation,  
186 the OCP of PS/MFC pilots significantly increased up to a maximum of *ca.* 700 mV and remained  
187 stable upon periodic measurement thereafter. This OCP increase is attributed to the  
188 development of an electroactive biofilm capable of acetate oxidation at the surface of the  
189 graphite rod. This is confirmed in cyclic voltammetry studies of the mature bioanodes (Figure  
190 1b, red curve) developed in PS/MFC1 which shows a sigmoidal oxidation curve with onset  
191 between -0.6 and -0.5 V vs Ag/AgCl 3 M KCl attributed to catalytic acetate oxidation. Additional  
192 redox peaks centered between -0.42 to -0.30 V are attributed to outer membrane  
193 cytochromes present in the electroactive biofilm [19], [15]. A further oxidation peak was  
194 observed between -0.2 V and -0.18 V vs Ag/AgCl 3 M KCl consistent with the presence of iron  
195 oxides surrounding the biofilm as previously reported [15].

196

197





198

199 **Figure 1.** a) Open circuit potential (OCP) of both PS/MFC Pilots (MFC1 and MFC2) obtained after 40  
 200 incubation days. b) Cyclic voltammogram recorded from the PS/MFC1 pilot from the first day of  
 201 incubation (black curve) and again after 90 days of incubation (red curve). c) Cyclic voltammograms of  
 202 bioanode PS/MFC1 before and after the addition of 10 mM acetate. d) SEM image of a mature mixed  
 203 bioanode developed from PS/MFC1.

204 At this point, such biofilms yielded reproducible voltammograms with the sigmoidal catalytic  
205 profile, in both pilots (PS/MFC1 and PS/MFC2; see supporting information Figure S4b)  
206 compared to the sterilized pilots that show no catalytic profile (see supporting information  
207 Figures S5, S6b) with an OCP around 400 mV compared to the PS/MFC that recorded 700 mV  
208 at the same period of incubation (see supporting information Figure S6a). The specificity of  
209 the acetate response was confirmed by an enhancement in the catalytic current upon re-  
210 addition of 10 mM acetate after 24h of constant turnover in PS/MFC1 (Figure 1c). The acetate  
211 dependence was also replicated on other independent pilots (see supporting information  
212 Figure S4a). These results suggest that the exoelectrogenic bacteria developed at the  
213 bioanode in PS/MFC pilots provided from the initial paleomarine inoculum. Moreover, a  
214 complete loss of catalytic activity was observed after the removal of the biofilm for DNA  
215 extraction (Supporting Information Figure S7), thus confirming that the catalytic response  
216 observed could be attributed to a biofilm developed on the electrode surface (see supporting  
217 information Figure S8). SEM imaging of the bioanode after glutaraldehyde fixation confirmed  
218 the presence of a mixed bacterial biofilm at the electrode surface. A representative image is  
219 shown in Figure 1d and indicates that bacteria grow across the graphite surface (Supporting  
220 Information Figure S9a). The control electrode which was not electrically coupled to a cathode  
221 through a resistor also shows significant biomass but not a continuous biofilm as same form  
222 as anodes operated as MFCs (Supporting Information Figure S9b-d and S10). This result is  
223 consistent with community analysis from 16S as discussed below.

224

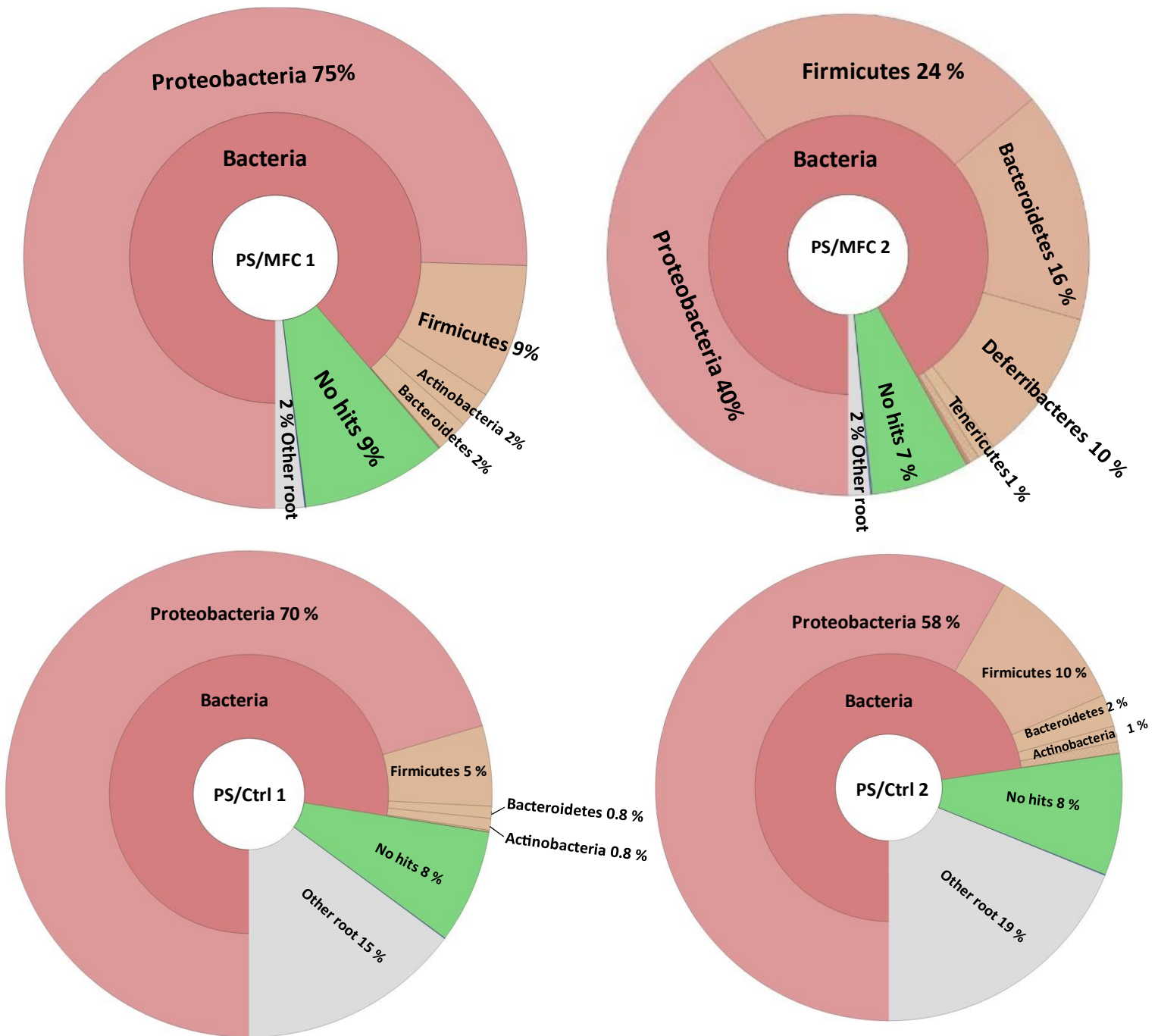
### 225 3.2. Extraction of the biofilm and community determination

226 Based on electrochemical experiments and SEM images, it is evident that a mixed community  
227 electroactive biofilm has developed on the surface of the graphite electrode. To enhance  
228 electron transfer efficiency and gain a better understanding of the electron transfer  
229 mechanism, it is crucial to identify and study the physiology of anodic microbial communities  
230 developed on the electrode surface, as this serves as a fundamental aspect in optimizing  
231 electron transfer processes and improving overall system performance [7].

232 To determine the nature of the biofilm colonizing the bioanode, 16S amplicon sequencing  
233 analysis was conducted on the PS/MFC pilots, SPS/MFC pilots, controls, and PS day 0. The  
234 sequencing analysis for all the pilots revealed the abundance of three phyla Proteobacteria,  
235 Firmicutes, and Bacteroidetes (Figure 2). These findings align with the initial composition of  
236 the marlstone samples before incubation (see Supporting Information Figure S11). Notably,  
237 we recorded the growth of *Deferribacteres* phylum at the PS/MFC (PS/MFC 2) with a relative  
238 abundance of around 10 %. This phylum was initially present in the marlstone samples prior  
239 to incubation with a low abundance of less than 0.1% and developed under the incubation  
240 conditions to reach 10%. The growth of this phylum suggests the development of  
241 exoelectrogenic bacteria at the anodic compartment such as those of the *Geovibrio* genus,  
242 capable of reducing metal ions. This genus has been found to have industrial applications,  
243 including the conversion of organic compounds to biofuels [20].

244 These results are not surprising, knowing that the Proteobacteria are the most abundant  
245 phylum in soil [21], which includes important exoelectrogenic bacteria such as those of the  
246 *Geobacter* genus, able to oxidize organic compounds completely to carbon dioxide with an  
247 anode serving as the sole electron acceptor [22] [23]. Likewise, in the Firmicutes phylum, some  
248 isolated members can transfer electrons to solid phases as electron acceptors [24], including  
249 iron and manganese, as part of a respiratory mechanism [25]. Bacteroidetes phylum has been  
250 associated with the anodic biofilm and reported as a fermentative phylum able to hydrolyze  
251 complex organics molecules like cellulose[26].

252



254 **Figure 2:** Relative abundance of bacterial communities (at the Phylum level) colonizing the electrode  
 255 from PS/MFC bioanodes (PS/MFC1 and PS/MFC 2) and from PS/Control (PS/Ctrl 1 and PS/Ctrl 2).

256

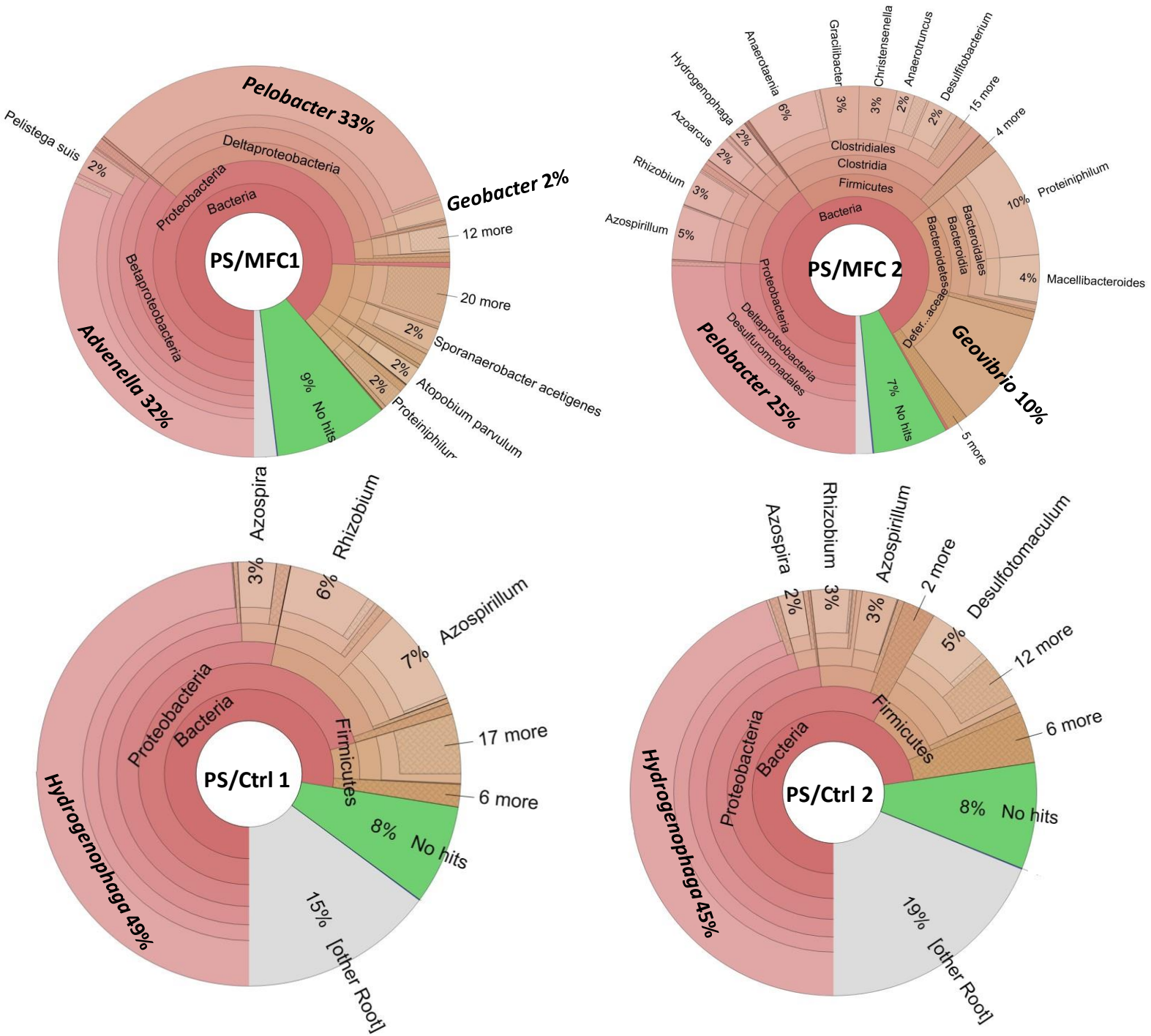
257 In more detailed sequencing data, we recorded the abundance of the *Pelobacter* genus in both  
258 PS/MFC duplicates, constituting approximately 33% and 25% of the microbial community  
259 recorded respectively in PS/MFC 1 and PS/MFC 2 (Figure 3). The abundance of *Pelobacter* at  
260 the anode surface is not surprising as *Pelobacter* is often isolated from anodic biofilms [27]  
261 including a recent study from our group [15]. In general, bacteria of the *Pelobacter* genus are  
262 not able to oxidize acetate and perform extracellular electron transfer simultaneously. While  
263 recent studies recorded the presence of some species of *Pelobacter* (*Pelobacter carbinolicus*)  
264 able to reduce Fe(III) and S(0) by an incomplete oxidation of organic substrates[28], they  
265 typically reduce smaller quantities of Fe(III) compared to *Geobacter* and *Desulfuromonas*  
266 species [29]. Indeed, *Pelobacter carbinolicus* is not capable of direct electron transfer to  
267 graphite anodes [30]. However, a recent publication in our team highlighted the presence of  
268 unidentified species of *Pelobacter* able to colonize the electrode and apparently transfer their  
269 electrons to the electrode directly [15]. Moreover, we registered the presence of two well-  
270 known exoelectrogenic bacterial genera, *Geobacter*, and *Geovibrio* with relative abundances  
271 of approximately 10% and 2% respectively, in PS/MFC 1 and PS/MFC 2. Previous studies have  
272 documented the occurrence of these genera in the anodic compartment, as they possess the  
273 ability to metabolize acetate and directly transfer electrons to insoluble electron acceptors  
274 [25]. Considering the low abundance (less than 1%) of the three genera in the initial sediment,  
275 we suggest that their development is favored through selective pressure under anaerobic  
276 conditions in the presence of acetate. We therefore attribute the observed catalytic behavior  
277 of PS/MFC pilots in acetate oxidation to the presence of a mixed biofilm rich in these known  
278 electricigens developed from a natural paleomarine sediment inoculum.

279 Considering the sequencing analysis, the presence of well-known exoelectrogenic bacteria in  
280 PS/MFC pilots is nonetheless relevant, as they may be implicated in anaerobic transformations  
281 of the marlstone samples. Community analysis of PS/Control (PS/Ctrl1 and PS/Ctrl 2) pilots  
282 incubated without external connection to a cathode showed radically different results to the  
283 PS/MFC pilots (Figure 2). Surprisingly, we recorded a large predominance of *Hydrogenophaga*  
284 in both PS/MFC duplicates, constituting approximately 49% and 45% of the microbial  
285 community recorded respectively in PS/Ctrl 1 and PS/Ctrl 2. According to previous studies, the  
286 *Hydrogenophaga* genus occurred often in the anodic biofilm community [31,32], developed  
287 under anaerobic conditions with suitable electron acceptors as nitrate or electrode in a  
288 Microbial Fuel Cell[32]. Moreover, *Hydrogenophaga* contains a species known as

289 *Hydrogenophaga electricum* that can develop under fixed flow of H<sub>2</sub> using a reactor connected  
290 to an electrolyzer to produce a stable concentration of H<sub>2</sub> [33]. Based on our findings and  
291 current understanding, the other bacteria observed in the PS/control samples do not possess  
292 a known capability for producing hydrogen from acetate. This leads to the hypothesis that  
293 hydrogen production may be attributed to unidentified bacteria or a symbiotic relationship  
294 between different bacteria present in the environment. Further investigations are currently  
295 underway to explore this possibility and shed more light on the mechanisms involved.

296 The low abundance of known exoelectrogenic bacteria at the electrode surface, can be  
297 explained by the presence of another electron acceptor used by bacteria instead of the  
298 electrode. To substantiate this hypothesis, we investigated the interface solution-sediment to  
299 explore the presence of a possible exoelectrogenic bacteria at the sediment surface. The  
300 sequencing results of the PS/MFC were surprising, indicating the abundance of *Advenella*  
301 *genus* (around 81% in PS/MFC1) known for their potential to degrade alkanes and diesel [34],  
302 and the development of *Azospirillum* (41% in PS/MFC 2) capable of nitrogen fixation [35]  
303 (Supporting Information Figure S12). However, we noted the absence of *Pelobacter*,  
304 *Geovibrio*, and *Geobacter* recorded previously at the surface of the bioanode, suggesting that  
305 this genus preferentially colonizes the electrode (Figure 2). In contrast, the PS/Ctrl pilots  
306 shows the abundance of the species *Hydrogenophaga* respectively around 40 % in PS/Ctrl 1  
307 and 28% in PS/Ctrl 2 (Supporting Information Figure S13). Moreover, the sequencing data of  
308 SPS/MFC pilots developed from the sterilized inoculum shows a mixed biofilm with the  
309 absence of well-known exoelectrogenic bacteria (Supporting Information Figure S14).  
310 Suggesting that the sterilization effectively eliminated the initial bacteria present in the  
311 paleomarine sediment and we attribute the development of the exoelectrogenic bacteria to  
312 the initial paleomarine inoculum.

313



314 **Figure 3:** Summary of community analysis (species levels) colonizing the PS/MFC bioanodes,  
 315 PS/Control bioanodes  
 316  
 317

318 **3.3 Bulk Chemical Changes in Sediment Samples**

319 Under anaerobic conditions, the marlstone incubated in a microbial fuel cell (MFC) system,  
320 using acetate as an electron donor and an electrode rod as an electron acceptor, promote the  
321 growth of exoelectrogenic bacteria, as previously discussed. In addition, we observed notable  
322 environmental changes marked by the appearance of black suspended aggregate (Supporting  
323 Information Figure S15). The diffractogram analysis of the initial sediment from the marlstone  
324 sample indicated the presence of various minerals such as gypsum ( $\text{CaSO}_4$ ), calcite ( $\text{CaCO}_3$ ),  
325 quartz ( $\text{SiO}_2$ ), and different clay minerals rich in iron like smectite, illite, kaolinite, and chlorite  
326 [13].

327 The X-ray diffraction performed on the black powder recovered from the PS/MFC shows the  
328 dissolution of the gypsum ( $\text{CaSO}_4$ , diffraction peaks G in Figure 4). Likewise, the clay mineral  
329 assemblage exhibited a broadening and shift in peak positions, indicating possible alterations  
330 in lattice parameters and crystalline structure. Additionally, the distribution of Fe in layer  
331 structure can affect the chemical reactivity of clay by affecting the layer charge which can  
332 change the properties of minerals in terms of cation exchange capacity and swelling [36] [37].

333 The detection of new mineral phases is difficult in our study as we used impure natural  
334 sediment without any previous treatment, which contains impurities and mixed elements  
335 (Figure 4).

336

337

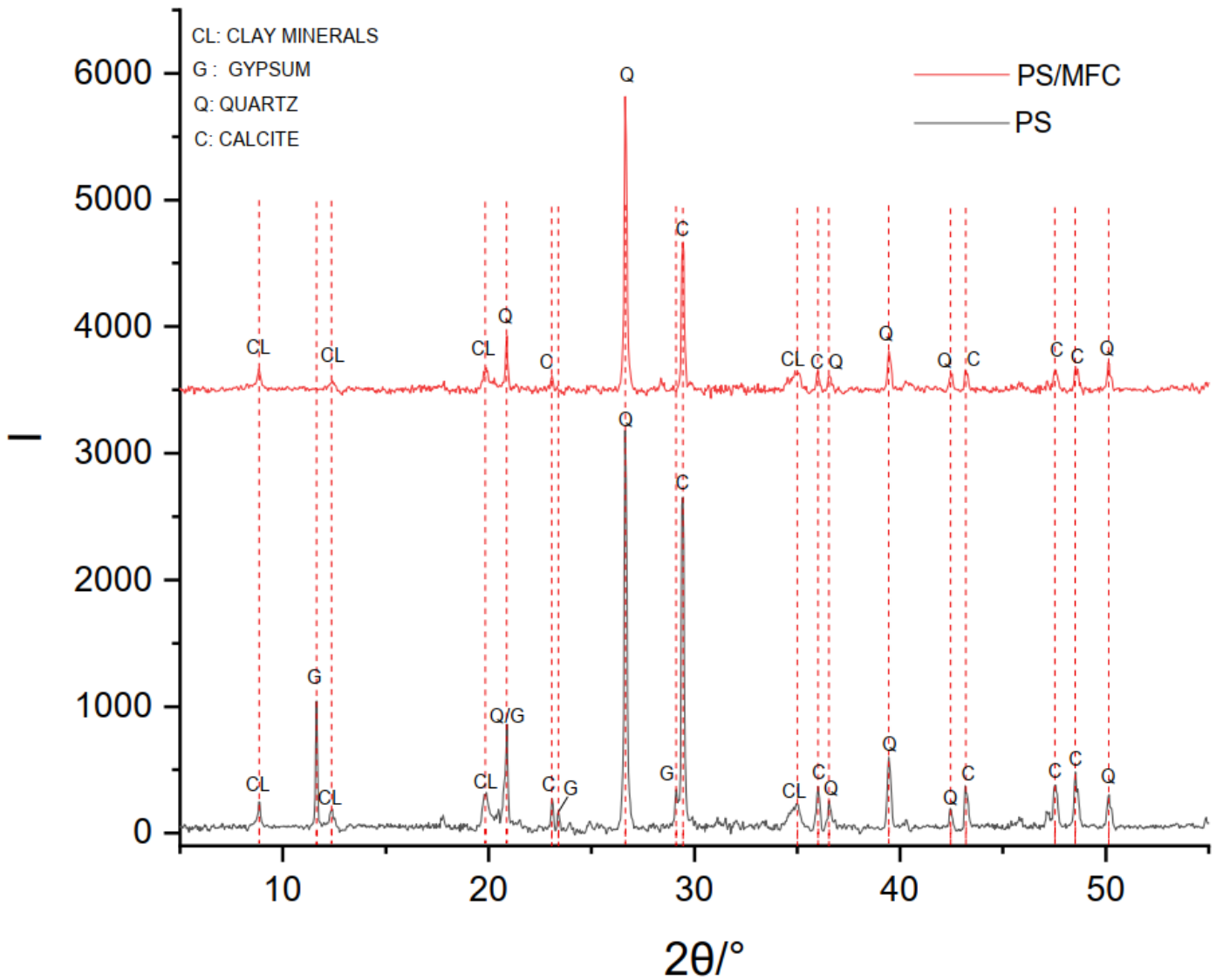
338

339

340

341

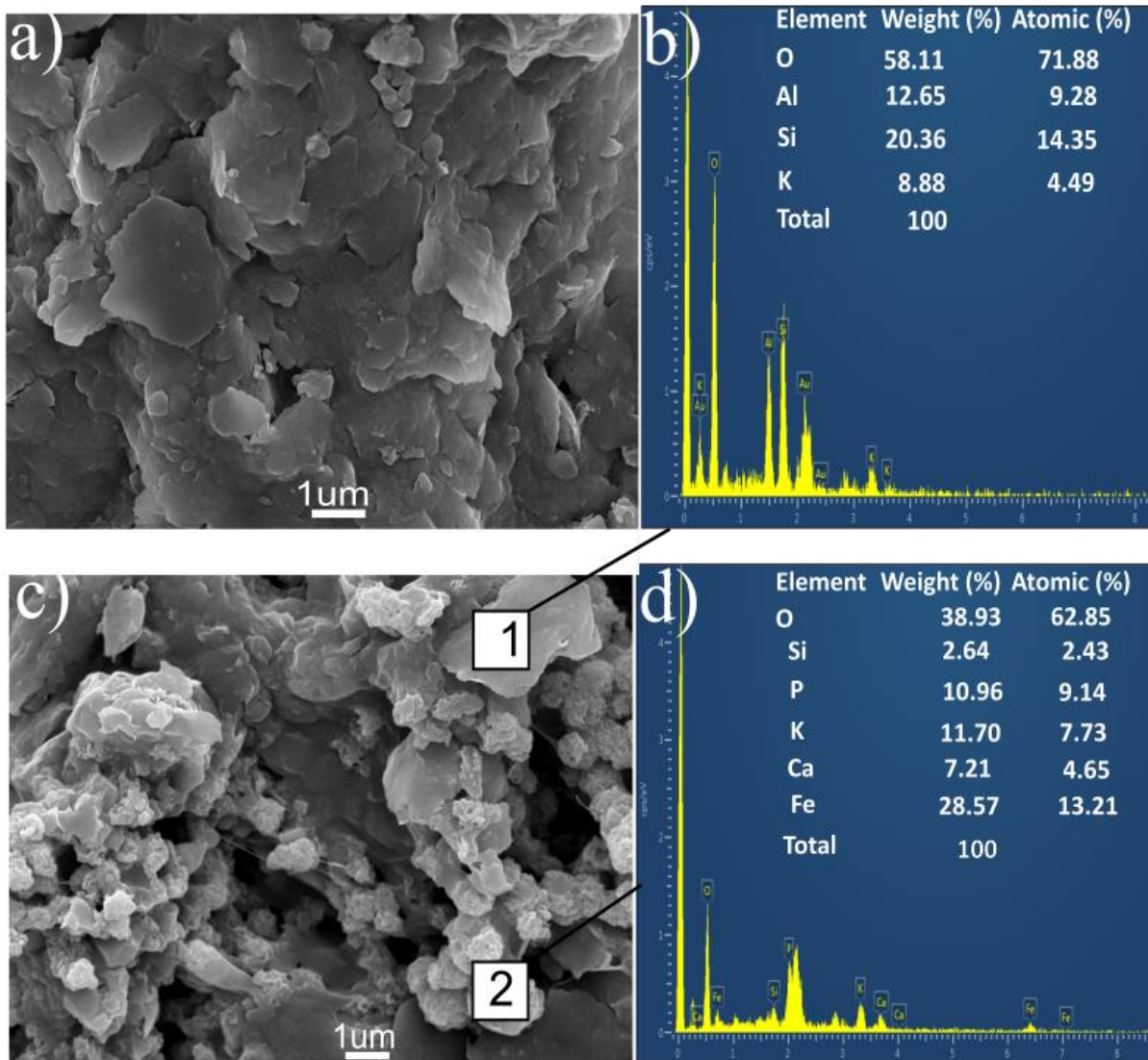




342 **Figure 4.** XRD analysis comparing the mineralogical assemblage in paleomarine sediment (PS)  
 343 before and after treatment (PS/MFC).

344

345 Yet, the SEM images show the presence of new precipitation rich in iron and phosphorus (from  
 346 phosphate buffer used as electrolyte) compared to the initial marlstone sample which is rich  
 347 in aluminosilicate (Figure 5, Supporting Information Figure S16). The particle size ranges of the  
 348 new precipitate phase and their low abundance would make this phase undetectable by XRD  
 349 as previously reported [38].

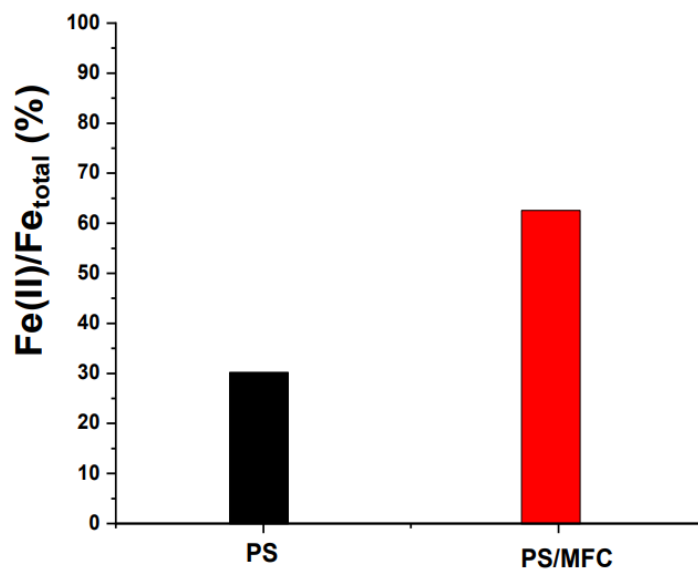


350

351 **Figure 5.** Scanning electron microscopy (SEM) images of the marlstones samples both before  
 352 (a) and after (c) incubation (PS/MFC). a) displays the structure of clay minerals present in the  
 353 PS before incubation. Small agglomerations with high concentrations of iron and potassium  
 354 are observed in c) as confirmed from energy-dispersive X-ray spectroscopy (EDS) analysis (b)  
 355 and d) that compares the initial composition of the PS (labeled as 1) with the composition of  
 356 a new precipitate (labeled as 2). This analysis demonstrates a significant enrichment of iron in  
 357 the newly formed precipitate.

358

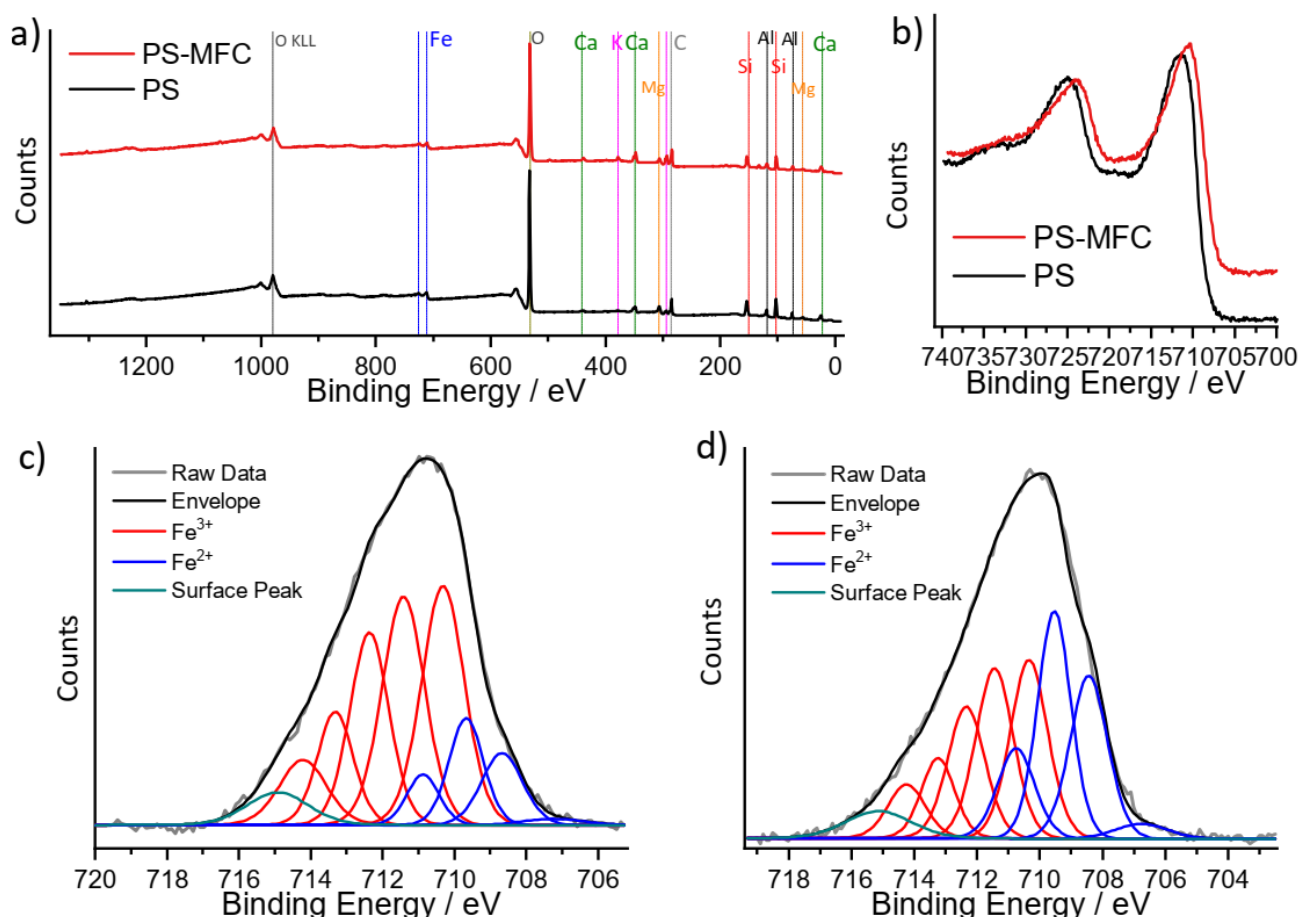
359 These results agree with our geochemical analysis of the powder before and after incubation,  
360 where we recorded an increase on  $Fe_{total}$  and Fe (II). The potential link between the  
361 development of exoelectrogenic bacteria in the PS/MFC system and the increase in the  
362 concentration of ferrous iron Fe (II) is likely related to the reduction of Fe(III) present on the  
363 clay minerals by the developed biofilm (Figure 6) followed by re-precipitation as oxide[39].  
364 The interaction between bacteria and clay minerals is well-known in geochemical phenomena  
365 [6]. The microorganisms have the ability to degrade and decompose clay minerals [40],  
366 promoting the conversion of smectite to illite and the biotransformation of chlorite to  
367 vermiculite [41]. Moreover, the bacteria have the ability to reduce structural iron Fe (III) of  
368 clay minerals which play a main role in iron cycles in marine sediments [42] to Fe(II) as  
369 documented for the species *Bacillus sp* isolated from clay minerals [43]. The distribution of Fe  
370 in the layered structure can affect the chemical reactivity of clay since reduction or oxidation  
371 of Fe will affect the layer charge and hence change the properties of minerals in terms of  
372 cation exchange capacity and swelling [44] [37]. According to the literature and our data, we  
373 hypothesize that the black powder developed in PS/MFC is related to the reduction of iron  
374 Fe(III) present in the marlstone samples to Fe(II) by the developed exoelectrogenic bacteria.



375 **Figure 6.** Iron content of the marlstone samples powder before (in black) and after  
376 incubation (in red)

377

378 After MFC operation there is an apparent increase in the contributions of potassium along  
 379 with the appearance of a small phosphorus contribution in the survey, consistent with the use  
 380 of potassium phosphate buffer in the electrolyte. No change in total iron content was found  
 381 after MFC operation, however, changes in the oxidation state of the iron are evident in the  
 382 high-resolution XPS spectra showing the Fe 2p<sub>3/2</sub> and 2p<sub>1/2</sub> peaks (Figure 7 b) which may be  
 383 attributed to the reduction of Fe(III) to Fe(II) by electroactive bacteria.



384  
 385 **Figure 7:** a) shows representative survey spectra of the PS and PS-MFC. Peaks associated to  
 386 selected assigned elements are indicated above the spectra in each case. b) High resolution  
 387 spectra in the region containing Fe 2p<sub>1/2</sub> and 2p<sub>3/2</sub> peaks after charge correction using  
 388 adventitious carbon in the C 1s envelope at 284.8 eV. c) and d) show deconvoluted high  
 389 resolution spectra in the Fe 2p<sub>3/2</sub> region. Spectra are shown after Shirley background  
 390 subtraction. Details of the assigned peak positions and full widths at half maximum are  
 391 reported in the supporting information Table S2.

392 The predominant elements detected in the survey are carbon, oxygen, silicon, and aluminum  
 393 with contributions from iron, calcium, magnesium, and potassium (Figure 7 a). The relative

394 proportions of each element quantified from the survey scans (Supporting Information Table  
395 S1) after accounting for relative sensitivity factors are broadly consistent with both EDS (Figure  
396 5) and chemical analysis results reported in Figure 6. After MFC operation there is an apparent  
397 increase in the contributions of potassium along with the appearance of a small phosphorus  
398 contribution in the survey, consistent with the use of potassium phosphate buffer in the  
399 electrolyte. No change in total iron content was found after MFC operation, however changes  
400 in the oxidation state of the iron are evident in the high resolution spectra showing the Fe  
401  $2p_{3/2}$  and  $2p_{1/2}$  peaks (Figure 7 b) which may be attributed to the reduction of Fe(III) to Fe(II)  
402 by electroactive bacteria.

403 Chemical analysis (Figure 6, Supporting Information Figure S17) confirmed the presence of  
404 both Fe (III) and Fe (II) species which may exist as ionic species within clay minerals [45,46]  
405 and also as oxide species, further complicating the deconvolution of high-resolution spectra  
406 shown in Figure 7 c) and d). Hence, deconvolutions of the  $2p_{3/2}$  peak were attempted based  
407 on characteristic multiplets for Fe (III) and Fe (II) reported in the literature[47–50], using the  
408 most prominent multiplet peaks associated to the Fe (III) and Fe (II) oxidation states. In  
409 addition, following the fitting protocol of Grosvenor et al. in reference[47], a ‘pre-peak’ which  
410 may also be associated to Fe(II) species and a ‘surface peak,’ which the authors attributed to  
411 surface effects of reduced coordination of Fe in crushed powder preparations, were found to  
412 be necessary to improve the fit. Good fits were obtained by applying this model to both PS  
413 and PS-MFC with similar values of the full width at half maximum (FWHM) and spacing  
414 between multiplets peaks, as reported in the Supporting Information Table S1 and S2. The  
415 most notable difference in the PS-MFC after MFC operation is the significant increase in the  
416 contributions of Fe(II) peaks in the region between 706-710 eV to the deconvolution.

417 Examining the relative peak areas for both samples yields estimates of Fe (II)/Fe(tot) of ca.  
418 18% in PS and 40% in PS-MFC, in agreement with chemical analysis methods which suggested  
419 that the Fe(II) proportion doubled in sediments isolated after 40 days of continuous MFC  
420 operation. As XPS experiments and chemical analyses were carried out on the same samples,  
421 the lower absolute value of the estimated Fe(II) content from XPS may be explained by the  
422 overlap of multiplets associated to the two Fe oxidation states.

## 423 **Discussion and Conclusions**

424 Exoelectrogenic bacteria development has been previously demonstrated for a wide variety  
425 of soil-based inocula, suggesting a broad predominance of these bacteria in anaerobic

426 environments where Fe(III) and other metal oxides found in common ores may serve as  
427 alternative electron acceptors. Here we have shown that such bacteria may occupy a similar  
428 niche in marlstone which possess comparably small levels of organic matter compared to soils  
429 but are rich in clay minerals and iron. Bacteria of the genus *Geovibrio*, *Geobacter*, well known  
430 for their electroactivity [20] [51] were the main electricigens identified and are proposed to  
431 explain the observed catalytic currents as well as the bulk modifications observed in the  
432 sediment inoculum in the MFC pilots. It is likely that the exo-electrogenic bacteria present in  
433 these sediments play a role in the cycling of Fe(III) present not only as trace oxide particles but  
434 also as ions encapsulated within the clay mineral interstices. This has implications for the  
435 structural modification of marlstone by the known metabolic processes of electroactive  
436 bacteria.

437 The sequencing data of the interface sediment solution shows the absence of exoelectrogenic  
438 bacteria, suggesting that these bacteria are only capable of developing into extensive biofilms  
439 under artificial selection conditions conducive to MFC development. Nonetheless, once  
440 developed such biofilms were apparently capable of dramatically affecting the oxidation state  
441 of all iron present in the clay mineral, a result which was not observed in control pilots not  
442 connected to an external cathode. We speculate that observed bulk changes in sediment color  
443 and composition identified via a combination of chemical and imaging analysis are directly  
444 related to Fe(III) to Fe(II) reduction followed by subsequent reprecipitation of Fe(II) as small  
445 aggregates identified by SEM-EDS. Further research is ongoing to evaluate whether direct or  
446 indirect electron transfer processes are responsible for these bulk modifications as well as  
447 tests of a range of clay mineral organic matter and iron concentrations. Nevertheless, the  
448 results presented here strongly suggest that electroactive bacterial activity can be implicated  
449 in the modulation of marine sediment structure and composition. It demonstrates that  
450 microbial communities and clay mineral assemblages mutually interact by exerting a selection  
451 pressure on the microbial community and reducing iron contained in clay minerals such as  
452 smectite, illite and chlorite.

453

454 **Declaration of Competing Interest**

455 The authors declare that they have no known competing financial interests or personal  
456 relationships that could have appeared to influence the work reported in this paper.

457 **Acknowledgment**

458 This study was supported by Université de Rennes and Region Bretagne for funding under  
459 grant SAD 2076 METOX. This project has received funding from the European Union's Horizon  
460 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement  
461 No 899546. This study was supported in part by the French National Research Agency 19-  
462 CE43-0013-01 CATHOMIX). The authors thank the GeT-Biopuces platform of INSA Toulouse  
463 for the 16S rRNA gene sequencing study and Biogeosciences laboratory, University Bourgogne  
464 Franche-Comté, Dijon for XRD analysis and also the National Service of Rocks and Minerals  
465 Analysis (SARM: <http://helium.crpq.cnrs-nancy.fr/SARM/>) in Nancy for all the chemical  
466 analyses. We would like to thank warmly Corinne Lagrost for help XPS analyses.

467 **Appendix A. Supplementary data**

468 Supplementary Figures and Tables can be found in the attached file including a closer view of  
469 Col de Pré-Guittard section, paleomarine sediment (marlstone) characterization, the bioanode  
470 setup and the voltammograms of the bioanode before and after biofilm removing, additional  
471 SEM images of the biofilms developed at the surface of the bioanode, supplementary data  
472 related to the Average repartition of bacterial communities (phylum) extracted from the  
473 interface solution-sediment and color alteration recorded at the PS/MFC after development  
474 of the exoelectrogenic bacteria , supplementary Different SEM image of the PS/MFC showing  
475 the presence of biogenic new precipitate rich on iron.

476

477 **References**

478 [1] J.M. Byrne, H. Muhamadali, V.S. Coker, J. Cooper, J.R. Lloyd, Scale-up of the  
479 production of highly reactive biogenic magnetite nanoparticles using *Geobacter*  
480 *sulfurreducens*, J. R. Soc. Interface. 12 (2015). <https://doi.org/10.1098/rsif.2015.0240>.

481 [2] S.E. Childers, S. Ciufu, D.R. Lovley, *Geobacter metallireducens* accesses insoluble  
482 Fe(III) oxide by chemotaxis, *Letters to Nature*. 416 (2002) 767–769.

483 [3] J.D. Coates, J. Woodward, J. Allen, P. Philp, D.R. Lovley, Anaerobic degradation of  
484 polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor  
485 sediments, *Appl Environ Microbiol*. 63 (1997) 3589–3593.  
486 <https://doi.org/10.1128/aem.63.9.3589-3593.1997>.

487 [4] D.R. Lovley, M.J. Baedeker, D.J. Lonergan, I.M. Cozzarelli, E.J.P. Phillips, D.I. Siegel,  
488 Oxidation of aromatic contaminants coupled to microbial iron reduction, *Letters to Nature*.  
489 339 (1989) 297–300.

490 [5] J.C.G. Walker, Suboxic diagenesis in banded iron formations, *Nature*. 309 (1984) 340–  
491 342.

492 [6] G.M. Gadd, *Metals, minerals and microbes: Geomicrobiology and bioremediation*,  
493 *Microbiology (N Y)*. 156 (2010) 609–643. <https://doi.org/10.1099/mic.0.037143-0>.

494 [7] B.E. Logan, B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman,  
495 W. Verstraete, K. Rabaey, *Microbial fuel cells: Methodology and technology*, *Environ Sci*  
496 *Technol*. 40 (2006) 5181–5192. <https://doi.org/10.1021/es0605016>.

497 [8] S.Z. Abbas, M. Rafatullah, N. Ismail, M.I. Syakir, A review on sediment microbial fuel  
498 cells as a new source of sustainable energy and heavy metal remediation: mechanisms and  
499 future prospective, *Int J Energy Res*. 41 (2017) 1242–1264. <https://doi.org/10.1002/er.3706>.

500 [9] L.M. Tender, S.A. Gray, E. Groveman, D.A. Lowy, P. Kauffman, J. Melhado, R.C. Tyce,  
501 D. Flynn, R. Petrecca, J. Dobarro, The first demonstration of a microbial fuel cell as a viable  
502 power supply: Powering a meteorological buoy, *J Power Sources*. 179 (2008) 571–575.  
503 <https://doi.org/10.1016/j.jpowsour.2007.12.123>.

504 [10] T. Ewing, P.T. Ha, H. Beyenal, Evaluation of long-term performance of sediment  
505 microbial fuel cells and the role of natural resources, *Appl Energy*. 192 (2017) 490–497.  
506 <https://doi.org/10.1016/j.apenergy.2016.08.177>.

507 [11] M. Feregrino-Rivas, B. Ramirez-Pereda, F. Estrada-Godoy, L.F. Cuesta-Zedeño, J.J.  
508 Rochín-Medina, Y.A. Bustos-Terrones, V.A. Gonzalez-Huitron, Performance of a sediment  
509 microbial fuel cell for bioenergy production: Comparison of fluvial and marine sediments |,  
510 *Biomass Bioenergy*. 168 (2023). <https://doi.org/10.1016/j.biombioe.2022.106657>.

511 [12] K.H. Wedepohl, Environmental Influence on the Chemical Composition of Shales and  
512 Calys, *Physics and Chemistry of the Earth*. (1971) 307–333.

513 [13] P. Corentin, J.F. Deconinck, P. Pellenard, F. Amédéo, L. Bruneau, E. Chenot, B.  
514 Matrimon, E. Huret, P. Landrein, Environmental and climatic controls of the clay mineralogy of  
515 Albian deposits in the Paris and Vocontian basins (France), *Cretac Res*. 108 (2020).  
516 <https://doi.org/10.1016/j.cretres.2019.104342>.

517 [14] J. D. Coates, J.P Phillips E, D. J Lonergan, H. Jenter, D. R. Lovley, Isolation of *Geobacter*  
518 *Species from Diverse Sedimentary Environments*, *American Society for Microbiology*. 62 (1996)  
519 1531–1536.

520 [15] T. Philippon, F.Z. Ait-Itto, A. Monfort, F. Barrière, J.A. Behan, Fe(III) oxide  
521 microparticles modulate extracellular electron transfer in anodic biofilms dominated by  
522 bacteria of the *Pelobacter* genus, *Bioelectrochemistry*. 151 (2023).  
523 <https://doi.org/10.1016/j.bioelechem.2023.108394>.

524 [16] A. Deeke, T.H.J.A. Sleutels, H.V.M. Hamelers, C.J.N. Buisman, Capacitive bioanodes  
525 enable renewable energy storage in microbial fuel cells, *Environ Sci Technol*. 46 (2012) 3554–  
526 3560. <https://doi.org/10.1021/es204126r>.

527 [17] M. Picot, L. Lapinsonnière, M. Rothballer, F. Barrière, Graphite anode surface  
528 modification with controlled reduction of specific aryl diazonium salts for improved microbial  
529 fuel cells power output, *Biosens Bioelectron*. 28 (2011) 181–188.  
530 <https://doi.org/10.1016/j.bios.2011.07.017>.

531 [18] A. Iannaci, A. Myles, T. Flinois, J.A. Behan, F. Barrière, E.M. Scanlan, P.E. Colavita,  
532 Tailored glycosylated anode surfaces: Addressing the exoelectrogen bacterial community via



533 functional layers for microbial fuel cell applications, *Bioelectrochemistry*. 136 (2020).  
534 <https://doi.org/10.1016/j.bioelechem.2020.107621>.

535 [19] K. Fricke, F. Harnisch, U. Schröder, On the use of cyclic voltammetry for the study of  
536 anodic electron transfer in microbial fuel cells, *Energy Environ Sci*. 1 (2008) 144–147.  
537 <https://doi.org/10.1039/b802363h>.

538 [20] F. Caccavo, J. J.D. Coates, *Geovibrio ferrireducens*, a phylogenetically distinct  
539 dissimilatory Fe(III)-reducing bacterium, *Arch Microbiol* . 165 (1996) 370–376.

540 [21] P.H. Janssen, Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and  
541 16S rRNA genes, *Appl Environ Microbiol*. 72 (2006) 1719–1728.  
542 <https://doi.org/10.1128/AEM.72.3.1719-1728.2006>.

543 [22] D.R. Bond, D.E. Holmes, L.M. Tender, D.R. Lovley, Electrode-reducing microorganisms  
544 that harvest energy from marine sediments, *Science* (1979). 295 (2002) 483–485.  
545 <https://doi.org/10.1126/science.1066771>.

546 [23] D.R. Bond, D.R. Lovley, Electricity production by *Geobacter sulfurreducens* attached  
547 to electrodes, *Appl Environ Microbiol*. 69 (2003) 1548–1555.  
548 <https://doi.org/10.1128/AEM.69.3.1548-1555.2003>.

549 [24] K.C. Wrighton, P. Agbo, F. Warnecke, K.A. Weber, E.L. Brodie, T.Z. DeSantis, P.  
550 Hugenholtz, G.L. Andersen, J.D. Coates, A novel ecological role of the Firmicutes identified in  
551 thermophilic microbial fuel cells, *ISME Journal*. 2 (2008) 1146–1156.  
552 <https://doi.org/10.1038/ismej.2008.48>.

553 [25] D.R. Lovley, D.E. Holmes, K.P. Nevin, Dissimilatory Fe(III) and Mn(IV) reduction, *Adv*  
554 *Microb Physiol*. 49 (2004) 219–286. [https://doi.org/10.1016/S0065-2911\(04\)49005-5](https://doi.org/10.1016/S0065-2911(04)49005-5).

555 [26] H. Rismani-Yazdi, S.M. Carver, A.D. Christy, Z. Yu, K. Bibby, J. Peccia, O.H. Tuovinen,  
556 Suppression of methanogenesis in cellulose-fed microbial fuel cells in relation to performance,  
557 metabolite formation, and microbial population, *Bioresour Technol*. 129 (2013) 281–288.  
558 <https://doi.org/10.1016/j.biortech.2012.10.137>.

559 [27] S. Freguia, E.H. Teh, N. Boon, K.M. Leung, J. Keller, K. Rabaey, Microbial fuel cells  
560 operating on mixed fatty acids, *Bioresour Technol*. 101 (2010) 1233–1238.  
561 <https://doi.org/10.1016/j.biortech.2009.09.054>.

562 [28] S.A. Haveman, R.J. DiDonato, L. Villanueva, E.S. Shelobolina, B.L. Postier, B. Xu, A. Liu,  
563 D.R. Lovley, Genome-wide gene expression patterns and growth requirements suggest that  
564 *Pelobacter carbinolicus* reduces Fe(III) indirectly via sulfide production, *Appl Environ Microbiol*.  
565 74 (2008) 4277–4284. <https://doi.org/10.1128/AEM.02901-07>.

566 [29] D.R. Lovley, E.J.P. Phillips, D.J. Lonergan, P.K. Widman, Fe(III) and S O Reduction by  
567 *Pelobacter carbinolicus*, *Appl Environ Microbiol*. 61 (1995) 2132–2138.  
568 <https://journals.asm.org/journal/aem>.

569 [30] H. Richter, M. Lanthier, K.P. Nevin, D.R. Lovley, Lack of electricity production by  
570 *Pelobacter carbinolicus* indicates that the capacity for Fe(III) oxide reduction does not  
571 necessarily confer electron transfer ability to fuel cell anodes, *Appl Environ Microbiol*. 73  
572 (2007) 5347–5353. <https://doi.org/10.1128/AEM.00804-07>.

573 [31] Z.I Kimura, S. Okabe, *Hydrogenophaga electricum* sp. nov., isolated from anodic  
574 biofilms of an acetate-fed microbial fuel cell, *J Gen Appl Microbiol*. 59 (2013) 261–266.

575 [32] Z.I. Kimura, S. Okabe, Acetate oxidation by syntrophic association between  
576 *Geobacter sulfurreducens* and a hydrogen-utilizing exoelectrogen, *ISME Journal*. 7 (2013)  
577 1472–1482. <https://doi.org/10.1038/ismej.2013.40>.

578 [33] E. Ehsani, C. Dumolin, J.B.A. Arends, F.M. Kerckhof, X. Hu, P. Vandamme, N. Boon,  
579 Enriched hydrogen-oxidizing microbiomes show a high diversity of co-existing hydrogen-  
580 oxidizing bacteria, *Appl Microbiol Biotechnol*. 103 (2019) 8241–8253.  
581 <https://doi.org/10.1007/s00253-019-10082-z>.

582 [34] H. Wang, S. Zhou, Y. Wang, D. Kong, X. Guo, J. Zhu, W. Dong, Z. Ruan, *Advenella*  
583 *alkanexedens* sp. nov., an alkane-degrading bacterium isolated from biogas slurry samples, *Int*  
584 *J Syst Evol Microbiol*. 66 (2016) 906–911. <https://doi.org/10.1099/ijsem.0.000811>.

585 [35] S.Y. Lin, Y.C. Liu, A. Hameed, Y.H. Hsu, W.A. Lai, F.T. Shen, C.C. Young, *Azospirillum*  
586 *fermentarium* sp. nov., a nitrogen-fixing species isolated from a fermenter, *Int J Syst Evol*  
587 *Microbiol.* 63 (2013) 3762–3768. <https://doi.org/10.1099/ij.s.0.050872-0>.

588 [36] S.N. Yong, S. Lim, C.L. Ho, S. Chieng, S.H. Kuan, Mechanisms of microbial-based iron  
589 reduction of clay minerals: Current understanding and latest developments, *Appl Clay Sci.* 228  
590 (2022). <https://doi.org/10.1016/j.clay.2022.106653>.

591 [37] L. Zhang, G.M. Gadd, Z. Li, Microbial biomodification of clay minerals, in: *Adv Appl*  
592 *Microbiol*, Academic Press Inc., 2021: pp. 111–139.  
593 <https://doi.org/10.1016/bs.aambs.2020.07.002>.

594 [38] D.G. Zavarzina, N.I. Chistyakova, A. V. Shapkin, A. V. Savenko, T.N. Zhilina, V. V.  
595 Kevbrin, T. V. Alekseeva, A. V. Mardanov, S.N. Gavrillov, A.Y. Bychkov, Oxidative  
596 biotransformation of biotite and glauconite by alkaliphilic anaerobes: The effect of Fe  
597 oxidation on the weathering of phyllosilicates, *Chem Geol.* 439 (2016) 98–109.  
598 <https://doi.org/10.1016/j.chemgeo.2016.06.015>.

599 [39] G.L. Li, C.H. Zhou, S. Fiore, W.H. Yu, Interactions between microorganisms and clay  
600 minerals: New insights and broader applications, *Appl Clay Sci.* 177 (2019) 91–113.  
601 <https://doi.org/10.1016/j.clay.2019.04.025>.

602 [40] B. Biswas, A. Chakraborty, B. Sarkar, R. Naidu, Structural changes in smectite due to  
603 interaction with a biosurfactant-producing bacterium *Pseudoxanthomonas kaohsiungensis*,  
604 *Appl Clay Sci.* 136 (2017) 51–57. <https://doi.org/10.1016/j.clay.2016.11.008>.

605 [41] G.J. Ross, H. Kodama, Experimental alteration of a chlorite into a regularly  
606 interstratified chlorite-vermiculite by chemical oxidation, *Clays Clay Miner.* 24 (1976) 183–190.

607 [42] T. Playter, K. Konhauser, G. Owttrim, C. Hodgson, T. Warchola, A.M. Mloszewska, B.  
608 Sutherland, A. Bekker, J.P. Zonneveld, S.G. Pemberton, M. Gingras, Microbe-clay interactions  
609 as a mechanism for the preservation of organic matter and trace metal biosignatures in black  
610 shales, *Chem Geol.* 459 (2017) 75–90. <https://doi.org/10.1016/j.chemgeo.2017.04.007>.

611 [43] P.J.G. J.W. Stucki, Microbial reduction of iron in nontronite, (1986).

612 [44] B. Müller, G. Défago, Interaction between the bacterium *Pseudomonas fluorescens*  
613 and vermiculite: Effects on chemical, mineralogical, and mechanical properties of vermiculite, *J*  
614 *Geophys Res Biogeosci.* 111 (2006). <https://doi.org/10.1029/2005JG000054>.

615 [45] N. Finck, M.L. Schlegel, A. Bauer, Structural iron in dioctahedral and trioctahedral  
616 smectites: a polarized XAS study, *Phys Chem Miner.* 42 (2015) 847–859.  
617 <https://doi.org/10.1007/s00269-015-0768-3>.

618 [46] N. Finck, M.L. Schlegel, K. Dardenne, C. Adam, S. Kraft, A. Bauer, J.L. Robert,  
619 Structural iron in smectites with different charge locations, *Phys Chem Miner.* (2019).  
620 <https://doi.org/10.1007/s00269-019-01028-y>.

621 [47] A.P. Grosvenor, B.A. Kobe, M.C. Biesinger, N.S. McIntyre, Investigation of multiplet  
622 splitting of Fe 2p XPS spectra and bonding in iron compounds, *Surface and Interface Analysis.*  
623 36 (2004) 1564–1574. <https://doi.org/10.1002/sia.1984>.

624 [48] A.K. Gupta, M. Gupta, Synthesis and surface engineering of iron oxide nanoparticles  
625 for biomedical applications, *Biomaterials.* 26 (2005) 3995–4021.  
626 <https://doi.org/10.1016/j.biomaterials.2004.10.012>.

627 [49] M.C. Biesinger, Accessing the robustness of adventitious carbon for charge  
628 referencing (correction) purposes in XPS analysis: Insights from a multi-user facility data  
629 review, *Appl Surf Sci.* 597 (2022). <https://doi.org/10.1016/j.apsusc.2022.153681>.

630 [50] N.S. McIntyre, D.G. Zetaruk, X-ray Photoelectron Spectroscopic Studies of Iron  
631 Oxides, *Anal. Chem.* 49 (1977) 1521–1529. <https://pubs.acs.org/sharingguidelines>.

632 [51] D.R. Lovley, Dissimilatory Fe(III) and Mn(IV) Reduction, 1991.  
633 <https://journals.asm.org/journal/mr>.

634  
635