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Electricity generation by microorganisms in the sediment-water interface of an extreme acidic microcosm

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Summary. The attachment of microorganisms to electrodes is of great interest for electricity generation in microbial fuel cells (MFC) or other applications in bioelectrochemical systems (BES). In this work, a microcosm of the acidic ecosystem of Río Tinto was built and graphite electrodes were introduced at different points. This allowed the study of electricity generation in the sediment/water interface and the involvement of acidophilic microorganisms as biocatalysts of the anodic and cathodic reactions in a fuel-cell configuration. Current densities and power outputs of up to 3.5 A/m^2 and 0.3 W/m^2, respectively, were measured at pH 3. Microbial analyses of the electrode surfaces showed that *Acidiphilium* spp., which uses organic compounds as electron donors, were the predominant biocatalysts of the anodic reactions, whereas the aerobic iron oxidizers *Acidithiobacillus ferrooxidans* and *Leptospirillum* spp. were detected mainly on the cathode surface. [**Int Microbiol** 2011; 14(2):73-81]

Keywords: microbial fuel cells · acidophiles · microcosm · electricity generation · Río Tinto

Introduction

Interest in the ability of microorganisms to attach to electrodes has grown exponentially over the last decade based on the potential for electricity generation in microbial fuel cells (MFC) as well as other applications in bioelectrochemical systems (BES) [7,23]. The main reason for this increased interest has been the discovery that many bacteria are capable of a direct exchange of electrons with electrodes, thereby obviating the need to add toxic redox mediators to the BES [26]. In spite of the considerable increase in the current and power densities recently achieved with MFC, their values are still several orders of magnitude below those of conventional fuel cells based on Pt electrocatalysts [40]. Nevertheless, MFC can be used for energy recovery in wastewater treatment or for power generation in remote applications [7,36]. In this context, there are several reports of MFC developed on sediment/water interfaces of aquatic environments. In this these fuel cells, a proton-exchange membrane is not needed for separating the anode from the cathode since the anode is placed in the anoxic sediment, and the cathode in the oxic water level. Energy is generated by the redox gradient

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through the interface, with the oxidation of organic and inorganic matter in the sediment anode mediated by several microorganisms and the reduction of oxygen at the cathode placed in the water level. Electron flow between anode and cathode via an external circuit is compensated by proton transport through the sediment/water interface [35]. Fuel cells of this design are reportedly sufficient to power marine-deployed electronic instrumentation [37]. Sediment MFC can be also constructed in the laboratory, where they can be employed to assess the performance and capabilities of microbial electrocatalysts, which is critical for the improvement of MFC technology [17].

The use of extremophiles in MFC is of particular interest because these microorganisms could serve as bioelectrocatalysts in systems able to operate under conditions of pH, salinity, or temperature that are more favorable for higher power production [5,30]. For example, the study of sediment MFC from acidic ecosystems is relevant because one of the main limitations of MFC technology are the pH gradients generated between the anode and cathode compartments during operation [3,11]. In many cases, the current density is limited by the cathodic reaction due to slow proton transport, as four protons are required for the reduction of each O_2 molecule [11,12]. Therefore, single- or double-chamber MFC that operate efficiently at low pH would be an important improvement. One of the natural acidic ecosystems is the Río Tinto (Huelva, Spain), where the average pH is 2.3 ± 0.6 and heavy metal concentrations but also microbial diversity levels are high [2,14,24,25]. The biooxidation of pyrite, which is the main mineral component of the Río Tinto ecosystem, has, over time, resulted in significant amounts of ferric iron and sulfates.

The abundance of ferric ions in the water acts as a buffer, maintaining the acidic pH of the river [14]. Microbial ecology studies have confirmed that 80% of the prokaryotic diversity in the water column corresponds to three bacterial genera, *Acidithiobacillus ferrooxidans*, *Acidiphilium* spp., and *Leptospirillum* spp., all of them conspicuous members of the iron cycle [14,15]: *A. ferrooxidans* oxidizes iron aerobically and reduces it anaerobically, whereas all *Leptospirillum* isolates from Río Tinto are aerobic iron oxidizers and all *Acidiphilium* isolates can use ferric iron as an electron acceptor, and organic compounds as electron donors [14,15,19,34]. A significant number of *Acidiphilium* and *Acidiphilium*-related bacteria carry out dissimilatory reduction of ferric iron under anoxic as well as microaerophilic conditions [19,34].

In a previous work we studied the interaction of pure cultures of *Acidiphilium* sp. from Río Tinto with carbon electrodes and analyzed the system's electrocatalytic properties with respect to the oxidation of organic matter [28]. Cells from the pure culture were able to colonize graphite felt electrodes and produce electrocatalytic currents of up to 3 A/m^2 in the absence of redox mediators, by oxidizing glucose even at saturating air concentrations and very low pH values [28]. Here we present the results of a study in which electricity was generated using sediment and water from the Río Tinto ecosystem. The physical and chemical parameters at the electrodes interfaces were measured and their respective bacterial populations were analyzed.

Materials and methods

Construction of a Río Tinto microcosm. Water and sediment samples from the Río Tinto were used to study this acidic ecosystem at a small scale. The microcosm was designed according to the proposed functional model of the Río Tinto ecosystem [13,29]. It was formed by a column of 70 cm height and 7 cm diameter, and by three methacrylate containers: containers 1 and 3 had dimensions of 10 × 12 × 17 cm, whereas those of container 2 were 31 × 51 × 26 cm (see Fig. 1). All containers were filled with sediments and water from two sites of Río Tinto. Those of container 1 were collected from sampling point JL (UTM coordinates: N4178250-29S0714879), which corresponds to the site where the Río Tinto meets the sewage waters from the town of Nerva; those from container 3 were collected from point 3.2 (UTM coordinates: N4177714-29S0714771), where an artificial dam of 6 m depth is located near the river source. Container 2 was filled with sediments and waters from both sites. The column contained water from both sites. All three containers and the column were connected by rubber tubes of 1 cm diameter and water was circulated by a peristaltic pump (Ismatec-Ecoline VC-280) in order to simulate the river conditions.

Physical-chemical variables. A Crison pH/mV-meter 506 was used to measure pH and redox potential. Conductivity and temperature were measured with an Orion-122 conductivity meter. Oxygen concentration was determined with a Simplair F-15 oximeter (Syland Scientific).

Chemical analyses. Fe^{2+} concentration was determined spectrophotometrically with di-2-pyridyl ketone benzoylhydrazone, as reported previously [32]. Total iron was determined by the addition to the tested solution of 72 mM hydroxylamine, which reduces Fe^{3+} to Fe^{2+}, and then the Fe^{2+} concentration was measured as indicated above. Heavy metal concentrations were determined by X-ray fluorescence reflection (TXRF) and inductively coupled plasma-mass spectrometry (ICP-MS) measurements performed at the Research Service of the Autonomous University of Madrid. Glucose and glycerol concentrations were measured using the kits Glucose MR Cromatest (Linear) and free glycerol reagent (Sigma), respectively. For this purpose, 10 g of sediment or 5 ml of water was placed in an extraction container with 5 ml of methanol and stirred for 12 h. The resulting extract was then filtered through a 0.22-µm filter (Millex GS, Millipore), left overnight at 37°C, and finally stored at 4°C until use.

Electrochemical measurements. The electrodes were graphite felt (RVG 4000, 9-µm fiber diameter, 3500 cm^2/g surface area, Le Carbon Lorraine) disks 1.3 cm in diameter and 1 cm in thickness (6.5 cm^2 total geometric area) or graphite bars (99.99% purity, low density graphite, Sigma)

15 cm in height and 0.6 cm in diameter (28.5 cm^2 total geometric area). Before use, the graphite felt disks were cleaned in 2 N sulfuric acid and then sterilized at 120ºC and 1 atm for 30 min; the graphite bars were sterilized in the same way but without previous treatment. Polarization curves were generated by connecting the anode and cathode with external resistances of different values (4–10,000 Ω) and, after a 5-min wait during which a pseudo steady state was reached, measuring the voltage difference for each case with a Keithley 2000 multimeter. The measured voltage difference was converted to current according to Ohm's law ($\Delta V = I \times R$). Current densities were obtained by dividing the cell current by the projected surface area of the anode.

Microbial cultures. Material scraped from the electrodes with a sterile razor blade was rinsed with PBS buffer and then inoculated aerobically into either Mackintosh medium [27] or a specific medium for growing *Acidiphilium* spp. [14].

Fluorescent in situ hybridization analysis (FISH). Samples from the electrodes were immediately fixed with 4% formaldehyde in minimal Mackintosh medium [27]. After 4 h, the samples were filtered through GTTP Millipore filters (pore size 0.22 μm) and washed with 8 ml of PBS buffer to eliminate excess formaldehyde and heavy metals. The filters were stored at –20ºC prior to hybridization. Hybridization and microscopic counting of hybridized and 4′,6′-diamidino-2-phenylindole (DAPI)-stained cells were performed as described previously [1,14]. The hybridization values were corrected by subtracting the signals obtained from the control probe Non338 [39]. The probes employed in this study were: Eub338, for all bacteria; the *Nitrospira* group probe Ntr712, used in conjunction with the competitor Ntr712c [9]; Thio 1, specific for *A. ferrooxidans*; and Acd638, specific for *Acidiphilium* spp. The sequences of the probes used in this work are listed in Table S1.

Total DNA extraction, PCR amplification, and denaturing gradient gel electrophoresis (DGGE). Cultures derived from the electrode scrapings were filtered for DNA extraction using the FastDNA kit for soils BIO101, as described by González-Toril et al. [15]. The 16S rRNA genes from mixed microbial DNA were PCR-amplified. To obtain an almost complete 16S rRNA gene, two oligonucleotide primer pairs, 8F and 1492R [21] for the domain Bacteria, were used as reported previously. Amplified 16S rRNA gene products (N1400 bp) were purified on a GeneClean Turbo Column (Q-Bio Gene), and cloned using the Topo TA Cloning Kit (Invitrogen) as previously described [13]. Sequencing reactions were run on an Applied Biosystems sequencer from the Genomics Unit of the Research Services of the Autonomous University of Madrid. DGGE, band excision, and reamplification were performed as previously described [31]. The resultant sequences were analyzed by BLAST [http:/ncbi.nlm.nih.gov/BLAST] and deposited in the GenBank data base of the National Center for Biotechnology Information (NCBI).

Results

Electrochemical study. Electricity production in the Río Tinto ecosystem was evaluated in the laboratory by building a microcosm as described in Materials and methods and in Fig. 1. The physical-chemical parameters of the laboratory system were confirmed to be similar to those of the Río Tinto ecosystem. The average pH, redox potential, and O_2 concentration of the water level were 3.4 ± 0.3, 520 ± 40 mV (vs. Ag/AgCl), and 6.3 ± 0.3 ppm respectively. These values well reproduce the acidic ecosystem of Río Tinto, with its high redox potential due to the high concentration of ferric ions at the oxic water surface [2,14,24,25]. Carbon electrodes were placed at different sites of the sediment and water; after 24 h, the open circuit potential (OCP) between different pairs of electrodes was measured. The OCP values ranged between 0 and 0.440 V. The best results of current generation were obtained with an anode (graphite bar) dipped

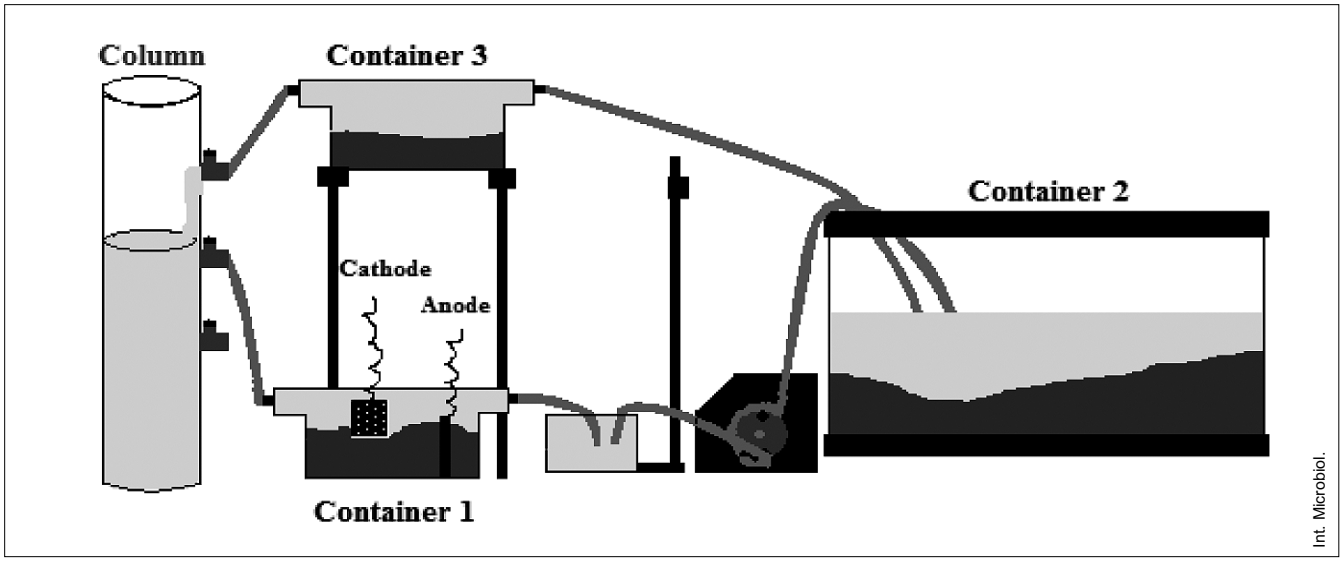

Fig. 1. Scheme of the microcosm of the Río Tinto.

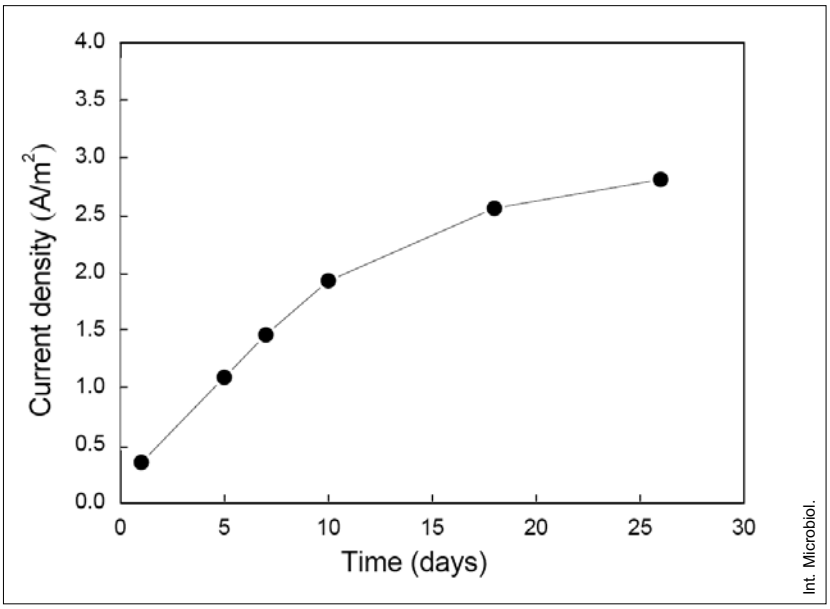

Fig. 2. Time dependence of the current density measured in the sediment microbial fuel cell with an external load of 10 Ω.

7 cm within the sediment of container 1 and a cathode (carbon felt disk) placed just above the water/sediment interface of the same container, at approximately 11 cm from the anode in the horizontal direction. All further studies presented herein were therefore done with these two electrodes. Figure 2 shows that the current density measured when both electrodes were connected with an external load gradually increased during 26 days. This slow increase of the current is typical of electrodes colonized by electrochemically active bacteria [4,11,28].

The polarization (current-voltage diagram) and power density curves measured after 26 days are shown in Fig. 3. It can be observed that voltage loss is dominated by ohmic resistance, as the voltage (V) decreased linearly with the current density (I/A) across almost the entire range (except at very low current densities). This polarization behavior is typical of sediment MFC because of the high internal resistance in these systems [18,22,37]. From the slope of the linear polarization curve an internal resistance ($R_{int} = -\Delta V/\Delta I$) of 33 Ω was obtained. The power curve ($P = V \times I/A$) indicated that

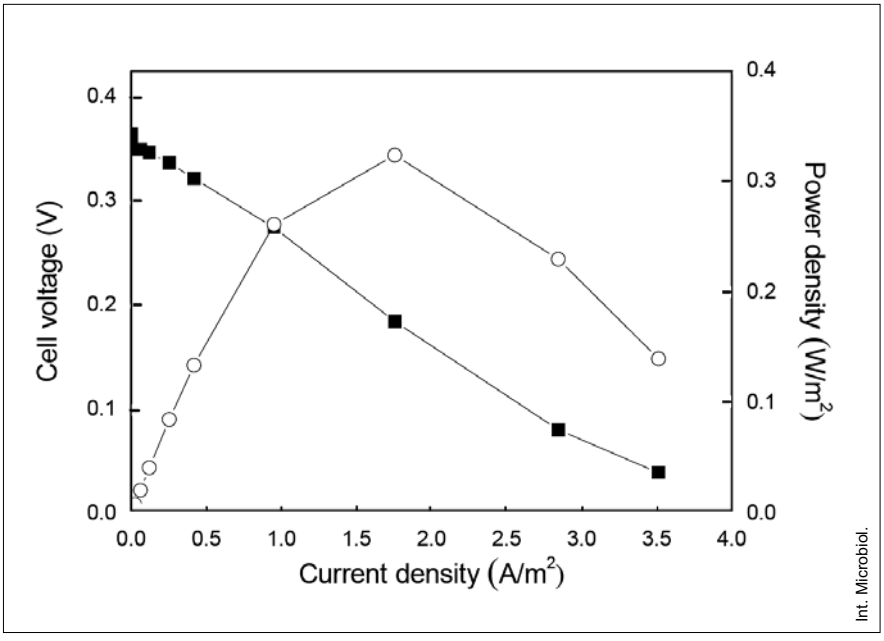

Fig. 3. Polarization (filled squares) and power density (open circles) curves of the sediment microbial fuel cell.

Table 1. Concentration of organic compounds in container 1[a]

	Glucose	Glycerol
Water (mg/l)	157 ± 5	74 ± 5
Sediment (mg/kg)	77 ± 10	56 ± 12

[a]Mean values from 3 measurements.

the maximum power output of the sediment MFC was 0.32 W/m^2. In fact, the sediment MFC was able to provide power to a small electronic device of 0.4 mW for several weeks. Polarization curves for other pairs of electrodes placed in container 1 are shown in Fig. S1 (supplementary information). Current production between pairs of electrodes in the other containers was much smaller (not shown).

Chemical analyses. The element composition in the water level of container 1 was measured (Table S2, supplementary information). As expected for water extracted from Río Tinto, the concentrations of Fe and S were very high (99 ± 1 and 309 ± 1 mM, respectively). Other metals such as Cu and Al were also very abundant (35.0 ± 0.1 and 108 ± 6 mM, respectively). In agreement with the high redox potential measured in the water column, 92% of the Fe content was in the ferric state. The content of organic compounds such as glucose and glycerol in the sediment and water of container 1 was measured because, in MFC, organic compounds are used as fuel. The microorganisms oxidize these compounds and electrons produced by their metabolic processes are subsequently transferred to the anode. Table 1 shows that a considerable amount of glycerol and glucose were present in the sediments, suggesting that they served as fuels of the anodic reaction.

Microbial analysis. The electrodes used in the electrochemical study were scraped and rinsed with PBS buffer. Taking into account the known prokaryotic diversity in Río Tinto [14], the microorganisms present in the buffer after rinsing the electrodes were inoculated in media supporting the growth of heterotrophic acidophiles and iron-oxidizing bacteria. Initially, the inoculants were grown on solid media under oxic conditions. The resulting colonies were then transferred to liquid medium with glucose as energy source to obtain cultures of heterotrophic acidophiles. The cultures established from the colonies were analyzed by in situ hybridization and all of them corresponded to *Acidiphilium* species.

Aliquots of the buffer used for rinsing the electrodes were inoculated in Mackintosh medium with Fe^{2+} as energy source. DGGE was then carried out, with the bacterial primers 341-GC and 907R, which are specific for iron-oxidizing microorganisms, used for gene amplification (Fig. S2, supplementary information). The DGGE bands obtained indicated the presence of two and three different species of microorganisms in the anode and cathode, respectively (Table 2).

The amplified 16S rRNA genes of the bacteria washed from the electrodes were inserted in a pGEMT easy vector and then cloned in *E. coli* cells. From each electrode, 96 bacterial 16S rRNA gene clones were obtained, which corresponded to 12 and 13 different species on the anode and cathode, respectively. The resultant sequences were analyzed by BLAST. The average size of the fragments was between 1100 and 1300 bp. The analysis indicated that several *Acidiphilium* species were present on the anode and cathode, whereas *A. ferrooxidans* and *Leptospirillum* species were present on the cathode (Table S3, supplementary information).

Once the presence of *Acidiphilium* spp., *A. ferrooxidans*, and *Leptospirillum* spp. on the electrodes was confirmed, they were quantified by the FISH method using a specific fluorescent probe for each of these microorganisms (Table S1 supplementary information). Their percentages relative to the total amount of bacteria on the electrodes were calculated by

Table 2. Analysis of the sequences obtained from the DGGE bands of the anode and cathode

DGGE bands	Gene bank accession number	Related BLAST sequences	Similarity (%)
[a]D1	HQ585859	*Acidithiobacillus ferrooxidans*	98
[a]D2	HQ585860	*Acidiphilium* sp. BGR	98
[b]D3	HQ585863	*Acidithiobacillus ferrooxidans*	99
[b]D4	HQ585861	*Acidiphilium cryptum*	100
[b]D5	HQ585862	*Acidiphilium* sp. BGR	97

[a]Anode. [b]Cathode.

Table 3. Quantification of bacteria attached to the electrodes by FISH

Electrode	Number of cells/cm^2 × 10^{-5}	Eub 338[a]	Acd 638[a]	Thio 1[a]	Ntr 712[a]
Anode	33 ± 2	78 ± 1	58 ± 1	11 ± 1	6 ± 1
Cathode	10 ± 1	83 ± 1	13 ± 1	37 ± 1	17 ± 2

[a]Percent cells detected with probe (mean values from 18 measurements).

performing hybridizations with probe Eub 338, a universal probe for bacteria. The results are presented in Table 3, which shows that a high percentage of live cells was extracted from the electrode surfaces. *Acidiphilium* spp. cells were dominant in the anode, as 58% of the attached cells were detected with the Acd 638 probe, whereas *A. ferrooxidans* and *Leptospirillum* spp. cells, detected with the Thio 1 and Ntr 712 probes, respectively, were much less abundant. In contrast, the most abundant microorganism on the cathode was *A. ferrooxidans*, with 37% of the attached cells detected by the Thio 1 probe. *Acidiphilium* spp. and *Leptospirillum* spp. cells were also detected in the cathode but in minor proportions.

Discussion

In this work we have shown that electricity can be generated in an acidic environment based on the involvement of acidophilic microorganisms as biocatalysts of the anodic and cathodic reactions. This is an important issue because MFCs that are able to operate at low pH are technologically advantageous, as the proton transport rate from anode to cathode increases and the kinetic barrier for O_2 reduction to H_2O at the cathode decreases, which leads to higher current and power densities [3,11]. In our system, we consistently measured current densities and a maximum power generation about 2–20 times higher than achieved with other sediment MFCs from aquatic environments in which the pH is close to neutral [10,18,33,37,38].

In sediment MFC the power output is typically several orders of magnitude lower than in other types of MFC, such as membrane and single-chamber air cathode types [23]. The main reason for the low output is the very high ohmic drop between anode and cathode. In our system, however, this drop was greatly reduced; indeed, the internal resistance was only 33 Ω, probably because of the short distance between the electrodes compared to those placed in seawater or in rivers and mostly because the high concentration of metallic ions and protons in the medium raised the ionic strength. In fact, other works on sediment MFC have shown that current and power densities increase with the increasing salinity of the aquatic medium and therefore with increasing ionic strength [10,37].

In addition, the high current densities measured in the microcosms suggest that the redox reactions at the anode and cathode had low kinetic barriers. Furthermore, our results indicate that acidophilic bacteria had catalyzed those reactions. First of all, the slow build-up of the cell current over several days is typical of the colonization of electrodes by electrochemically active microbes [4,11,28]. Second, we identified and quantified the acidophilic microorganisms that had colonized the anode and cathode surfaces. The dominant species on the anode surface belonged to the *Acidiphilium* genus. This result was expected since in a previous report we showed that a pure culture of *Acidiphilium* sp. from Río Tinto colonized graphite felt electrodes and electrocatalyzed glucose oxidation in the absence of redox mediators [28].

In the microcosm, a significant amount of glucose and glycerol were detected in the sediment where the anode was placed, which could be expected given that the sediment was collected at the point where the Río Tinto meets the sewage wastewater (of domestic and industrial origin) coming from the town of Nerva. It is well known that *Acidiphilium* spp. are able to use organic compounds such as glucose and glycerol as electron donors [19,20,28]. The reducing equivalents obtained by glucose oxidation can be transferred directly to the electrode (Fig. 4), as was shown in a previous study [28]. Nevertheless, other organic compounds might also have contributed to the power generation in the microcosm.

Minor quantities of *A. ferrooxidans* and *Leptospirillum* spp. were detected on the anode. The former species can reduce ferric iron under anoxic conditions using sulfur as the sole energy source [29]. Lovley and coworkers have reported that pure cultures of *Desulfobulbus propionicus* are able to oxidize elemental sulfur with an electrode serving as electron acceptor, suggesting that the reaction would be an important biological process in the anode of marine sediment fuel cells [16]. Given the large amount of sulfur in our system, this reaction might have also contributed to the anodic current. However, it remains to be determined whether pure cultures

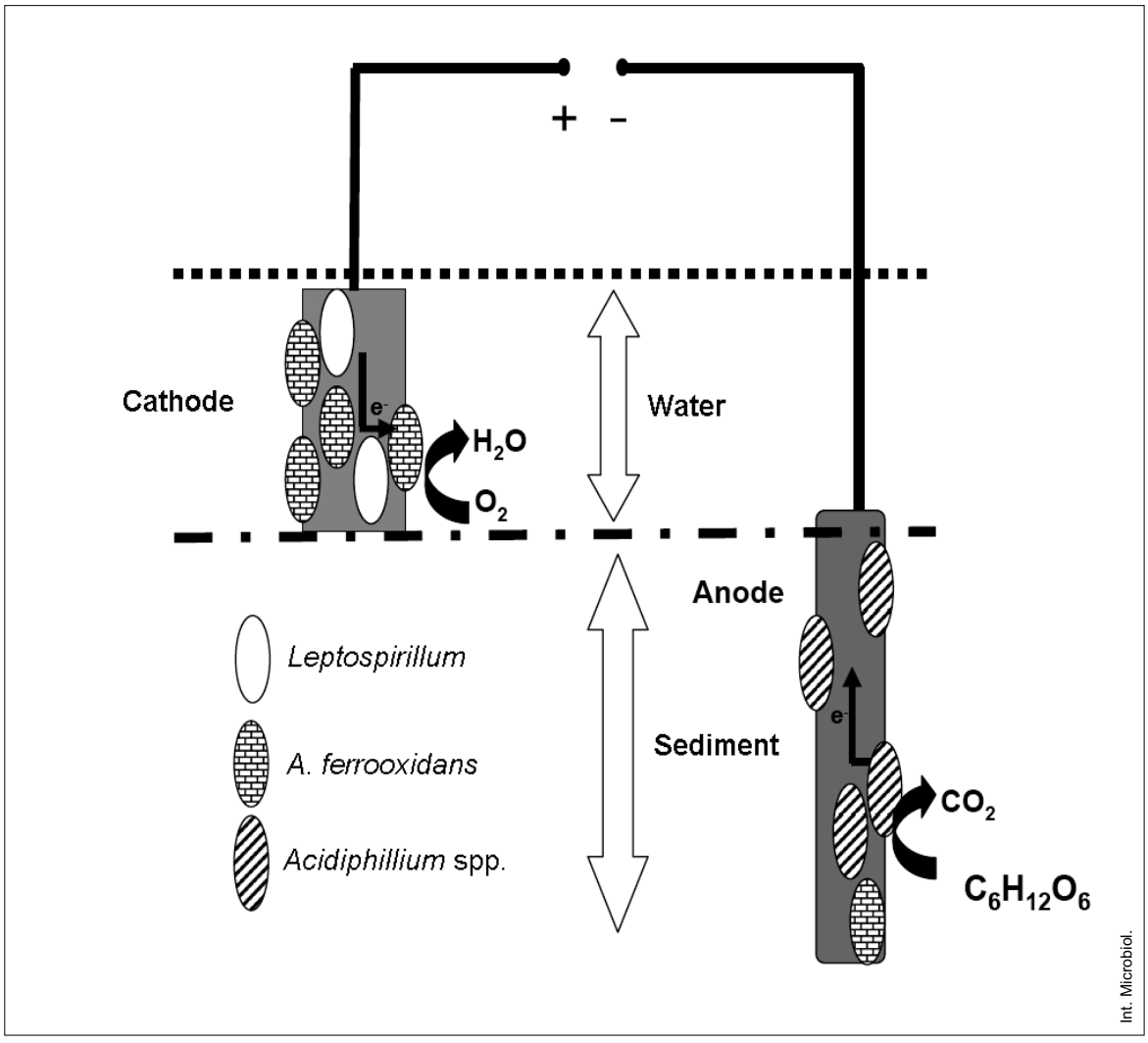

Fig. 4. Scheme of the anodic and cathodic main reactions in the sediment microbial fuel cell.

of *A. ferrooxidans* are able to produce electricity from sulfur. In any case, most probably the main fuels used in the anode reaction were organic compounds, due to the much higher proportion of *Acidiphilium* spp. cells detected on the anode and to the fact that the best results of electricity generation were measured when the electrodes were placed in container 1 and not in container 3, which had sediments and water from a site near the river source and therefore a much lower content of organic compounds [25]. Moreover, in a previous report we showed that *Acidiphilium* sp. cells from Río Tinto are able to produce high electrocatalytic currents from glucose oxidation in the absence of redox mediators [28]. The current density values measured with the pure culture in that work were of the same order of magnitude as those measured in the sediment MFC in the present study (Fig. 2).

The aerobic iron oxidizers *A. ferrooxidans* and *Leptospirillum* spp. were detected mainly on the cathode surface. The former species predominated and was probably the main biocatalyst of the cathodic reaction (Fig. 4). We recently reported that *A. ferrooxidans* cells immobilized on a carbon electrode from a pure culture catalyze oxygen reduction without the need for added redox mediators, resulting in current densities of the same order of magnitude as in the present sediment MFC [6]. A minor proportion of cells attached to the cathode belonged to the *Acidiphilium* genus. As these microorganisms are able to reduce ferric iron [19,28] or carbon electrodes [28] under oxic conditions, their presence in the cathode, although minor, might have been responsible, at least in part, for the decreased cathodic potential and thus the lower cell voltage.

In summary, the results of this work show that acidophilic bacteria from Río Tinto can serve as biocatalysts for the anodic and cathodic reactions of a MFC operating at low pH. High current densities and power output were measured in a sediment MFC configuration. Presumably, with adequate engineering of MFC using these acidophilic biocatalysts, power outputs comparable to those obtained with single-chamber air cathodes, which rely on the use of Pt as cathode electrocatalyst, may be obtained.

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Suplementary information. Additional supplementary information is found in the online version of this article: Table S1 (probes sequences for FISH experiments), Table S2 (element composition in container 1 solution), Table S3 (16S rRNA clones identified on the anode and cathode), Fig. S1 (polarization curves for different pairs of electrodes in container 1), Fig. S2 (DGGE fingerprints of 16S rRNA).

Competing interests. None declared.

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Supplementary Table S1. FISH probes for bacteria quantification one electrodes

Probe	Target	Sequence (5_ to 3_)	% FM[a]	Specificity
Eub338	16s	GCT GCC TCC CGT AGG AGT	35	*Bacteria* domain
Acd638	16s	CTC AAG ACA ACA CGT CTC	20	*Acidiphilium* spp.
Thio1	16s	GCG CTT TCT GGG GTC TGC	35	*Acidithiobacillus* spp.
Ntr712[b]	16s	CGC CTT CGC CAC CGG CCT TCC	35	*Nitrospira* group
Non338	–	ACT CCT ACG GGA GGC AGC	35	Negative control

[a]Percent (vol/vol) formamide in the hybridization buffer.

[b]Used together with competitor probe Ntr712c (50-CGC CTT CGC CAC CGG TGT TCC-30)

Table S2. Element composition of container 1 solution

Element	Concentration (ppm)
Al	2900 ± 150
Si	1270 ± 90
P	452 ± 16
S	9881 ± 23
Cl	51 ± 3
Ar	21 ± 1
K	0.9 ± 0.1
Ca	314 ± 1
Sc	1.7 ± 0.4
V	1.5 ± 0.2
Mn	243.0 ± 0.8
Fe	5529 ± 14
Co	8.4 ± 0.7
Ni	4.9 ± 0.1
Cu	2223 ± 6
Zn	550 ± 1
Ga	0.9 ± 0.1
Ge	< 0.1
As	26.6 ± 0.2
Br	0.50 ± 0.05
Rb	< 0.6

Table S3. 16S rRNA clones recovered from electrode surfaces

Clones	Electrode	Related BLAST sequences	% Similarity
1	Anode	*Acidiphilium sp. DC2*	96
2	Anode	*Acidiphilium cryptum JF-5*	98
3	Anode	*Acidiphilium* sp *SB8*	95
4	Anode	*Acidiphilium sp. DC2*	97
5	Anode	*Acidiphilium sp. DC1*	97
6	Anode	*Acidiphilium sp. DX1-7*	97
7	Anode	*Acidiphilium sp. DC2*	97
8	Anode	*Acidiphilium sp. SB6*	96
9	Anode	*Acidiphilium sp. DC2*	96
10	Anode	*Acidiphilium sp. DBS4-1*	95
11	Anode	*Acidiphilium sp. DC1*	98
12	Anode	*Acidiphilium sp. DC1*	97
13	Cathode	*Acidiphilium sp. SB8*	98
14	Cathode	*Leptospirillum sp.*	97
15	Cathode	*Acidiphilium sp. DC2*	97
16	Cathode	*Acidithiobacillus ferrooxidans*	96
17	Cathode	*Acidiphilium sp. DC1*	96
18	Cathode	*Acidiphilium sp. DC2*	97
19	Cathode	*Acidiphilium sp. SB8*	97
20	Cathode	*Acidiphilium sp. BGR*	97
21	Cathode	*Acidiphilium sp. DC1*	96
22	Cathode	*Acidiphilium sp. DX1-4*	96
23	Cathode	*Leptospirillum ferriphilum*	96
24	Cathode	*Acidiphilium sp. SB8*	97
25	Cathode	*Acidiphilium sp. DC1*	98

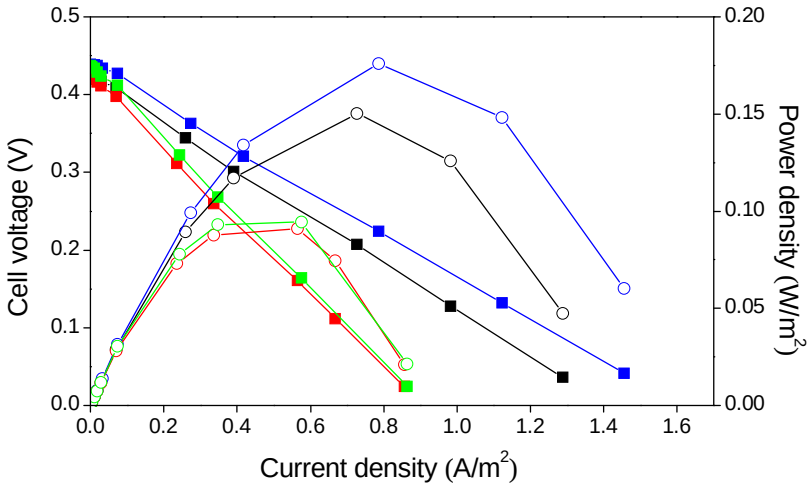

Fig. S1. Polarization (filled squares) and power density (open circles) curves measured with 4 different pairs of electrodes after 7 days of being placed in the sediment or water region of container 1 of the microcosms. The blue points correspond to the pair of electrodes of Figs. 2-4 and Fig. S2.

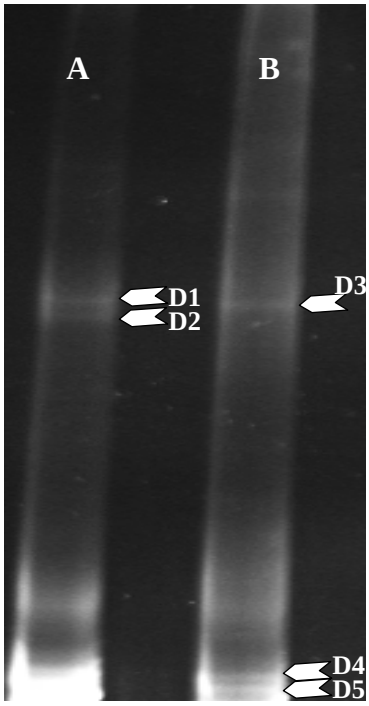

Fig. S2. DGGE fingerprints of 16S rRNA (marked by arrows) obtained by using universal primers for members of the domain *Bacteria*. (A) Anode. (B) Cathode.