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Archaea-based microbial fuel cell operating at high ionic strength conditions

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Abstract In this work, two archaea microorganisms (*Haloferax volcanii* and *Natrialba magadii*) used as biocatalyst at a microbial fuel cell (MFC) anode were evaluated. Both archaea are able to grow at high salt concentrations. By increasing the media conductivity, the internal resistance was diminished, improving the MFC's performance. Without any added redox mediator, maximum power (P_{max}) and current at P_{max} were 11.87/4.57/0.12 μW cm^{-2} and 49.67/22.03/0.59 μA cm^{-2} for *H. volcanii*, *N. magadii* and *E. coli*, respectively. When neutral red was used as the redox mediator, P_{max} was 50.98 and 5.39 μW cm^{-2} for *H. volcanii* and *N. magadii*, respectively. In this paper, an archaea MFC is described and compared with other MFC systems; the high salt concentration assayed here, comparable with that used in Pt-catalyzed alkaline hydrogen fuel cells, will open new options when MFC scaling up is the objective necessary for practical applications.

Keywords Electricity generation · MFC · *Escherichia coli* · *Haloferax volcanii* · *Natrialba magadii* · Nafion

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Introduction

A microbial fuel cell (MFC) is a device that converts chemical energy stored in organic substances or other reduced compounds into electrical energy by using microorganisms as biocatalysts. Microbial fuel cells have had a long history in the academic world, from the first description of the phenomena by Potter (1911), when he placed a platinum electrode into cultures of yeast or *E. coli* and showed that a potential difference was generated. Later, MFCs were rediscovered by Bennetto (Bennetto 1984; Allen and Benneto 1993).

Currently, there are several factors limiting the performance of MFCs and inhibiting the progress of applying MFCs in practice. These limiting factors include the activity of biocatalysts (microorganisms), electrodic reactions (both in cathode and anode), internal resistance and reactor design, among others. In the last 10 years, a new paradigm about extracellular electron transfer without the assistance of extracellular (added or produced by bacteria) redox mediators (as flavins) has taken into consideration new limiting factors and mechanisms. Direct electron transfer via outer-surface c-type cytochromes, long-range electron transfer via microbial nanowires and electron flow through a conductive biofilm matrix containing cytochrome have been proposed (Baron et al. 2009; Lovley 2011). Besides, the chemical and physical working conditions of MFCs (at least at the bio-anode compartment) are dictated by the nature of the biological component, a key element of MFCs. Depending on the microorganism and growth conditions, changes in the external chemical and physical conditions can bring about alterations in several primary physiological parameters, inhibiting growth and metabolism, and eventually causing the death of the microorganisms used as biocatalysts at the anode.

In the last few years, several MFC designs have been assayed, in which multiple combinations of electrode material, microorganism and other parameters were evaluated, as discussed in recently published reviews (Rabaey and Verstraete 2005; Bullen et al. 2006; Davis and Higson 2007; Debabov 2008; Osman et al. 2010; Lovley 2011).

A previous study (Jang et al. 2004) showed that an MFC could be operated increasing the salt concentration at the cathode compartment, without affecting the survival of the microbial communities at the anode. The study showed that when NaCl concentration at the cathode was increased from 0.1 to 1 M, the produced current increased from 3.5 up to 7.7 mA. Also, Liu et al. (2005) proved that by increasing the anode ionic strength (IS) from 100 to 400 mM of NaCl, the internal resistance (R_{int}) was lowered and the maximum power density was increased. However, electricity production at MFCs, as far as we know, has been only previously linked to the metabolic activity of only very few extremophiles, salt-tolerant microorganisms. Arsenate respiring bacteria isolated from moderately hypersaline Mono Lake (*Bacillus selenitireducens*) was previously used, with a maximum power of 18.5 mW m^{-2} (Miller and Oremland 2008).

The objective of this research was to investigate the performance (by means of polarization curves) of halophile archaea used at MFC anode and the effect of high salt concentration both at the anode and cathode compartments. We hypothesize that such an increase at the media conductivity would improve the MFC's performance (electricity production) by reducing R_{int}, among other possible factors. To achieve this, two halophilic archaea were used and the results obtained were compared with a bacterial strain, using an identical hardware (*H. volcanii*, *N. magadii* and *E. coli*, respectively). *E. coli* was used previously by other authors in mediated and non-mediated MFCs (Ieropoulos et al. 2005) and it is worthy of comparison studies.

We evaluated these microorganisms at non-mediated (more precisely, non-added mediator) and mediated MFCs, where a mediator (neutral red, NR) was used to improve electron transport between the oxidative microbial metabolism and the anode surface. Also, the use of NR allows us to compare the different MFCs in a condition where the current is not limited by naturally occurring mediators or mediator-like substances that could be presented in the culture media produced by the microbial strains used. NR was used because it has a redox potential of −325 mV, similar to that of NADH (−320 mV vs. SHE), and a structure similar to that of flavins. Its redox potential suggests that NR could interact with metabolic steps prior to respiratory chains (McKinlay and Zeikus 2004). The MFCs described here are based on plain Toray® carbon paper electrodes, and Nafion® was used as a proton transporter membrane.

We show in this work that an archaea microorganism can be used as a biocatalyst in MFCs, and electricity generation is possible. Furthermore, the effect of high conductivity in both current production and internal resistance is shown. Previously published works, where biofilm-forming electrogenic bacteria were used, showed serious scaling-up limitations. Here, we open new possibilities for the design and operation of MFCs.

The new and amazing possibilities of *H. volcanii* and other extreme microbial physiologies could be a key to increase the maximum current density and power obtained with MFCs. The use of an added mediator allowed us to compare both ionic strength conditions; for potential applications, many naturally occurring or microbially synthesized compounds can serve as electron carriers.

Experimental

Microbial strains and microbiological methods

H. volcanii strain DS70 (DS2 cured of pHV2, Wendoloski et al. 2001) was grown aerobically at 35°C, until an OD (600 nm) of ca. 1 was reached. Growth medium Hv-YPC contains (gL^{-1}): yeast extract (5), peptone (1), casamino acids (1), NaCl (144), $MgSO_4$ $7H_2O$ (21), $MgCl_2$ $6H_2O$ (18), KCl (4.2), $CaCl_2$ (0.35) and Tris–HCl (1.9), pH was adjusted to 7.0.

N. magadii (ATCC 43099) was grown aerobically at 37°C with shaking at 200 rpm. Growth medium composition was (gL^{-1}): yeast extract (5), NaCl (200), Na_2CO_3 (18.5), sodium citrate (3), KCl (2), $MgSO_4$ $7H_2O$ (1), $MnCl_2$ $4H_2O$ (3.6×10^{-4}) and $FeSO_4$ $7H_2O$ (5×10^{-3}), with pH adjusted to 10 (modified from Tindall et al. 1984). The optical density of the cultures was spectrophotometrically measured at $\lambda = 600$ nm until it reached an OD of ca. 0.5.

Escherichia coli K-12 derived strain (ATCC 15153) was used. It was grown aerobically at 37°C until an OD (660 nm) of ca. 1.0 was reached. Tryptic Soy Broth (DIFCO) was used as culture media, dissolving 30 g in 1 L of distilled water, and adjusted to pH 7.2 if necessary. The broth prepared in this way contains (gL^{-1}), pancreatic digest of casein (17), enzymatic digest of soybean meal (3), NaCl (5), K_2HPO_4 (2.5) and glucose (2.5).

Microbial fuel cell hardware

The MFC used had two compartments with a volume of one-liter, separated by 4 cm^2 of Nafion® 115 membrane (from FuelCellStore, San Diego, CA, USA). The cell was made on 6-mm-thick transparent acrylic, and had a lid with

six 0.6-cm-diameter holes, allowing connections for electrodes, gas bubbling and sample removal/reagent addition. Before inoculation with the microbial biocatalyst, the anolyte solution was purged with N_2 for 20 min to remove oxygen. The catholyte solution was bubbled continuously with air to allow mixing. The electrode separation was 2 cm. Before its use, the cell was sterilized by immersion overnight into 10% v/v H_2O_2, followed by distilled (double osmosis) sterile water rinse.

Electrodes

MFC cathode and anode were made of plain carbon paper TGP-H-030 (Toray®, Tacoma, WA, USA), with a density of 0.40 g cm^{-3} and a porosity of 80%. The geometrical area of anodes and cathodes was 10 cm^2. Before their use, they were cleaned by consecutive immersion during 1 h in 1 mol L^{-1} HCl and NaOH, rinsed exhaustively and stored in distilled sterile water.

Catholyte and anolyte composition

Haloferax volcanii anolyte was the Hv-YPC growth medium. In some experiments, a final concentration of 0.1 mM in NR was used as redox mediator. The ionic strength (IS) was ca. 2.68 M. The catholyte was modified *H. volcanii* growth medium Hv-YPC; yeast extract, peptone and casamino acids were not included, but contained ferricyanide (8.4 gL^{-1}) and the IS was ca. 2.72 M.

Natrialba magadii anolyte was *N. magadii*-grown medium. In some experiments, a final concentration of 0.1 mM in NR was used as mediator; IS was ca. 3.63 M. The catholyte was modified *N. magadii* growth medium; yeast extract was not included. The ferricyanide concentration was 8.4 gL^{-1} and IS was ca. 3.67 M.

The *E. coli* anolyte contained (gL^{-1}), glucose (5), Na_2HPO_4 (6), yeast extract (5), KH_2PO_4 (3), NH_4Cl (1), NaCl (0.5), $MgSO_4$ $7H_2O$ (0.12) and $CaCl_2$ (0.01) dissolved in distilled water, pH adjusted to 7.0. In some experiments, the final concentration of 0.1 mM in neutral red (NR) was used as mediator. The ionic strength was ca. 92.0 mM. The catholyte contained (gL^{-1}), Na_2HPO_4 (6), KH_2PO_4 (3), NaCl (0.5) and ferricyanide (8.4); The pH was adjusted to 7.0 and IS was ca. 125.0 mM.

MFC setup and operation

The MFC was operated at 37°C with continuous air purging (cathode) to provide agitation. N_2 purging (anode) was used during measurements and 20 min before measurements or start-up. After purging, N_2 was used to provide agitation; no mechanical or magnetic stirring was used. The pellet obtained by centrifugation of 400 (*E. coli* and *H. volcanii*) or 800 (*N. magadii*) mL culture was used as inoculum (start-up) at the anode compartment. The inoculated MFCs were allowed to stabilize overnight, with an external resistor (RL) of 4.7 kΩ, used in continuous operation. Before performing voltammetric or polarization curve studies, the RL was disconnected for 6 h to allow the system to reach open circuit (OC, without any external resistor) potentials. The experiments were usually carried out for 7 days. The polarization curves presented here represent typical experiments acquired when the OC and P_{max} reached stable and elevated (plateau) values.

MFC analysis

To study the behavior of the MFC and its electrical characteristics, measurements were carried out, intercalating different external resistors (RL). Polarization curves were obtained using RLs from 100 kΩ to 2.3 Ω; the potential E was measured by a digital tester with PC recording capabilities (Fluke 289). Current (I) production was calculated using Ohm's law ($I = VR^{-1}$), where V is the voltage and R the resistance. Current density, j (A cm^{-2}), was calculated as $j = IS^{-1}$, where S is the geometrical (projected) surface area of the anode electrode. Power density, P (W cm^{-2}), was calculated as $P = IVS^{-1}$. Internal resistance (R_{int}) was calculated from the slope of the plots of V and I, using $V = E_{cell} - IR_{int}$, where E_{cell} is the electromotive force of the cell (Logan 2008). We eliminate the data from the regions I and III (where polarization behavior is dominated by activation potential and mass transfer overpotentials) to construct quasi-linear plots for computing R_{int}.

Electrochemical studies

Cyclic voltammetry at low scan rate (1 mV s^{-1}) using a standard three-electrode system was used to search for possible redox mediators at *H. volcanii* culture media or microbial culture; phosphate buffer (100 mM, pH 7) was used so as to investigate possible redox substances at the Toray paper electrode. The window potential applied was from −400 to +500 mV; in order to obtain voltammograms in static conditions, N_2 was bubbled for only 10 min; following another 10 min (quiet time), the CVs were initiated.

A plain Toray paper anode was used as working electrode (WE), stainless steel wire as counter electrode (CE) and Ag/AgCl (KCl saturated) as reference electrode (RE). To investigate the NR reaction at the Toray electrodes, at the high IS used and in the presence of *H. volcanii*, CVs between −700 and +700 mV were performed. N_2 was used to remove oxygen and CVs at 50, 100, 200 and 400 mV s^{-1} were performed. All CVs were carried out in

quiet solutions; before the beginning of the experiment, and the WEs were poised for 1 min at the initial potentials. We used a potentiostat TEQ 03 (Ing. Sobral, La Plata, Argentina) with data acquisition and control via proprietary software.

Results and discussion

Polarization and power curves

Figure 1 shows typical polarization and power curves for two of the three strains evaluated; we present four independent experiments with each strain (different inoculations) for *H. volcanii* and *E. coli* with and without an added redox mediator (neutral red). Numerical results can be observed in Table 1. When *H. volcanii* was used as an anodic biocatalyzer, the maximum power density (P_{max}) without an added mediator was 11.87 ± 0.54 μW cm^{-2}, almost a 100-fold increase with respect to *E. coli*. We applied an independent two-sample *t* test to compare the P_{max} obtained with both microbial strains, which provides evidence that the means are significantly different at $p \leq 0.01$, showing that the two strains produce different power output. The same trend was observed when the current density (*j*) was compared, and values of 0.58 ± 0.16 and 49.67 ± 0.81 mA cm^{-2} were obtained for *E. coli* and *H. volcanii*, respectively. *j* values presented are the ones obtained at P_{max}, as customary and data presented are averaged $\pm SD_{(n-1)}$.

Also in Table 1, we show the effect of NR addition. Here, the maximum power increases approximately five times in both *E. coli* and *H. volcanii* MFCs, showing that charge transport between microbial cells and electrodes could be improved by an external mediator.

Furthermore, in Table 1, our results are compared to the ones published by other authors. Our results show that *P* and *I* produced by our mediated archaea-based system were exceptionally superior to other mediated systems, reaching values approaching the highest standards established recently by non-mediated biofilm-based MFCs (Ishii et al. 2008). Although our non-mediated MFCs could be comparable concerning their electrical performance with other MFCs designs (Table 1), several critical factors (geometrical design, electrode size, membrane, etc.) forbid a direct comparison with other published data. However, when comparisons are made using the results obtained in this work, by means of the same MFC hardware, valid conclusion can be elaborated. It may be noted that R_{int} (one important performance-limiting factor) is strongly influenced by MFC setup, geometry, electrodes, etc., and that the data presented by other authors are only partially comparable.

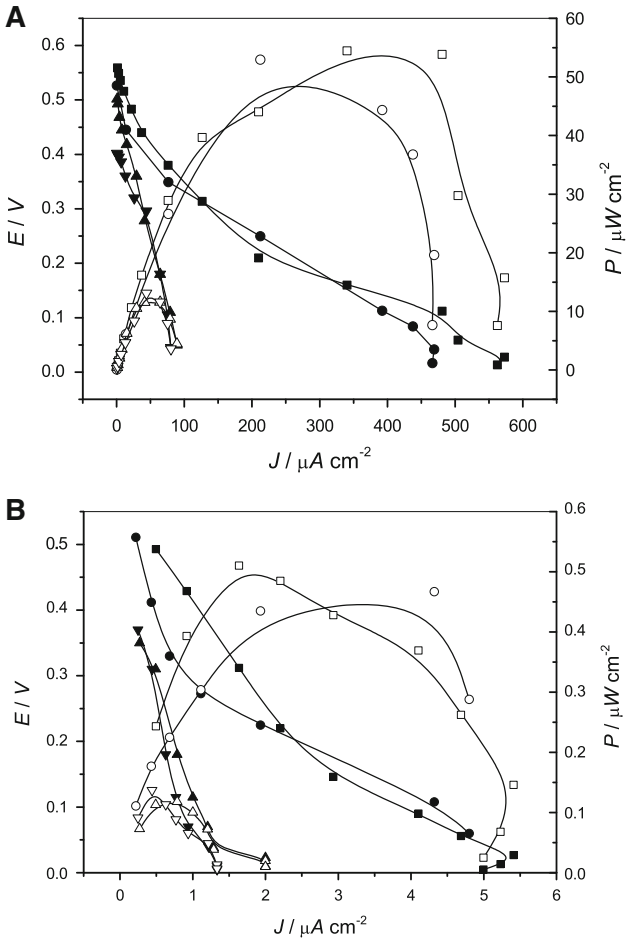

Fig. 1 MFC power density (*open symbols*) and voltage (*filled symbols*) as a function of current density (normalized to total geometrical electrode area). **a** *H. volcanii* without neutral red (*triangles*) and with neutral red (*circles* and *squares*). Two independent experiments are plotted. **b** *E. coli* without neutral red (*triangles*) and with neutral red (*circles and squares*). Two independent experiments are plotted

Some authors have obtained very interesting results for axenic cultures. Rabaey et al. 2005 reported a maximum of 88 mW m^{-2} for *P. aeruginosa* without an added mediator (this bacterium produces pyocyanin, which in this work is reported to be necessary for efficient electron transfer). Also, a maximum of 91 mW m^{-2} for *E. coli* with redox mediator has been reported (Park and Zeikus 2003). However, the comparison between different MFC setups is only partially accurate. Park et al. used *E. coli* in his setup to produce about 19 times more power than in ours, so we can assume that this difference is related mainly to the setup, and also that the effect of a Mn^{4+}–graphite anode and a Fe^{3+}–graphite cathode could in part be responsible. We hypothesize that, in such optimized MFC, *H. volcanii* could produce higher power densities.

We show that by adding a soluble mediator to halophile archaea (*H. volcanii*), the MFC's performance is improved

Table 1 MFC maximum power density (P_{max}), and R_{int} for different MFC systems

Biocatalyzer	Mediator	R_{int} (Ω)	P_{max} (mW m^{-2})	IS (mM)	pH	References
H. volcanii	NM-MFCs	447 ± 46	118.7 ± 5.4	2680	5.9	This work
H. volcanii	NR	66 ± 10	509.8 ± 36.6	2680	5.9	This work
N. magadii	NM-MFCs	962	45.7	3635	10	This work
N. magadii	NR	1038	53.8	3635	10	This work
E. coli	NM-MFCs	2433 ± 17	1.21 ± 0.08	92	7.0	This work
E. coli	NR	708 ± 14	4.71 ± 0.33	92	7.0	This work
E. coli[a]	NR	11160	12.7	300	7.0	Ieropoulos et al. (2005)
Domestic wastewater inocula	NM-MFCs	161	720	100	7.0	Liu et al. (2005)
		91	1100	200		
		83	1200	300		
		79	1330	400		
Sludge inocula	NM-MFCs	1087	44.4	100[b]	7.0	Oh and Logan (2006)
		625	75.6	400[b]		
Geobacter metallireducens[c]	NM-MFCs	19920	2.2	158	7.0	Min et al. (2005)
		1286	40	158		

NM-MFCs are non-mediated MFCs, where direct electron transfer from bacteria to anode is postulated to occur. IS is the estimated ionic strength of the anolyte solution

[a] Recalculated data considering anode geometrical area (10 cm^2); the indicated P_{max} corresponds to an RL of 10 kΩ

[b] IS was estimated from data presented by the authors

[c] Data obtained from 2 types of MFC, salt bridge (R_{int} = 19920 Ω) and membrane-based MFC with lower R_{int}

almost a 100 times with respect to a non-halophilic bacterium (*E. coli*). Co-culture of *H. volcanii* and a mediator-producing halotolerant/halophile bacterium (or other combinations) could make the incorporation of any redox mediator unnecessary, an obvious problem when real-world applications are prospected. Also, it is known that wastewater and sediment contain many naturally occurring substances (humic acids, iron, sulfide ions), which are known to facilitate electricity generation (Reimers et al. 2001).

Complementary work was done using a haloalkaliphilic archaea at extreme pH (pH 10), *N. magadii* (Fig. 2; Table 1). In this experiment, 800 mL of *N. magadii* culture was used as anodic microbial suspension and the polarization curves were measured after 2, 24 and 48 h. Then, an external mediator was added at 0.1 mM final concentration, and a final polarization curve was measured after 4 h. The absorbance of the anodic microbial suspension was 0.81, 0.96 and 1.01 for 2, 24 and 48 h, respectively. The experiment showed that P_{max} increased from 1.01 to 1.92 and 4.57 μW cm^{-2} as incubation time and absorbance increased. The increase in current and power production is likely related to the metabolic behavior of the archaea cells, given that the absorbance increased only slightly. The addition of an external mediator in this alkaline system (pH 10) slightly increased the values obtained previously without redox mediator. Using this system, it could be possible that other current limitations occur (as proton

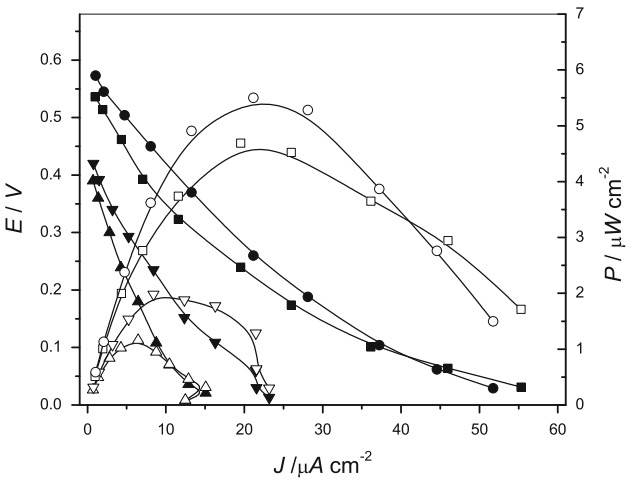

Fig. 2 MFC power density (*open symbols*) and voltage (*filled symbols*) as a function of current density (normalized to total geometrical electrode area). *N. magadii* without neutral red at increasing absorbance, (*upper triangle*, *lower triangle*, *squares*) and with neutral red (*circles*)

availability) which are stronger limitations to current production. Also, the differential membrane/metabolic characteristics of *N. magadii* could be responsible for this effect. Remarkably, the power and current production in *N. magadii*, although it was the highest IS used in this work, was (without NR) ca. half of that obtained with *H. volcanii*. This effect could be related to the different apparent ionic mobility of H^+ in water, which is about 6–7

times more than that of Na^+ (Wraight 2006). Given the alkaline pH used in this experiment (pH 10) and the high NaCl concentration (200 gL^{-1}), Na^+ must be important in charge transfer processes both inside and outside the Nafion membrane.

Here, we compared three microbial strains using an identical hardware, demonstrating that the power and current were increased, and R_{int} decreased when high IS and a halophile microorganism were used. The use of halophilic archaea as MFC anode biocatalyst improves the current and power. This is probably an effect of the lower internal resistance of the halophilic, no mediator-added MFC based on *H. volcanii* (ca. 447 Ω).

Probably, these effects mainly depend on the anolyte and catholyte IS, and are poorly related to the microbial physiology of the microbial strains used, given the low growth and metabolic rates of *H. volcanii*, when compared with *E. coli*. The results obtained with *N. magadii* also show a positive effect of increased IS, but a negative effect of alkaline pH (pH 10) is possibly related to the different mobility of the ionic charge transporters at the anodic microbial suspension. However, more detailed experiments involving other halophile archaea are necessary to probe this hypothesis. When mediated systems were compared, the effect of NR was significant with *H. volcanii* and less so for *N. magadii* cells. It is possible that NR works better as electron shutter for *H. volcanii* than for *N. magadii*, but we do not have enough data to speculate about this phenomenon. Therefore, experiments involving other documented microbial mediators (methylene blue, humic acid) could be useful to understand the difference between the two halophilic archaea assayed here.

Internal resistance

Internal resistance (R_{int}) is a key performance driver of fuel cells (Barbir 2005). In mediated MFCs, ohmic resistance (R_Ω) is usually the most important contributing factor to R_{int}. The three sources of ohmic voltage loss are: (a) resistance to ion migration within the electrolyte, (b) resistance to electron transport within the fuel cell components (electrodes, gas diffusion layer, current collectors) and (c) contact resistances (Logan 2008). The salt concentration we used was comparable with alkaline fuel cells, 6.6 M KOH is habitually used (Burchardt et al. 2002), allowing very low R_{int} (less than 1 Ω cm^{-2}). Increase of NaCl is generally used at the electrolyte to improve the mass transfer of charged particles (Gil et al. 2003). The increase in the fuel cell performance with *H. volcanii* seems to be related to the increased mass transfer of charge transporters and to the increased proton availability in the cathode (pH decrease from 7.0 to 5.9 in *H. volcanni* MFC). MFCs were reported to have better performance using marine water and sediment when compared with wastewater-based ones (low IS), as reported previously (Tender et al. 2002; Bond et al. 2002). The effect of increased ionic strength was also assayed by Liu (Liu et al. 2005), where the ionic strength was increased from 100 to 400 mM, showing a noticeable power increase at high IS.

Electricity production at MFCs has been previously linked to the metabolic activity of only very few salt-tolerant bacteria (Miller and Oremland 2008), by using arsenate-respiring bacteria isolated from moderately hypersaline Mono Lake (ML, *Bacillus selenitireducens*) and salt-saturated Searles Lake, CA (SL, strain SLAS-1). When pure culture bacteria were used, very low current was obtained for both strains, 49 and 59 μW m^{-2}, respectively. When the bacteria were assayed at MFC together with lake sediment, which could have some naturally occurring redox mediator, significantly more power was produced. Also, in other experiments, more power is produced in MFCs with ML sediment (18.5 mW m^{-2}, salinity 90 gL^{-1}) than with SL sediment (1.2 μW m^{-2}, salinity 346 gL^{-1}). Although these results appear not to be consistent with our hypothesis (high power production at high salinity/IS), the highest power production at the Mono Lake is consistent with the following facts: microbial activities are greater in the Mono Lake, *B. selenitireducens* grows faster than strain SLAS-1, and inorganic electron donors, especially sulfide, are present in the Mono Lake sediment. Also, the presence in ML of a wider range of anaerobic bacteria capable of efficiently transferring electrons to the anode could be a possible reason.

The physical design of non-mediated, biofilm-based MFCs, where the distance between bioelectrochemical reactions and the anode is minimal (these reactions occurring mainly at the biofilm layer), allow lower R_{int} and higher currents. At the high salt concentrations used here, we achieved non-mediated (non-added mediator) and mediated MFCs with R_{int} comparable with a previously published work (Table 1). Our MFCs are expected to have relatively high R_{int}, considering the distance between electrodes and the presence of a Nafion membrane. High IS and neutral or acidic pH are effective ways to improve MFC performance, by lowering R_{int} considerably. Also, the incorporation of NR has the same effect at normal or high IS (Table 1). At pH 10, the effect over R_{int} is less, perhaps related to the lower proton availability.

The R_{int} in mediated MFCs, as the ones used here (dual-chamber, plain carbon electrodes, Nafion membrane), are usually in the kΩ range (Table 1, *E. coli*, Ieropoulos et al. 2005). However, using high IS in combination with the halophile *H. volcanii*, we were able to obtain values compared to those obtained in non-mediated MFCs. But any comparison is in some way obscured by the high

influence MFC design has over the majority of described performance factors, including internal resistance. To overcome this problem, we compare our archaea MFC with respect to a more widely studied *E. coli* MFC.

Anode and cathode potentials and pH

When the two halophilic archaea MFCs investigated here are compared, better performance could be expected to match with *N. magadii* anodes, given the highest IS of the anolyte and catholyte solutions; instead, its performance was significantly poor. This effect was also observed recently (Veer Raghavulu et al. 2009), where the effect of pH 6, 7 and 8 was assayed, finding lower P_{max} at alkaline conditions (pH 8) and higher ones at acidic conditions. This phenomenon was attributed to the effective extracellular e^- transfer at acidophilic pH compared to basic operations, or well related to a higher activity of intracellular e^- carriers. Also, Akiba et al. (1987) found 10 times less current when using alkaline microorganisms, which agree with our data (Akiba et al. 1987). But He et al. (2008) found better performance at pH 8–10, attributed to the cathodic reaction (air cathode) that was improved by increasing pH. In the mentioned work, the electrochemical impedance spectroscopy data demonstrated that the polarization resistance of the cathode was the dominant factor limiting power output. In our setup, using a ferricyanide cathode, we assured that the cathode and cathode-related reactions did not limit the current and power production, allowing us to focus our study at the anode reactions. Given the described conditions, probably the low performance of *N. magadii* MFC (when compared with *H. volcanii*) could be related to the low H^+ availability at pH 10 or to the biology of this archaea (metabolism, enzymes, membrane characteristics), or both.

The potentials of anode and cathode were measured with respect to a saturated Ag/AgCl reference electrode for *H. volcanii* MFC. When measured at OC or connecting RLs of 1195, 100.1 and 11.7 Ω, E_a (anodic peak potential) was −198, −140, 52 and 191 mV and E_c (cathodic peak potential) was 300, 301, 274 and 239 mV. The data showed that anodic limitations were more important when j was increased, an effect that was observed previously (Jadhav and Ghangrekar 2009). The pH values remain almost constant in the *E. coli* and *N. magadii* MFCs, but in the *H. volcanii* MFC the pH diminished during the first 24 h, reaching a value of 5.9, both at the anode and cathode compartments. During the anaerobic incubation, the accumulation of organic acids produced by the microbial strains assayed is expected, but a pH change was only evident in *H. volcanii* MFC. Probably, the buffer capacity at this MFC (lower than in *E. coli* MFC) was not sufficient to avoid pH changes.

Cyclic voltammetry studies

Electrochemical studies are usually carried out using thoroughly cleaned glass material, milli-Q water and high-purity metallic electrodes. When low-purity carbon electrodes and complex media, including living microbial cells, are electrochemically studied, the data obtained have inevitably more uncertainty, and, in some aspect, must thus be considered speculative. We evaluated our data following classical CV interpretations; comparing the results obtained using plain Toray paper electrodes at 1 mV s^{-1} on plain phosphate buffer, *H. volcanii* culture media and *H. volcanii* culture, without added NR (Fig. 3). It can be seen that both have a redox couple with an anodic peak (E_a) at ca. 128 mV and a midpoint potential of ca. 100 mV ($E_a + E_c/2$). This couple is apparently reversible, given that the anodic and cathodic currents are ca. 2.55 μA, and the potential difference between these two peaks are ca. 58 mV ($\Delta E_p = E_a - E_c$); both are reversibility criteria for one electron reaction. In this region, the plain buffer CV also shows an anodic peak at ca. 148 mV. These peaks present in all the CVs in Fig. 3 may be related to material adsorbed or included at Toray paper electrodes, given that this material is relatively "dirty" when compared, e.g., with glassy carbon, a more pure electrochemical-grade electrode material. Plain buffer CV does not show any cathodic peak; also, this voltammetry does not show any other feature at negative potentials. *H. volcanii* culture also shows a second quasi-reversible redox couple, with an E_a at ca. −264 mV, midpoint potential of ca. −300 mV and a ΔE_p of ca.

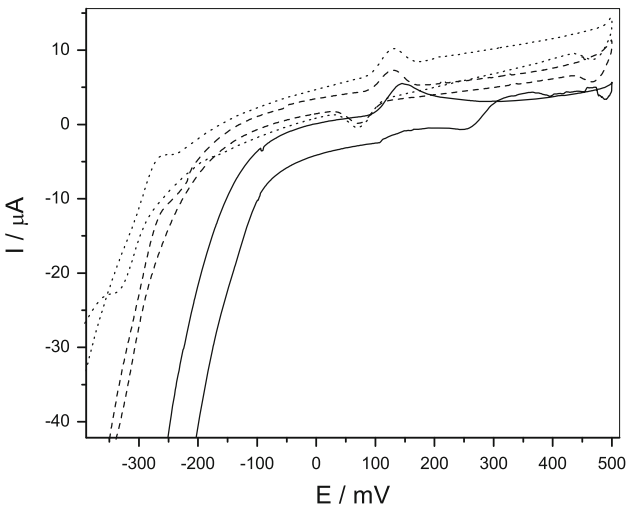

Fig. 3 MFC cyclic voltammetry of Toray paper anode. Plain phosphate buffer, 100 mM (*solid line*); *H. volcanii* sterile culture media (*dashed line*); *H. volcanii* culture (*dotted line*). No NR was added in any experiment; scan rate 1 mV s^{-1}

71 mV. At media and buffer CV, this redox couple does not seem to be present. This quasi-reversible couple could correspond to redox-active pigments, redox-active proteins or other substances produced by *H. volcanii* during growth and excreted/secreted actively by the cells, or else released from dead cells. The available information is insufficient to determine their nature.

Besides, the sterile medium shows some less evident redox couple at the same potentials; this means that, although in the experiments no NR was added, the current produced, at least in part, is probably assisted by chemical mediators. Other authors have found that quasi-reversible couples at CV increased with incubation time, related probably to secreted or biofilm accumulation of electrochemical mediators (Cercado-Quezada et al. 2010). These mediators, if produced at limited quantities at *H. volcanii* MFCs, probably limited the power and current produced, as evident when NR was incorporated.

The current observed at non-mediated *H. volcanii* MFCs are probably related to this electroactive substance, or with other compound in the media. Sulfate is important in the culture media (21 gL^{-1}) and it is well known that sulfur compounds are naturally present mediators at, for example, sedimentary MFCs. Also, Mn and Fe compounds have been proposed as mediators (Lowy et al. 2006). We do not have enough information to assign these peaks to a single redox substance, but it is evident that the current production in *H. volcanii* is assisted by external mediators. During *H. volcanii* growth, a redox soluble mediator could be produced; this strain produces carotenoid pink pigments, which are proposed as a shield against ultraviolet light. *Haloferax volcanii* contained 0.04% carotenoids of dry weight (Roslashnnekleiv and Liaaen-Jensen 1995). Some of these pigments could have redox properties and could be responsible for the peaks observed with a midpoint potential of −296 mV. Endogenous mediators can be produced by biofilm-growing bacteria, as demonstrated recently by Marsili et al. (2008), where flavins secreted into the growth media for *Shewanella* are responsible for at least a part of the charge transfer to the electrode, a phenomena also reported by other authors for the same bacteria (Von Canstein et al. 2007).

The behavior of NR over Toray paper electrodes and high IS was also investigated. CVs performed after 10 min of N_2 purge are shown in Fig. 4. The NR present at the *H. volcanii* growing media show a quasi-reversible behavior, with a midpoint potential of −442 mV. Previous studies (Park and Zeikus 2000) have shown a value of −524 mV (both vs. $Ag/AgCl_{sat}$) using fine-woven graphite felt as electrodes and with "normal" (ca. 100 mM) IS conditions.

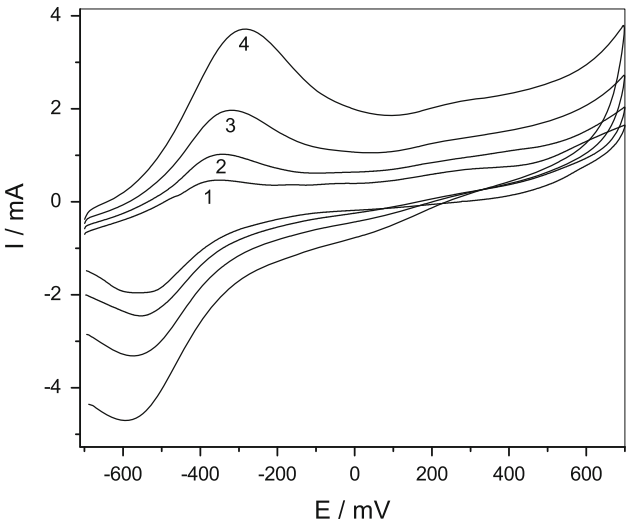

Fig. 4 MFC cyclic voltammetry of Toray paper anode. *H. volcanii* culture with added NR at increasing scan rate, 50 (1), 100 (2), 200 (3) and 400 (4) mV s^{-1}

Conclusions

In MFCs, the anode contains microorganisms capable of oxidizing organic material and releasing electrons and protons. While hydrogen fuel cells use high conductive electrolytes, almost all the work published in the field of MFCs have been limited so far to relatively low ionic strength anodic microbial suspensions, given the concentration limits imposed by the physiology of the microorganisms used.

The present study demonstrates that more than 0.1 or 0.5 W m^{-2} (non-mediated or mediated systems, respectively) can be achieved at the high ionic strength conditions used. The increase in power production seems related to the increased mass transfer of charge transporters and to the increased proton availability in the electrolyte, permitted by the amazing physiology of the *H. volcanii* archaea. Also, we show that neutral or acidic conditions are more favorable than alkaline ones, at least using our setup. The new and amazing possibilities of *H. volcanii* and other extreme microbial physiology could be a key to increase the maximum current density and power obtained with MFCs. The use of added mediators allowed us to compare both ionic strength conditions; for potential applications, many naturally occurring or microbially synthesized compounds can serve as electron carriers.

The approach used here for the first time could be a key to non-biofilm based MFC, allowing practical scaling-up. The requirement of the physical contact of the involved cells with the electrode (restricted to few microbial layers) limits the achievable density of active cells and thus the achievable power density. Moreover, high volumes of brine (around 70 gL^{-1} of salinity) are produced by reverse

osmosis installations around the world (mainly for drinking water production). These brines are simply pumped again into the sea, but they could be used in high IS MFC wastewater depurating installations. The high salt concentration assayed here, comparable with that used in Pt-catalyzed alkaline hydrogen fuel cells, and the use of extremophiles to cope with these conditions are new options to increase power production and MFC scaling-up necessary for practical applications.

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