Extremophiles (2008) 12:837–848
DOI 10.1007/s00792-008-0191-5

ORIGINAL PAPER

Electricity generation by anaerobic bacteria and anoxic sediments from hypersaline soda lakes

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Received: 1 July 2008 / Accepted: 1 September 2008 / Published online: 3 October 2008

Abstract Anaerobic bacteria and anoxic sediments from soda lakes produced electricity in microbial fuel cells (MFCs). No electricity was generated in the absence of bacterial metabolism. Arsenate respiring bacteria isolated from moderately hypersaline Mono Lake (*Bacillus selenitireducens*), and salt-saturated Searles Lake, CA (strain SLAS-1) oxidized lactate using arsenate as the electron acceptor. However, these cultures grew equally well without added arsenate using the MFC anode as their electron acceptor, and in the process oxidized lactate more efficiently. The decrease in electricity generation by consumption of added alternative electron acceptors (i.e. arsenate) which competed with the anode for available electrons proved to be a useful indicator of microbial activity and hence life in the fuel cells. Shaken sediment slurries from these two lakes also generated electricity, with or without added lactate. Hydrogen added to sediment slurries was consumed but did not stimulate electricity production. Finally, electricity was generated in statically incubated "intact" sediment cores from these lakes. More power was produced in sediment from Mono Lake than from Searles Lake, however microbial fuel cells could detect low levels of metabolism operating under moderate and extreme conditions of salt stress.

Keywords Hypersaline · Microbial fuel cell · Electricity · Arsenate

Communicated by T. Matsunaga.

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Abbreviation
MFC Microbial fuel cell

Introduction

Microbial metabolism in extreme environments can be weak and difficult to detect (Thauer et al. 1977; Oren 1999; Amend and Shock 2001). However, it is in just such environments that life may have originated and where it persists (Nisbet and Sleep 2001). Much of what we know about the biogeochemical processes occurring in extreme environments comes from laboratory studies using highly manipulated samples. For instance, studies with microorganisms isolated from sediment or water are far removed from in situ conditions. Electrochemical methods, including microbial fuel cells (MFCs), offer the possibility of studying microbially mediated redox reactions in nearly intact conditions. However, MFCs are typically used for other purposes; e.g. demonstrating power production during waste conversion or generating electricity in remote locations (see Rabaey and Verstraete 2005; Lovley 2006; Logan and Regan 2006a, b for reviews). Here we show that MFCs may be useful tools for studying the role of microbes in the terminal electron accepting processes occurring in the sediments of alkaline, hypersaline lakes.

Microbial fuel cells operate like a battery, with an anode and cathode separated by a cation exchange membrane. They rely on electron transferring bacteria (exoelectrogens) to catalyze the conversion of chemical energy to electricity. Bacterial oxidation of organic matter, H_2, or other reduced compounds provides electrons to the anode and thence through an electrical circuit to the cathode. Reduction of oxygen to water takes place at the cathode and consumes the

electrons, completing the circuit. Not all bacteria are exoelectrogens, and many of those with the potential to make electricity cannot transfer electrons efficiently to the anode without an exogenous electron shuttle or mediator (Park and Zeikus 2000). Nonetheless, electricity production using mediator-less MFCs is common (Reimers et al. 2001; Kim et al. 2002; Chaudhuri and Lovley 2003; Rabaey et al. 2004). High coulombic efficiencies (up to 89% of the electrons available) have been reported during power production from acetate using thermophilic microbes in mediator-less MFCs (Jong et al. 2006; Wrighton et al. 2008). However, electricity production has not previously been linked to the metabolic activity of haloalkaliphiles or other salt-tolerant microorganisms.

Dissimilatory arsenate reduction is an important electron accepting process in both moderately hypersaline Mono Lake (salinity 90 g l^{-1}) and extremely hypersaline (salt-saturated) Searles Lake (salinity 346 g l^{-1}). For example, arsenate reduction in the anoxic water column of Mono Lake (As 0.2 mM) consumed 14% of the electrons supplied by primary production (Oremland et al. 2000), surpassed only by aerobic oxidation and sulfate reduction. In Searles Lake (As 3.9 mM) arsenate reduction was the dominant anaerobic terminal electron accepting process, as both methanogenesis and sulfate reduction were inhibited by the high salt content in the sediments (Oremland et al. 2005; Kulp et al. 2006, 2007). In this paper, we show that cultures of arsenate respiring bacteria from these two environments generate similar levels of electrical power in microbial fuel cells. Further, we report that MFCs are capable of detecting electricity generated by micro-organisms in sediment slurries and in intact sediments from moderate and extreme hypersaline environments.

Materials and methods

Sediments and bacterial strains

Anoxic sediments were collected from the bottom of Mono Lake (ML) and Searles Lake (SL) California for use directly in sediment fuel cells and for preparing sediment slurries. Sediment organic carbon content was 3.5% in ML (Miller et al. 1993) and 0.5% in SL (TR Kulp, personal communication). Sediments were stored anaerobically at 4°C in completely filled glass jars sealed with metal lids for 4 years (ML) and 2 years (SL) before use. Such long storage times could change the proportions of anaerobic bacteria from their in situ values. Cultures of two arsenate respiring bacteria previously isolated from these lakes, namely *Bacillus selenitireducens* and candidatus *Halarsenatibacter silvermanii* strain SLAS-1, were maintained in our laboratory (Switzer Blum et al. 1998; Oremland et al. 2005; Switzer Blum et al. in preparation). *Bacillus selenitireducens* is a low G + C gram-positive non-spore-forming rod, while strain SLAS-1 is a gram-negative motile rod which occupies a deeply branched bacterial clade within the order Halanaerobiales. Cultures maintained on electron acceptors other than arsenate (e.g. nitrate) for any time were transferred and grown on 5 mM arsenate at least twice before use.

Artificial lake water used to prepare sediment slurries was also the mineral salts media used in MFC experiments. Artificial ML lake water prepared at the ambient ML salinity of 90 g l^{-1} was composed of the following (concentrations are g l^{-1} and are shown in parentheses): NaCl (75), $(NH_4)_2SO_4$ (0.1), KH_2PO_4 (0.08), K_2HPO_4 (0.15), $MgSO_4{\cdot}7H_2O$ (0.025), Na_2CO_3 (10.6), and $NaHCO_3$ (4.2). The conductivity of this solution, measured after 100-fold dilution, was 126 mS cm^{-1}. Artificial SL lake water was prepared at 0.9× the ambient SL condition of salt saturation (346 g l^{-1}) in order to prevent precipitation. This 302 g l^{-1} solution was composed of the following (concentrations are g l^{-1} and are shown in parentheses): NaCl (151), Na_2SO_4 (84), K_2SO_4 (25), $(NH_4)_2SO_4$ (0.05), KH_2PO_4 (0.08), K_2HPO_4 (0.15), $MgSO_4{\cdot}7H_2O$ (0.025), Na_2WO_4 (0.075), Na_2SeO_4 (0.00001), Na_2CO_3 (36), and $NaHCO_3$ (6). The conductivity of this solution, measured after 100-fold dilution, was 395 mS cm^{-1}. All media were adjusted to pH 9.8 with 0.1 M NaOH prior to use. Sediments were slurried (1:6) with artificial lake water under flowing nitrogen. Conductivities of sediment slurries were 3% (ML) and 14% (SL) greater than artificial lake water.

Description of MFCs

The three types of fuel cells employed in this study relied on the same external electrical circuit, including a fixed resistor (510 Ω) for determining power production. Voltage and current were measured hourly using an Agilent 3497A Data Acquisition/Switch unit. Power (Watts = Volts × Amps) was normalized to anode surface area and reported as power density (μW m^{-2}). Anode chamber and ambient temperatures were measured hourly using calibrated thermistors. At least two experiments were conducted for each environment (2) and each type of fuel cell (3) and results presented are representative. No electrode catalysts were used and no mediators were added to the anode chamber.

Culture MFC

Experiments with cultures of anaerobic bacteria were conducted in fuel cells made of two flow-through polycarbonate cylinders (10.8 cm diameter × 3.9 cm thick, 0.4 l) separated by a Nafion 450 cation exchange membrane (Dupont, Wilmington, DE), and sealed using neoprene

O-rings. Electrodes were constructed from solid graphite discs (Graphite Engineering, Greenville, MI; 2.5 cm diameter × 1.3 cm thick) connected to 14 AWG plastic coated stainless steel wire using conductive epoxy. The MFC (Fig. 1a) was designed with external reservoirs for sparging the anolyte and catholyte with N_2 and O_2, respectively ($\sim$30 ml min^{-1}), before and during experiments. Catholyte sparging maintained aerobiosis while anolyte sparging removed O_2 which may have diffused across the membrane. External sparging also allowed us to keep a single liquid phase in the electrode chambers. Initially, identical media (1.0 l artificial lake water) were provided to the anode and cathode. Media were circulated by peristaltic pump ($\sim$40 ml min^{-1}) through Norprene tubing to provide an active chamber residence time of about 10 min. Prior to inoculating the anode chamber with washed cells, the anolyte was amended with 5 or 10 ml of 2.5% cysteine-HCl, a reducing agent, and 5 ml of trace element solution (Widdle et al. 1983) required for growth. Electron donor (5 mmoles lactate) was added initially and one or more times during each experiment. Arsenate (3 or 5 mmoles) was added near the end of the experiment to serve as a competing alternative electron acceptor to the anode. Liquid samples were collected by syringe through septa attached to T-connectors in the tubing between the MFC and the peristaltic pump. Unfiltered samples for optical density (0.5 ml) were transferred to glass cuvettes and measured spectrophotometrically by absorbance at 680 nm wavelength. Additional aliquots (0.5 ml) were collected for direct counts of bacteria by fluorescence microscopy (Hobbie et al. 1977).

Slurry MFC

Experiments with sediment slurries were conducted in fuel cells made of glass tubes (5 cm diameter × 15 cm long, 0.3 l) connected in an H-shape (Thrash et al. 2007, Fig. 1b) and separated by a Nafion 450 cation exchange membrane (3.0 cm diameter). The tubes were closed at the top using rubber stoppers which allowed 14 AWG plastic coated stainless steel wire to pass through to connect with a solid graphite cuboid electrode (2.3 cm × 1.2 cm × 7.0 cm) and which also provided a gas-tight seal. Several glass ports held crimp-sealed rubber stoppers which were aligned vertically to provide access to gas (0.1 l) and liquid (0.2 l) phases during the experiment. Gases were added to or sampled from the anode chamber by syringe through the upper-most port. Mixing was achieved by placing the cell on a reciprocal (200 rpm) shaking platform.

Sediment MFC

A thick-walled (0.7 cm) polycarbonate cylinder (7.7 cm diameter × 15 cm, 0.7 l) with a sealed bottom contained the membrane-less sediment water microcosm MFC (Fig. 1c). Graphite disc electrodes (see open system, above) were secured near the center of the cylinder using plastic coated 14 AWG stainless steel wire. Mixed sediment (0.3 l) from ML and SL was transferred to the bottom half of the cell in an anaerobic glove box containing 90% N_2, 5% CO_2 and 5% H_2. Artificial lake water (0.3 l, see above) was slowly dispensed over the sediment to avoid stirring, and the sediment MFC was removed from the glove box and assembled for operation in a fume hood. Parafilm covered the open top of the cylinder in an effort to reduce evaporation. A gas dispersion tube bubbled air at 5 ml min^{-1} through the catholyte to maintain aerobiosis in the upper-half of the cell. The anode had a 0.003 cm groove cut in the upward facing surface which secured a loop of 0.005 cm diameter silastic tubing for diffusing H_2 to the electrode surface. The silastic tubing was routed horizontally to the anode from the cylinder wall along the coated steel wire and was plugged with a 25 g. needle connected to a 0.5 ml glass syringe when not in use. Hydrogen was introduced at various times by flowing gas through the loop of silastic tubing (20 ml min^{-1}) for several hours, or for extended periods (several days) by pressurizing the tubing to 10 kPa and capping the end. This allowed H_2 to diffuse though the tubing wall to sites very close to the electrode surface. Later, H_2 (0.5 ml) was added directly to the sediment near the anode using a glass syringe and 25 g needle.

Analytical

Samples of anolyte were collected from culture and slurry experiments. Aliquots (1 ml) for HPLC analysis of dissolved arsenate, arsenite, lactate, acetate and formate as well as for IC analysis of sulfate were filtered using Spin-X centrifuge filter tubes (0.2 μm; Corning Inc., Corning, NY) and stored at −80°C for up to 2 months prior to analysis (Kulp et al. 2007, Hoeft et al. 2004, 2007). Additional aqueous samples (1 ml) were collected from slurry experiments by syringe for total reduced sulfur (TRS) analysis (Fossing and Jørgensen 1989). TRS in these samples was fixed at the time of sampling by immediately reacting with 0.25 ml 10% zinc acetate contained in 4 ml Vacutainer vials and stored at 4°C for up to 3 months before analysis. TRS was reduced to sulfide using chromium and extracted in HCl under flowing nitrogen. Liberated H_2S was trapped in zinc acetate, analyzed spectrophotometrically (Cline 1969), and reported as sulfide. The detection limit for sulfide was 0.1 mM or 0.03 mmoles. Gas samples were collected from the anode chamber headspace of slurry experiments. Methane was analyzed by FID gas chromatography (Miller et al. 1993) and H_2 was analyzed by TCD gas chromatography (Oremland 1983).

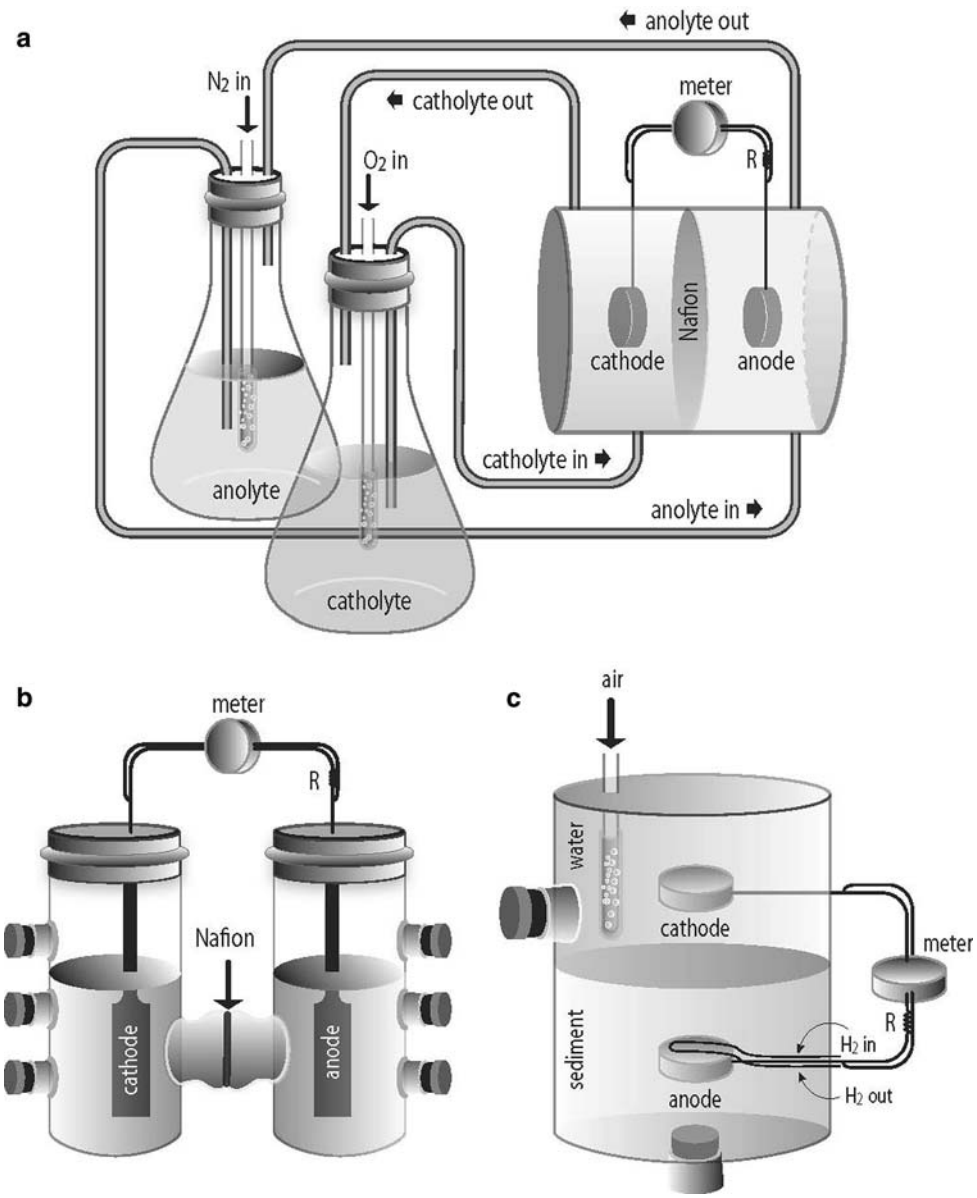

Fig. 1 Illustration of the three types of MFCs: **a** culture MFC, **b** slurry MFC and **c** sediment MFC. Catholytes were kept aerobic by flushing with O_2 (**a**) or air (**c**) or by free exchange with the atmosphere through a vent needle in (**b**). Anolyte in (**a**) was purged with N_2; otherwise anaerobiosis was maintained by the reducing power of anoxic sediment

Results

Experiments with anaerobic, haloalkaliphilic bacteria

Arsenic respiring haloalkaliphiles were first tested for their ability to produce electricity. Mono Lake (ML) and Searles Lake (SL) media were highly conductive and provided low internal electrical resistance, facilitating measurement of power production in fuel cells. No power was produced initially in MFCs either before or immediately after amendment with bacteria (Figs. 2a, 3a). A transient rise in power was observed in both abiotic experiments (not shown) and in bacterial culture experiments following the addition of reducing agent (cysteine-HCl) to the anode. This spike resulted from the rapid chemical oxidation of this compound as opposed to its utilization as a substrate (Logan et al. 2005). A delay in sustained power production corresponded to a lag phase in growth of both cultures. Conversely, the rapid rise in power after 4 days (*B. selenitireducens*) and 24 days (strain SLAS-1) coincided with a notable increase in optical density (Figs. 2c, 3c) indicative of growth.

Details of the timing and response of added bacteria, electron donors and electron acceptors were different for each incubation and bear explaining. Cysteine-HCl (5 ml) was added at the start (day 0) of both experiments. Washed cells of *B. selenitireducens* (Fig. 2a) was introduced on day 1, and no further addition of cells or reductant occurred. Similarly, washed cells of strain SLAS-1 were added at day 1 (Fig. 3a), however because no activity was observed in the first 9 days, an additional 10 ml cysteine-HCl, along

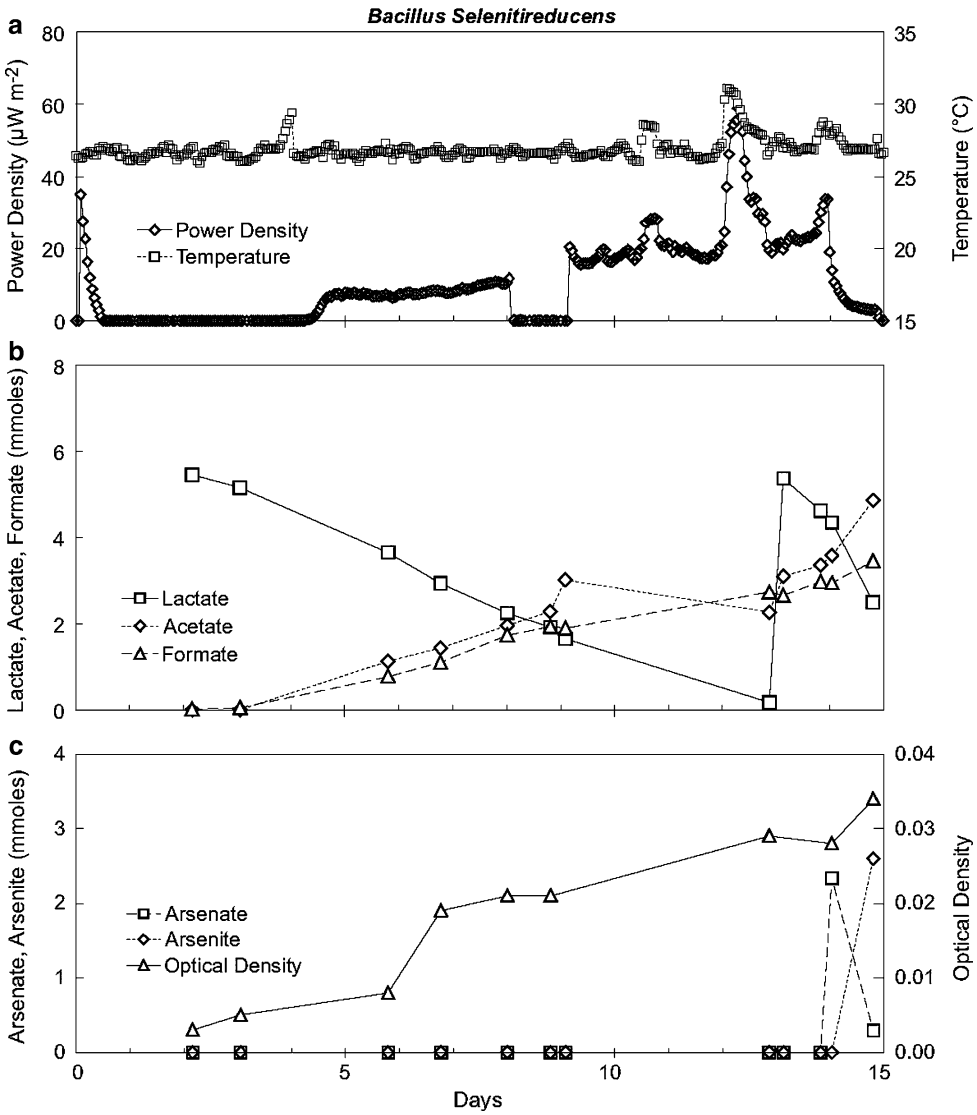

Fig. 2 Incubation of *B. selenitireducens* over 15 days in culture MFC with initial addition of 5 mmoles lactate. Power density and temperature in **a** using left and right *y*-axes, respectively. The circuit was deliberately opened on day 8 for 1 day. Arsenate was added on day 14 and O_2 added on day 15. Oxidation of lactate to acetate and formate is shown in **b** while optical density and reduction of arsenate to arsenite are shown in **c**

with 20 ml of actively growing SLAS-1 containing some lactate, acetate and both As(V) and As(III), was added at day 10. Peak power production (49–59 μW m^{-2}) occurred later and was similar in both experiments. Electrical output was dependant on the fuel cell temperature. Positive excursions in MFC temperature occurred when air conditioning was reduced on weekends and coincided with large, transient increases in power along any point in the power profiles. Diurnal temperature and power fluctuations were also observed.

The rate of lactate oxidation and the final products differed in experiments with the two bacterial cultures. *B. selenitireducens* oxidized lactate (5 mmoles) to equal amounts of acetate and formate within twelve days (Fig. 2b). A second addition of lactate (5 mmoles) on day 13 was more rapidly consumed. In contrast, only a small decrease (~1 mmole) in lactate and a corresponding smaller increase (<1 mmole) in acetate were observed during 40 days incubation with strain SLAS-1 (Fig. 3b) and no formate was formed.

Arsenate was added near the end of each experiment; at day 14 (3 mmoles, Fig. 2c) and at day 33 (5 mmoles, Fig. 3c). All of the added arsenate was reduced to arsenite within one day by *B. selenitireducens* whereas only 1 mmole arsenate was reduced to 1 mmole arsenite by strain SLAS-1. An immediate decrease in power was observed upon arsenate addition to both cultures (90% drop for *B. selenitireducens*, 80% drop for strain SLAS-1); however current continued to be generated by the cells. Power production was completely eliminated only when O_2 was introduced to the anode.

Experiments with sediment slurries

Dilute sediment slurries were next tested to determine if microbes were present that were capable of producing

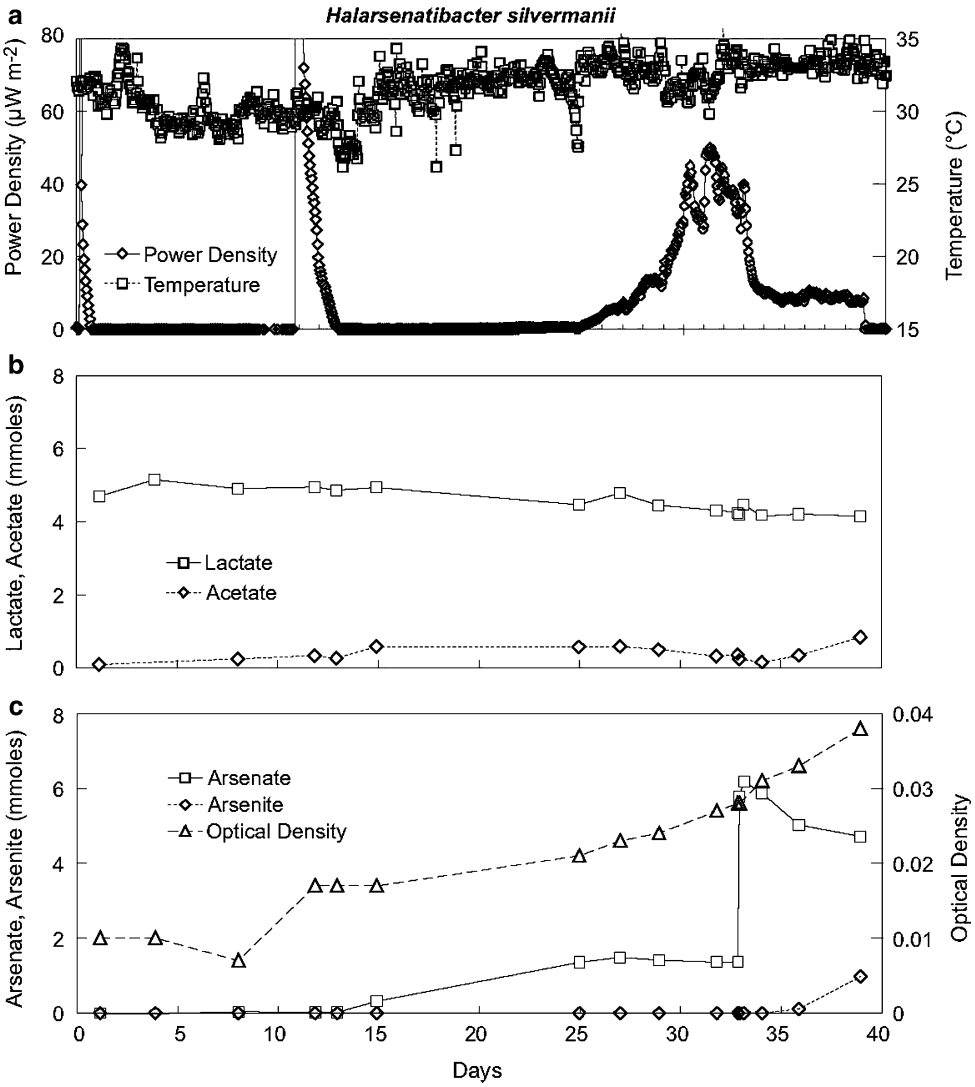

Fig. 3 Incubation of strain SLAS-1 over 40 days in culture MFC with initial addition of 5 mmoles lactate. Power density and temperature in **a** using left and right *y*-axes, respectively. Arsenate was added on day 33 and O_2 added on day 40. Oxidation of lactate to acetate is shown in **b**, while optical density and reduction of arsenate to arsenite are shown in **c**

electricity. Power produced by sediment slurries (Figs. 4a, 5a) followed similar patterns to power produced during incubations of cell suspensions of the two haloalkaliphiles. Diurnal fluctuations and weekend excursions in MFC temperatures were similarly reflected in power output. There was a delay (39 days) in power production at the start of the experiments with SL only. However, the most striking difference was that power production in the ML slurry was three orders of magnitude greater than the SL slurry. Power continued to increase at the end of both slurry experiments.

Lactate was neither added to the ML slurry nor detected, however acetate (~0.5 mmoles) was present throughout the incubation (Fig. 4b). Lactate (5 mmoles) was added to SL on day 20, and subsequently decreased by 2.9 mmoles over the next 10 weeks (Fig. 5b); however less than half as much acetate (1.2 mmoles) was produced during this time. Sulfate derived from the sediment (~5 mmoles) was present initially in the ML slurry and an additional 5 mmoles was added at day 14. There was no obvious sulfate consumption at either of these levels and no decrease in power output upon further addition of sulfate. Therefore, sulfate was not a significant alternative electron acceptor to the anode. However, sulfide increased in ML from an initial value near 0.2 mmoles to 0.4 mmoles at the end; hence some sulfate reduction must have occurred. Sulfate in SL (180 mmoles; derived from both sediment and artificial lake water) remained constant throughout the entire 13-week incubation (data not shown) and sulfide production was never detected in SL. Arsenate was added to the SL slurry (3 mmoles, day 69) which resulted in an immediate 50% decrease in power. The added arsenate (2.3 mmoles) was almost entirely reduced to arsenite (2.0 mmoles) over the final 3 weeks incubation.

Hydrogen, a readily utilizable source of electrons, was added at various times to the headspace of ML and SL slurries (Figs. 4c, 5c). However, consumption of added hydrogen had no impact upon power production. A

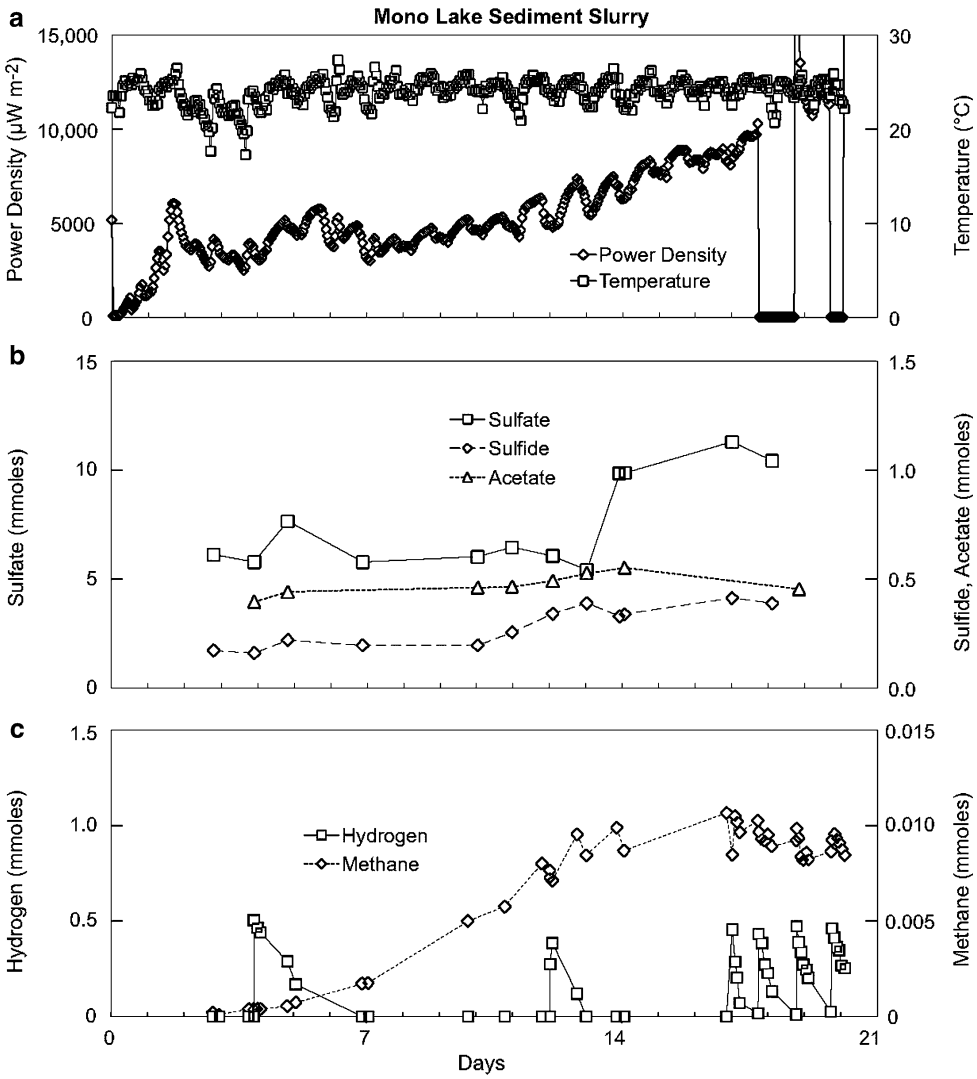

Fig. 4 Incubation of ML sediment slurry over 21 days in slurry MFC with no added lactate. Power density and temperature in **a** using left and right *y*-axes, respectively. The circuit was deliberately opened on days 18 and 20 for 24 and 8 h, respectively, to compare H_2 consumption rates with open and closed circuits. Sulfate, sulfide and acetate concentrations are shown in **b**, while H_2 consumption and CH_4 production are shown in **c**

separate experiment in ML examined the impact upon hydrogen uptake of removing the anode as electron acceptor by opening the circuit (days 18 and 20). No pattern was observed in the rate of hydrogen removal on those days compared with days 17 and 19 when the circuit was closed (Fig. 6). Further, the cumulative consumption of hydrogen (3 mmoles in ML and 1.5 mmoles in SL) had no observable impact upon the production of sulfide or methane.

Experiments with intact sediment columns

Mixed whole sediments from hypersaline soda lakes were tested to demonstrate electricity production in nearly unaltered conditions. Electricity generation commenced immediately in sediment fuel cells (Fig. 7a, b) and could be sustained for over 3 months (Fig. 7c). Power generally decreased over the first 4–5 days and stabilized thereafter. Diurnal temperature fluctuations (2–4°C range) had a noticeable effect upon power output. Both temperature and power fluctuations diminished when sediment fuel cells were placed in a water bath (Fig. 7c). In addition, increasing the temperature of the water bath (from 30 to 35°C) increased the amount of electricity generated by SL sediment. Power produced by ML sediment (Fig. 7a) was comparable to power produced by other sediment fuel cells (Reimers et al. 2001) however the amount of power produced in SL sediment was ~3 orders of magnitude lower (Fig. 7b, c). Addition of hydrogen to sediment near the anode, either directly or by diffusion through the silastic tubing, did not stimulate power production in ML or SL sediment.

Discussion

All of the MFC experiments reported herein produced electricity using Mono and Searles Lake sediment and

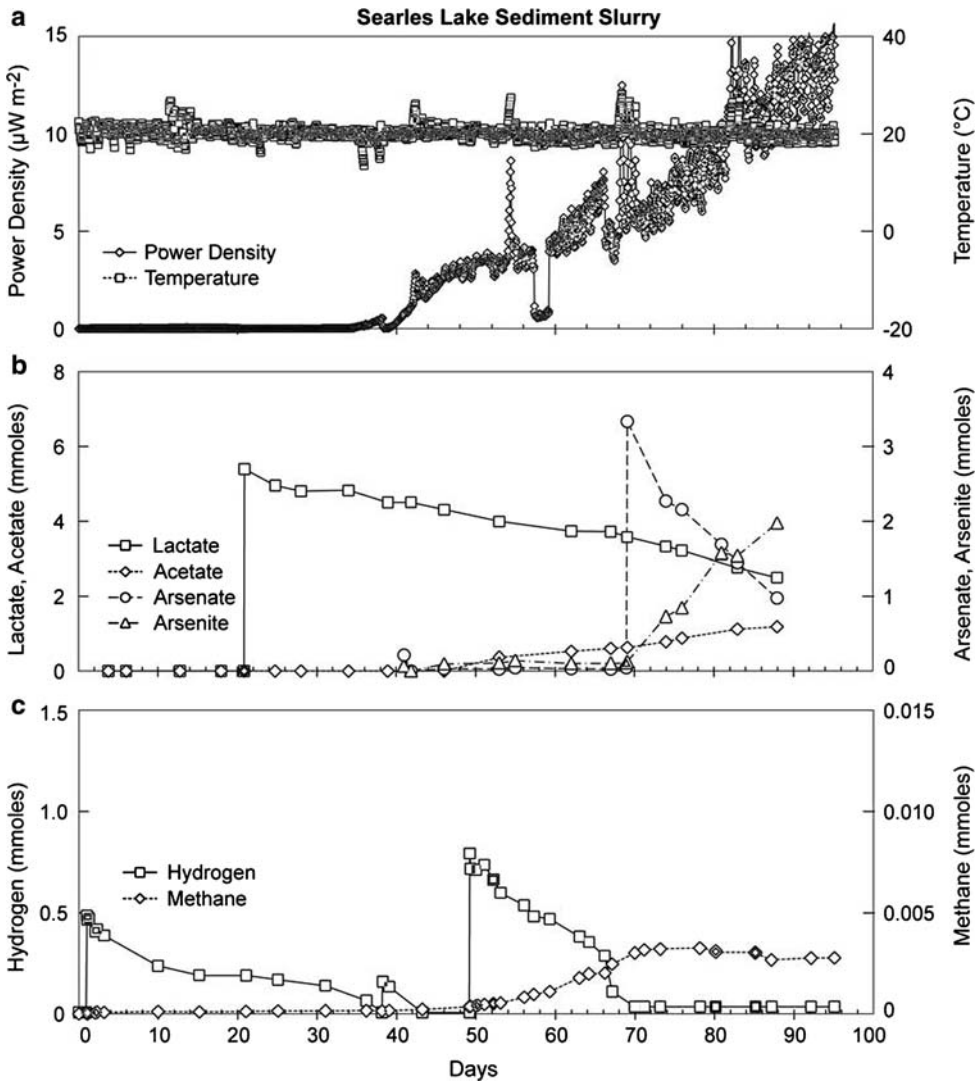

Fig. 5 Incubation of SL sediment slurry over 94 days in slurry MFC with lactate added on day 20. Power density and temperature in **a** using left and right *y*-axes, respectively. Lactate oxidation to acetate, and arsenate reduction to arsenite are shown in **b** while H_2 consumption and CH_4 production are shown in **c**

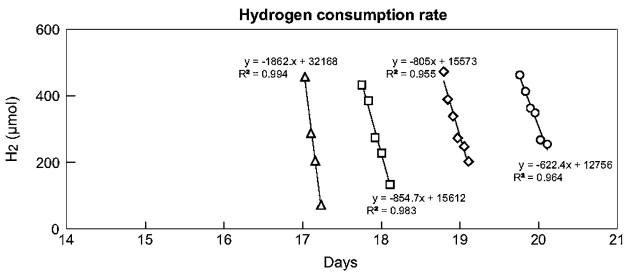

Fig. 6 Hydrogen consumption rate in ML sediment slurry during the final week of incubation in type-b MFC. The slope, *y* intercept and R^2 value of the linear regression of H_2 amounts (*symbols*) over time are presented with each uptake experiment conducted with the circuit closed (days 17 and 19) and with the circuit open (days 18 and 20). Multiplying the slope by 5 gives the uptake rate in μmoles $liter_{slurry}^{-1}$ d^{-1}

bacteria. Thus, MFC technology is sufficiently sensitive to detect weak in situ microbial metabolism in hypersaline environments (Oren 1999; Oremland et al. 2005; Kulp et al. 2006). No power was produced prior to growth or metabolism of arsenate respiring bacteria. Power increased as bacteria grew and decreased when an alternative electron acceptor to the anode was provided (Figs. 2a, 3a). Power also increased with elevated temperature and was likely a response to increased microbial metabolism as temperatures approached the optimum for each bacterium (30°C for *B. selenitireducens* and 44°C for strain SLAS-1; Switzer Blum et al. 1998; Switzer Blum et al. in preparation). Exogenous electron shuttles (Bond and Lovley 2002) were not added in our experiments to facilitate electron transfer from bacteria to the anode, hence power densities appear low.

Electricity generation was coupled to heterotrophic metabolism in anaerobic cultures. Lactate was oxidized to acetate plus other products using either the anode or arsenate as the electron acceptor. In previous batch cultures, washed cells of *B. selenitireducens* and strain SLAS-1 achieved equimolar conversion of lactate to acetate

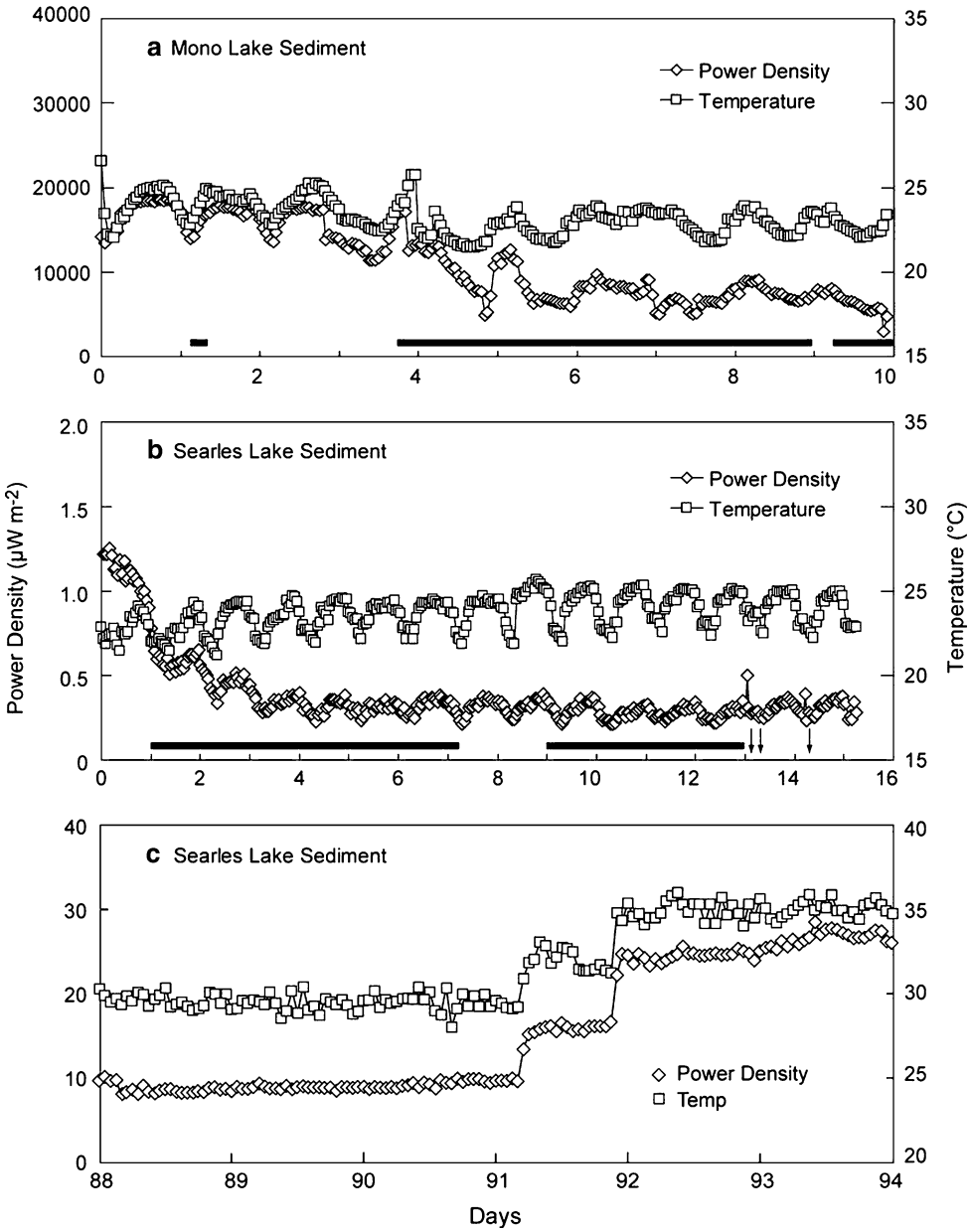

Fig. 7 Incubation of whole sediment from ML (**a**) and SL (**b**, **c**) in sediment MFC. Power density and temperature are shown using left and right *y*-axes, respectively. Addition of H_2 by diffusion through silastic tubing is indicated by *solid horizontal bars* near the *bottom* of the frame (**a**, **b**). Addition of H_2 by direct injection of 0.5 ml by syringe is shown indicated by *arrows* in **b**

(>90%) plus CO_2 (<10%; Switzer Blum et al. 1998; Oremland et al. 2005). In contrast, MFC conversion recoveries (acetate produced/ lactate consumed) were 60% for *B. selenitireducens* and 75% for strain SLAS-1. Sufficient formate was formed by *B. selenitireducens* to balance the lactate consumed in the MFC, however formate was not a product in incubations with strain SLAS-1. Hence, lactate was oxidized more completely (to formate or perhaps CO_2) by bacteria in the MFC utilizing the anode as an electron acceptor than by these same bacteria in batch experiments using soluble electron acceptors.

Bacteria grew slowly in the MFC culture incubations. Cell densities of *B. selenitireducens* increased eightfold (2.5×10^6 to 2.0×10^7 cells cm^{-3}) over 2 weeks, while strain SLAS-1 merely doubled (5×10^6 to 1.0×10^7 cells cm^{-3}) over 6 weeks. In contrast, *B. selenitireducens* and strain SLAS-1 grew 18- and 3.5-times faster, respectively, in earlier batch culture studies using lactate as electron donor and arsenate as electron acceptor (Switzer Blum et al. 1998; Switzer Blum in preparation). Hence, much of the remaining energy (i.e. electrons not devoted to cell growth) derived from the oxidation of lactate should have been available to the anode. However, the amount of electron flow diverted to the fuel cell was quite small in both cases. For example, total electrical output (integrated amps × time, divided by 96.5×10^4 coulombs per equivalent of electrons) was only 0.09 meq e^- for *B. selenitireducens* and 0.17 meq e^- for strain SLAS-1.

Comparing this with the number of electrons available from oxidizing lactate to acetate ($4e^-$), lactate to formate ($6e^-$), or lactate to CO_2 ($8e^-$) and subtracting the number of electrons consumed by arsenate reduction ($2e^-$) yields coulombic efficiencies of 0.3% and 6.7% for incubations of *B. selenitireducens* and strain SLAS-1, respectively. These low efficiencies imply that natural mediation of electron transport to the anode by these bacteria was limited. The remaining electrons generated by lactate oxidation were presumably used for cell growth or consumed by electron acceptors other than the anode and added arsenate (e.g. sulfate). Alternatively, diffusion of O_2 from the cathode across the relatively large membrane, although minimized by N_2 stripping of the anolyte, may have provided additional electron acceptor to arsenate and the anode. Facultative growth with up to 10 % O_2 has been demonstrated for *B. selenitireducens* (Switzer Blum et al. 1998) however strain SLAS-1 does not grow with either 5 or 10% O_2 (Switzer Blum et al. in preparation). Strain SLAS-1 remains to be tested for growth at lower O_2 levels.

Power density increased at the later stages of incubations with *B. selenitireducens* and strain SLAS-1 and decreased only when a competing alternative electron acceptor (i.e., arsenate) was added. Increased power production with growth, sensitivity to temperature, and most importantly the response to an alternative electron acceptor all suggest that the bacteria studied have the ability to transfer electrons to the anode surface, or that they excrete reduced electron shuttles that are subsequently oxidized at the anode (Rabaey et al. 2004). Maximum power densities in our culture incubations (6×10^{-5} W m^{-2}) were low compared with other MFCs operated at lower salinities using mesophilic bacteria (4.3 W m^{-2}, Rabaey et al. 2004). Somewhat less power has been reported for thermophilic bacteria (1.0 W m^{-2}, Jong et al. 2006). However, thermophilic and halophilic microorganisms provide greater tolerances to fluctuations in temperature and chemical composition, which makes them useful candidates for operating in MFCs under varying conditions.

Power production increased 2000-fold in ML slurries over pure cultures of *B. selenitireducens*. Lactate was absent in ML slurries, however other organic electron donors were available including acetate (0.5 mmoles) and various undetermined organic compounds present in particulate and soluble organic matter from Mono Lake sediment (Reed 1977). In addition, inorganic electron donors, especially sulfide (>0.2 mmoles), were present in ML sediment slurry. Hence, increased electricity production likely resulted from microbial metabolism of endogenous reduced compounds in Mono Lake sediment. The exoelectrogens present in ML sediment may have been better able to direct electrons to the MFC anode than *B. selenitireducens* alone, accounting for greater power. Alternatively, the presence in ML sediment of extracellular electron mediators such as quinones (or sulfide) may have facilitated electron transfer to the anode by these microbes. Conversely, salt-saturated Searles Lake was not likely conducive to other bacteria capable of oxidizing available organic electron donors, including added lactate. This is consistent with previous findings that high salt content in Searles Lake inhibited sulfate reduction and methanogenesis while supporting arsenate reduction (Oremland et al. 2005; Kulp et al. 2006, 2007). In addition, the absence of sulfide or other potential electron mediators from SL sediment may have restricted the transfer of electrons to the anode by exoelectrogens.

Hydrogen is a common electron donor used for metabolism by numerous anaerobic prokaryotes (Lovley and Goodwin 1988). Our choice of a closed system in which to incubate sediment slurries allowed us to monitor consumption of added hydrogen and to look for products of the most likely dissimilatory processes, methanogenesis and sulfate reduction. These are the dominant terminal electron accepting processes in Mono Lake sediment (Oremland and Miller 1993) however they occur feebly in Searles Lake sediment (Oremland et al. 2005; Kulp et al. 2006). Rates of hydrogen uptake follow this pattern, with removal of millimolar levels of H_2 on a daily time scale by ML slurries (Fig. 4c) but on a monthly time scale by SL slurries (Fig. 5c). Methanogenesis was not important, resulting in production of only 0.01 mmoles CH_4 in ML and less than half that amount in SL slurries. Sulfate reduction was significant in ML, resulting in a doubling of sulfide (from 0.2 to 0.4 mmoles) over 18 days incubation. This could account for removal of 1.6 mmoles of H_2 or about half of the 3 mmoles of H_2 consumed during the incubation. In SL slurries, sulfate reduction was non-detectable and methane production (0.005 mmoles) could only account for 0.3% of the 1.5 mmoles H_2 removed. Therefore, either the sulfide produced was oxidized at the anode or processes other than sulfate reduction and methanogenesis removed much of the H_2 in sediment from both lakes.

Hydrogen consumption by ML and SL slurries did not stimulate electricity production. This result was unexpected because hydrogen stimulated arsenate reduction in Searles Lake sediment slurries (Oremland et al. 2005) and suggests that hydrogenotrophs in Mono and Searles Lake sediment are not exoelectrogens. In addition, hydrogen uptake rates were unaffected by the selection of electron acceptor; no pattern emerged in the rates of H_2 removal in ML slurries with the circuit open or closed (Fig. 6). This is because the anode was a minor sink for electrons in ML slurries compared with other dissimilatory processes noted above. In the case of SL slurries, coulombic efficiency was threefold greater than in experiments with strain SLAS-1. Here, the total current integrated over 95 days yields

2.5 meq e^- transferred to the anode. Oxidation of lactate to acetate and CO_2 (no formate was observed) provided 18.4 meq e^- of which 3.9 meq e^- were consumed by arsenate reduction ($2e^-$). Hence, out of a possible 14.5 meq e^- available, 17.2% were used to reduce the anode. The remaining 12 meq e^- (82.8%) was presumably consumed by other terminal electron accepting processes, possibly including aerobic respiration using O_2 which may have diffused across the membrane from the cathode.

In MFC experiments with intact sediment, electricity production began immediately. Somewhat more power was produced in ML sediment than in experiments using ML sediment slurry (Table 1). However, as was observed with the slurry experiments, dramatically more power (four orders of magnitude) was produced in ML than SL sediments. Thus, ML sediment may harbor a community of exoelectrogens, some of which are more efficient than *B. selenitireducens* at transferring electrons to the MFC anode. Alternatively, ML sediment likely contained organic or inorganic compounds which could act as electron shuttles or electron donors. Addition of hydrogen had no effect upon electricity production in either sediment (Fig. 7a, b).

Table 1 highlights the major differences between ML and SL sediment and slurries. When no additional electron acceptors other than the anode were provided, significantly more power was produced in experiments with ML sediment (including slurries) than with SL sediment. This is consistent with previous findings that microbial activities were greater in Mono Lake than in the higher salinity Searles Lake (Kulp et al. 2006). Greater electrical output in ML may be due to (1) more substrates other than lactate to act as electron donors in ML sediments, (2) the presence in ML of exogenous electron mediators, or (3) the presence in ML of a wider range of anaerobic bacteria capable of efficiently transferring electrons to the anode. Regardless, a clear biologically driven production of electricity also occurred with SL sediment, which represents an environmental extreme.

Table 1 Summary of results from two lakes, three MFCs

Culture or sediment	Max. power ($\mu W\ m^{-2}$)	Lag time (days)	e^- donor/e^- acceptor added
Mono Lake			
B. selenitireducens	59	4	Lactate/As(V)
Mono slurry	11,800	0	H_2/SO_4^{-2}
Mono sediment	18,500	0	H_2/none
Searles Lake			
Strain SLAS-1	49	24	Lactate/As(V)
Searles slurry	15	35	Lactate + H_2/SO_4^{-2}
Searles sediment	1.2	0	$H_2 + S^{-2}$/none

Our observation that all of the microbes and sediments tested produced electricity supports the utility of MFCs to detect and study microbial metabolism in extreme environments such as alkaline, hypersaline lakes. We suggest that the basic concept of MFCs as life detectors could be applied to the search for extant microbial life on Mars and other planets in our solar system. Miniaturization of the hardware involved and improvement in detection efficiency could make MFC technology a practical approach to detecting extant life in hypersaline brines in the Martian regolith or beneath the ice caps of Europa, Callisto, and other satellites that are suspected of harboring saline, liquid oceans.

Acknowledgments We thank Suresh Seshadri and Martin Buehler of JPL for their enthusiastic support of this project. The following people helped in the laboratory: Jodi Switzer Blum, Stacy Bennett, Shelley Hoeft, Tom Kulp, Shaun Baesman, John Duff, and Larisa Yunerman. Thank you also to Charlie Ogle, Kelly Wrighton, and Cameron Thrash for assistance with microbial fuel cells. Kelly Wrighton and Chad Saltikov reviewed an early draft of this manuscript. Financial support was provided by USGS National Research Program, and NASA ASTID 2003.

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