Influence of Substrate on Electron Transfer Mechanisms in Chambered Benthic Microbial Fuel Cells

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This research investigated whether the addition of an exogenous electron donor would affect power production in laboratory-scale benthic microbial fuel cells (BMFC) by differentially influencing microbially mediated electron transfer processes. Six BMFCs were operated for over one year in a temperature-controlled laboratory. Three BMFCs relied on endogenous electron donors, and three were supplemented with lactate. The supplemented BMFCs generated more cumulative charge, but did not generate higher average current between periods of lactate enrichment. Coulombic efficiencies during the lactate treatments ranged from 25 to 65% suggesting that lactate utilization was variably coupled to power production. Cumulative electron flux resulting from lactate additions and chemical changes within the anode chamber, as well as a difference in the anode-hosted microbial communities indicated that lactate supplementation promoted sulfate reduction. After the addition of molybdate to suppress sulfate reduction and sulfur disproportionation, all BMFCs continued to produce current, but no longer responded to lactate additions. Chemical data support a two-step cycle in which endogenous organic carbon and/or supplemented lactate fuel sulfate reduction resulting in sulfide and simple organic molecules (such as acetate) that can act as the electron donors for the BMFC.

Introduction

A defining characteristic of microbial fuel cells (MFCs) is the utilization of electrons liberated by microbial redox processes. This utilization requires a mechanism for transferring available electrons between microorganisms and the electrodes of an MFC. Due to the fundamental importance of electron transfer processes at the anode, this topic has received considerable attention in the MFC literature. Two main types of electron transfer are direct electron transfer (DET) and mediated electron transfer (MET) (*1*). DET refers to cases where electrons are transferred directly from the microbial cell to the electrode via membrane-bound proteins, such as multiheme cytochromes (*2*−*4*), and/or via electrically conductive appendages often called nanowires (*5, 6*). MET refers to cases where electrons are transferred to the electrode via an electrochemically active compound, which could be a metabolite produced by microorganisms (e.g., sulfide or reduced iron compounds) (*7*−*10*), or an endogenous redox mediator (e.g., phenazines, quinones, and flavins) (*11*−*14*). An important distinction between DET and MET is that MET requires diffusion of redox mediators whereas DET does not rely on diffusive transport, even if outer membrane c-type cytochromes in the extracellular domain are behaving as redox mediators (*15*).

The mechanism that mediates electron transfer at the anode of a benthic microbial fuel cell (BMFC) likely influences the performance of the BMFC due to differing efficiencies and geochemical consequences (*8, 16*). For example, a process coupled to lactate oxidation by an organism (or group of organisms) using an anode as the terminal electron acceptor can be written as

$$CH_3CHOHCOO^- + 6H_2O \rightarrow 3HCO_3^- + 14H^+ + 12e^- \quad (1)$$

In eq 1, 12 electrons may be transferred to the circuit for every molecule of substrate oxidized, and the production of protons would likely result in a decrease in pH near the anode. An alternative mechanism is a sulfur-mediated process. It can be described by two simplified reactions:

$$2CH_3CHOHCOO^- + 3SO_4^{2-} \rightarrow 6HCO_3^- + H^+ + 3HS^- \quad (2)$$

$$3HS^- \rightarrow 3S^0 + 3H^+ + 6e^- \quad (3)$$

In eqs 2 and 3 only three electrons are passed to the circuit through sulfide oxidation for every molecule of substrate oxidized. In some cases microbes capable of sulfur disproportionation have been isolated from BMFC anodes (*8, 17*). Sulfur disproportionation could yield an additional six electrons to the circuit and help to buffer the pH, but the extent to which it occurs remains unknown.

The objective of this investigation was to better understand the degree to which direct oxidation of organic electron donors and sulfur-mediated oxidation of organic electron donors are influential in power production by BMFCs. Specifically we were interested in determining if we could promote one anode process over another by stimulating microbial communities capable of oxidizing an exogenous electron donor. These experiments illustrate the performance and efficiency of six laboratory-scale BMFCs operated for over one year, of which three were supplemented with an exogenous electron donor and three relied on endogenous electron donors. The effects of the exogenous electron donor (lactate) were examined in terms of short- and long-term gains in power production and for chemical impacts within the anode chambers.

Experimental Section

BMFC Design and Operation. Six BMFCs were constructed based on a design developed in the course of field experiments (*18, 19*). Sandy sediment was collected from the intertidal zone in Yaquina Bay, OR, and homogenized by mixing in a 20-L bucket. Each BMFC consisted of an 8-L plastic beaker with 4 L of homogenized sediment overlain by approximately 3 L of seawater. An acrylic core tube (70 cm^2 cross-sectional area) pushed approximately 10 cm into the sediment served as the anode chamber (Figure 1). The top of the core tube extended approximately 4 cm above the sediment and was

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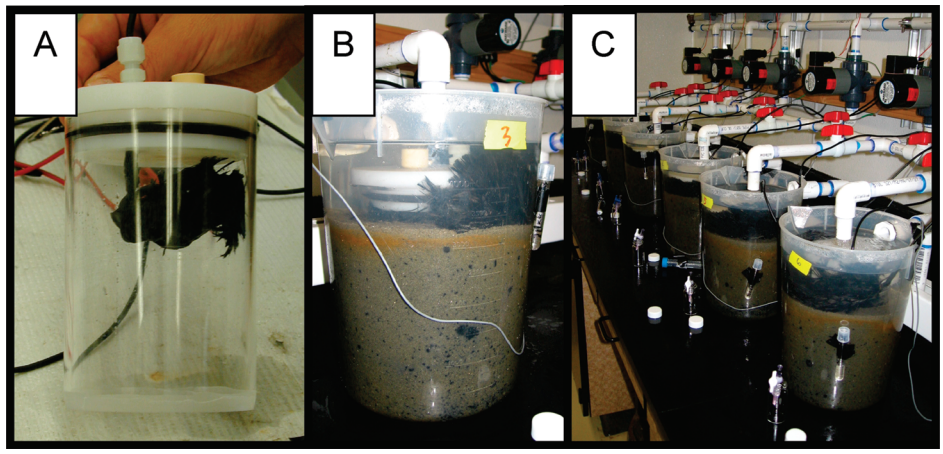

FIGURE 1. Photographs of laboratory BMFC construction. (A) Core tube and anode prior to insertion into sediment. (B) Complete BMFC showing sediment, cathode in overlying water, and anode chamber. (C) Six BMFCs in climate-controlled laboratory with seawater circulation system. Black spots in the sediment are pockets of organic material, and a layer of iron oxide in each container indicates the iron reduction zone.

capped with an O-ring sealed lid resulting in a chamber volume above the sediment of approximately 150 mL. Carbon-fiber brush anodes (*20*) were positioned inside each chamber and wired through a bulkhead fitting in the lid. Each anode was approximately 10 cm long with an estimated surface area of 2.6 m^2 (derived from manufacturer supplied value of 26 m^2 per m of length). Cathodes consisted of 20-cm lengths of the carbon-fiber electrode (5.2 m^2) positioned in the seawater overlying the sediment in each container. Each BMFC had a Ag/AgCl (3 M KCl) reference electrode (Microelectrodes, Inc., Bedford, NH) positioned in the overlying seawater. This seawater was refreshed and aerated approximately every 5 min by a seawater circulation system, and all BMFCs were operated at approximately 10 C in a refrigerated laboratory.

Whole-cell potential (E_{cell}), anode potential (E_{anode} vs Ag/AgCl) and current (I) were monitored and logged every 10 min with a multichannel datalogger (Agilent Technologies, Santa Clara, CA). Cathode potential (E_{cath}) was calculated according to $E_{cath} = E_{cell} + E_{anode}$. All BMFCs were operated at a constant E_{cell} of 0.4 V controlled by a custom designed potentiostat (NW Metasystems, Bainbridge Island, WA). The potentiostat operates as an automatic variable external resistor that adjusts the external resistance (and thus the current) to maintain a predefined E_{cell}. If E_{cell} falls below the predefined voltage, then the potentiostat opens the circuit to allow the redox gradient to recover. The selection of 0.4 V was driven by the results of other studies that have shown maximum power at this potential (*15*). This choice resulted in relatively constant anode potentials of about 0.0 V (vs Ag/AgCl).

Each anode chamber had a septum allowing sample collection and/or supplementation. BMFCs 1, 3, and 5 were not supplemented during the course of the experiment. BMFCs 2, 4, and 6 were supplemented with sodium lactate approximately every two weeks during the course of the experiment. We selected lactate as an electron donor because it is a known substrate for some dissimilatory metal reducing bacteria (*21*) and sulfate reducing bacteria (SRB) (*22*−*26*). Typical injections were 1 mL of 35 mM lactate producing a concentration of approximately 0.23 mM in the anode chamber. During days 240−320 we varied the amount of lactate injected into BMFC 4 to give a concentration range inside the chamber of 0.12−3.8 mM. Near the end of the experiment we made a series of injections containing molybdate (to a concentration of 50 mM inside the chambers), a specific inhibitor of sulfate reduction (*27*) and sulfur disproportionation (*28*). Molybdate alone was added to BMFCs 3 and 5, while lactate (to a concentration of 0.23 mM) was co-injected with the molybdate in BMFCs 4 and 6. We also conducted injections without molybdate to investigate the stirring effect of the injections themselves.

Fluid samples were intermittently collected from each anode chamber with a syringe and a No. 18 needle inserted through a septum. Approximately 10 mL was collected and subsequently partitioned for sulfide, sulfate, dissolved organic carbon (DOC), total alkalinity, pH, iron, and molybdenum analyses according to standard methods (described in detail in Supporting Information).

Samples for microbial community analysis were collected from the anodes of BMFCs 1 and 2 by transferring the entire BMFC to a N_2 flushed glovebag, opening the anode chamber lid, and clipping approximately 1 g of fibers with flame-sterilized shears. Samples were placed in WhirlPak bags and flash frozen by immersing them in liquid N_2 and then stored at −50 C for later analysis. Clone library construction is described in Supporting Information.

Results and Discussion

All BMFCs produced current for more than one year (Supporting Information, Figure S1). Table 1 summarizes performance in terms of cumulative charge passed by each cell, energy, and energy density. It is notable that an average of 31 ± 4 mW·hr of energy was produced during the course of the experiment from sand with only endogenous electron donors.

Effects of Lactate Addition. Supplementation with lactate resulted in short-term peaks in current but did not yield a long-term benefit. After the lactate was consumed, current declined to levels similar to those in the unsupplemented cells. The cumulative charge passed by supplemented BMFCs relative to unsupplemented BMFCs was consistent with the amount of added lactate, but not a clear indicator of the electron transfer process at the anode. For example, BMFC 6 received 46 1-mL injections of 35 mM lactate solution totaling 1.6 mmol of added lactate. If the anode community were capable of direct lactate oxidation with 100% Coulombic efficiency (see eq 1), BMFC 6 would pass ca. 19 mmol of electrons in excess of the amount passed attributable to other processes ("background current"). The average electron flux due to background current from the two unsupplemented cells with the same period of operation was about 21 mmol. We then predict that BMFC 6 would pass about 40 mmol of electrons if all the lactate were oxidized with 100% Coulombic efficiency. Rather, the measured flux was 33.4 mmol implying a 65% Coulombic efficiency for the added lactate. Alternatively, if lactate stimulated SRB leading to an anode process

TABLE 1. Summary of Power Production from Laboratory-Scale BMFCs

BMFC	total lactate added (mmoles)	no. of days operated	cumulative electrons passed (mmol e^-)	cumulative current efficiency[a] %	energy (Whr)	energy density[b] (Whr m^{-2})
1	0	372	18.4		0.20	28
2	1.4	372	25.9	45	0.28	40
3	0	482	18.3		0.20	28
4	2.3	482	36.9	58	0.40	57
5	0	482	24.0		0.25	35
6	1.6	482	33.4	65	0.35	50

[a] Cumulative current efficiency calculated relative to the lactate supply as described in section titled "Effects of Lactate Addition". [b] Energy density based on cross-sectional area of chamber.

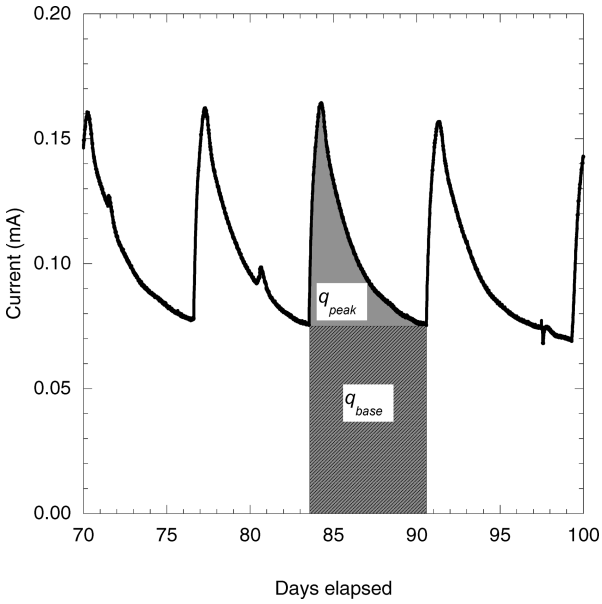

FIGURE 2. Illustration of Coulombic efficiency calculations. Area under curve was integrated to give q_{peak}. q_{base} was subtracted from q_{peak} to determine the amount of charge passed due to the lactate injection. This charge was divided by the number of electron equivalents added as lactate to give efficiency.

consistent with eq 3, then we would expect an excess of 5 mmol over the cumulative electron flux due to background current. The excess was more than double that amount. Thus, if we invoke the metabolic process indicated by eq 3 it must have also been accompanied by another process such as sulfur disproportionation to account for the excess cumulative electron flux.

Coulombic Efficiency in Cells with Lactate Supplements. We also calculated the efficiency of lactate conversion to current for individual injections (as in Figure 2). The area under the current vs time curve was used to determine the total amount of charge passed during one injection cycle (q_{total}). The baseline charge (q_{base}) was the area of a rectangle defined by the baseline current observed on the plot multiplied by the time between injections. The amount of charge resulting from each lactate injection (q_{peak}) was the difference between q_{total} and q_{base}. We calculated the Coulombic efficiency for 5 peaks from the early (day 40−75), middle (day 110−150), and late (day 195−230) periods (15 peaks total) from BMFC 4. By this method, the average Coulombic efficiency of the lactate injections was 34 ± 4%. There was no measurable difference among the different periods examined, suggesting that there were not discernible changes in gross microbial community activity. The observed discrepancy between this method of determining Coulombic efficiency and the method based on cumulative electron flux likely resulted from the frequency of lactate injections. In cases where the current was not fully returned to the baseline level prior to an injection, the contribution of baseline current would be overestimated and the contribution from the lactate injection would be underestimated.

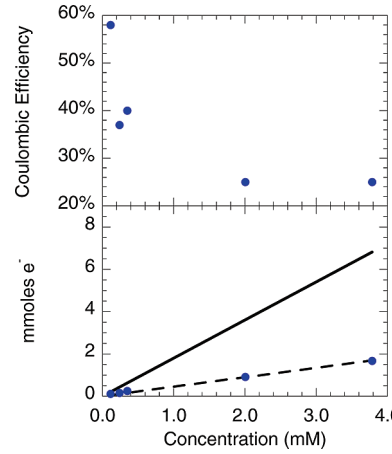

FIGURE 3. Coulombic efficiency and cumulative electron flux as a function of lactate concentration in the anode chamber of BMFC 4. Efficiency (upper panel) decreased with increasing concentration suggesting lactate fueled alternative processes that did not contribute to current production. Lower panel shows cumulative amount of electrons passed as a result of lactate injections. Symbols indicate the observed flux of electrons, the solid line represents the expected flux according to eq 1, and the dashed line represents the expected flux according to eqs 2 and 3.

Effect of Varying Lactate Concentrations. During days 240−320 the concentration of lactate in BMFC 4 supplements was varied. As shown in Figure 3, the Coulombic efficiency (c.f. Figure 2) decreased with increasing lactate concentration. The Coulombic efficiency ranged from 25% with a lactate concentration of 3.8 mM to 58% with a lactate concentration of 0.12 mM. The inverse relationship between efficiency and lactate concentration suggests (1) lactate supplements promoted biomass growth or metabolism that did not necessarily contribute to current generation (e.g., fermentation) and (2) at higher lactate concentrations, current was limited by some factor other than the concentration of the electron donor. Figure 3 also shows q_{peak} (c.f. Figure 2) as a function of lactate concentration and the q_{peak} that would be expected based on the reactions shown in eqs 1−3. At the higher concentrations of lactate, the observed electron flux was consistent with sulfate reduction to sulfide and subsequent oxidation of sulfide to elemental sulfur (25%).

Current Density. The maximum current density in these laboratory BMFCs was 0.07 mA·m^{-2} anode surface area or 27 mA·m^{-2} sediment. These values are at least an order of magnitude less than our previous in situ experiments that used the same electrode materials and similar BMFC design. In Yaquina Bay we achieved current densities of 0.45−0.73 mA·m^{-2} anode (580−950 mA·m^{-2} footprint) and in Monterey

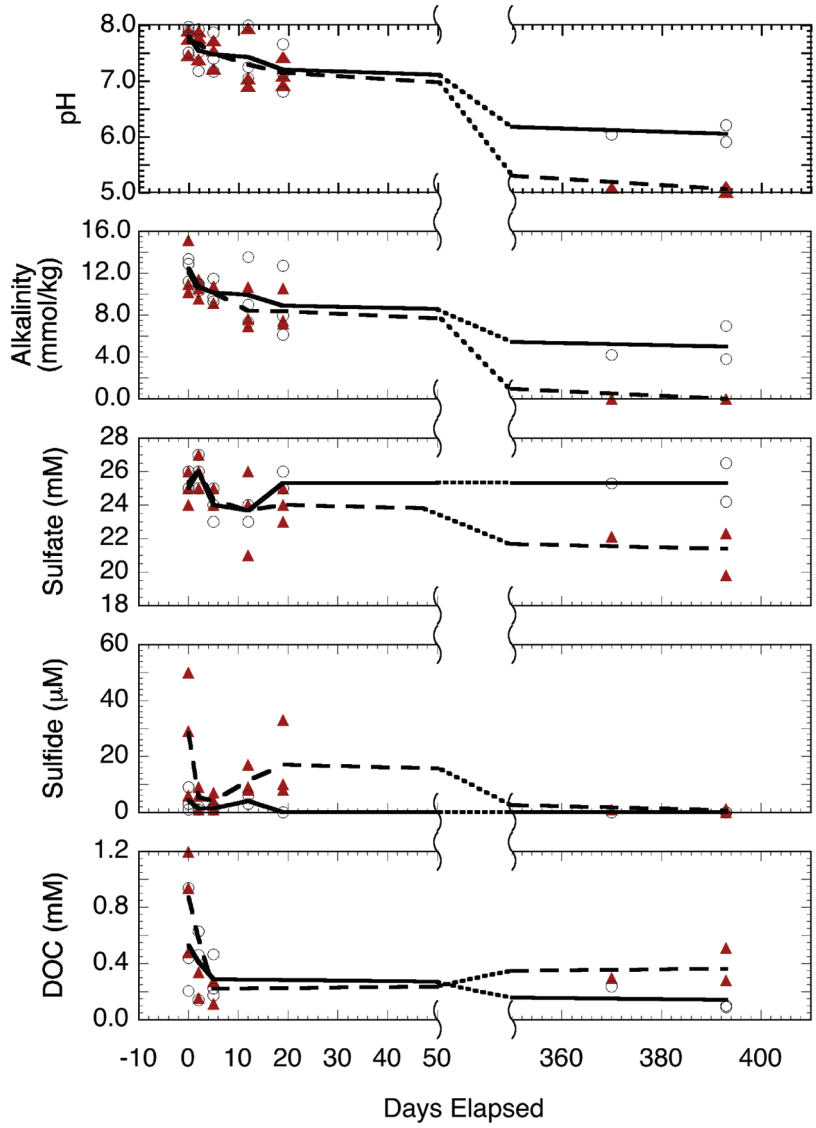

FIGURE 4. Summary of fluid chemistry from unsupplemented (open circles) and supplemented (filled triangles) BMFCs. Solid lines indicate the average for all unsupplemented results and dashed lines indicate the average for all supplemented results.

Canyon we achieved 1.8 $mA \cdot m^{-2}$ anode (350 $mA \cdot m^{-2}$ footprint) (*18, 19*). We speculate that the reason for the low current density in these laboratory BMFCs under the conditions of the lactate additions is due to the accumulation of reaction products (specifically proton accumulation in the anode chamber).

Chemical Consequences of BMFC Operation. Figure 4 summarizes the results of the chemical analyses of anode fluid samples taken from the anode chambers near the beginning and end of the experiment. Samples were collected when supplemented BMFCs were close to baseline conditions, usually at least one week after the most recent lactate injection. During the early phase of the experiment, there was no apparent difference in fluid (anolyte) composition between the supplemented and unsupplemented BMFCs (except for detectable sulfide in the supplemented cells, days 10−20). Toward the conclusion of the experiment, supplemented BMFCs generally had lower pH, alkalinity, and concentrations of sulfate, but higher DOC concentrations. The DOC concentrations do not show an increase resulting from repeated lactate injections suggesting that lactate was completely oxidized. As shown in Table 2, the concentration of iron in the anode chambers was extremely enriched relative to seawater values late in the experiment. The enrichment of iron could result from speciation changes tied to shifts in pH, Eh, and available ligands due to BMFC operation.

TABLE 2. Summary of Iron and Molybdenum Analyses

		Fe (μM)		
BMFC	pH[b]	before MoO_4^{2-} additions (day 393)	after MoO_4^{2-} additions (day 460)	Mo (mM)
3	6.2	38	9	30
4[a]	5.1	3807	118	35
5	5.9	211	22	35
6[a]	5.0	3682	51	44

[a] BMFCs 4 and 6 received lactate supplements. [b] pH data are from the same date as initial Fe samples. "before" samples were filtered, "after" samples were unfiltered.

The role of proton transport in anode biofilms has received considerable attention in the literature and has been implicated as one of the factors that limit current (*29, 30*). In other recent MFC studies, pH was manipulated and current production decreased in treatments outside of circum-neutral pH (*31, 32*). Conversely, in this experiment current production was enhanced by lactate supplementation and pH decreased relative to unsupplemented BMFCs. The low pH is not

TABLE 3. Summary of 16s rRNA Clone Libraries from BMFCs 1 and 2

phylum	order	proportion of phylotypes found BMFC 1	BMFC 2
Alphaproteobacteria		8%	
Deltaproteobacteria			
	Desulfuromonales	25%	22%
	Desulfobacterales	8%	44%
	Desulfovibrionales	2%	
	Syntrophobacterales	2%	
Epsilonproteobacteria		5%	2%
Gammaproteobacteria		11%	4%
Flavobacteria		23%	16%
Bacterioidetes		2%	2%
Sphingobacteria		7%	4%
other		8%	6%

surprising given that the production of protons results from oxidation of electron donors at the anode surface. However, the accumulation of protons in excess of the buffering capacity of the anolyte (or sediments underlying the chamber) could have effects that could alter anode mechanisms. According to Finster et al. (*28*) the growth range for a sulfur disproportionating organism (*Desulfocapsa* sp.) is pH 6.0−8.2. At the low pH that occurs in these BMFCs, sulfur disproportionation might be inhibited which would limit current and potentially result in a build-up of elemental sulfur on the anodes resulting in passivation of the surface. Furthermore, Biffinger et al. (*31*) showed that low pH inhibits *Shewanella* sp. from excreting riboflavin (which could serve as a redox mediator) resulting in lower current production. By analogy, the low pH in these BMFCs could have a similar inhibitory effect on other microbially produced redox mediators.

In addition, the low alkalinity observed in these BMFCs suggests processes other than sulfate reduction followed by sulfide oxidation at the anode contribute to current production. Even in the presence of an anode as an electron sink, we would expect a net increase in alkalinity as a result of sulfate reduction according to eqs 2 and 3. Alkalinity in seawater is a measure of the availability of substances that will react with hydrogen ions (*33*). In the case of these BMFCs the low alkalinity would likely curtail the transport of protons out of the anode biofilm.

Phylogenetic Analyses of Anode Communities. Table 3 summarizes the 16s rRNA clone libraries constructed from samples of the anodes from BMFCs 1 and 2. Phylogenetic identification does not equate to function but, when considered with electrochemical data and other studies from the literature, it does implicate possible processes. The communities from the two anodes were similar, but BMFC 1 (which was not supplemented) showed slightly higher diversity. *Deltaproteobacteria* was the dominant class in both samples, representing 37% and 66% of the phylotypes found in BMFC 1 and 2, respectively. In BMFC 1, however, *Deltaproteobacteria* were dominated by phylotypes within the order desulfuromonales whereas BMFC 2 was dominated by phylotypes of the order desulfobacterales. Organisms within desulfuromonales are known to oxidize organic acids using Fe(III) or an electrode as the terminal electron acceptor (*17, 34*). In contrast, some organisms within desulfobacterales are known for their ability to disproportionate sulfur (*28, 35*). The phylogenetic communities from the respective BMFCs suggest that the supplemented BMFCs were more reliant on a sulfur cycle (eqs 2−3) than the unsupplemented BMFCs. We speculate that the unsupplemented BMFCs relied on a cycle in which complex organic material was broken down by various hydrolytic enzymes and fermentation processes,

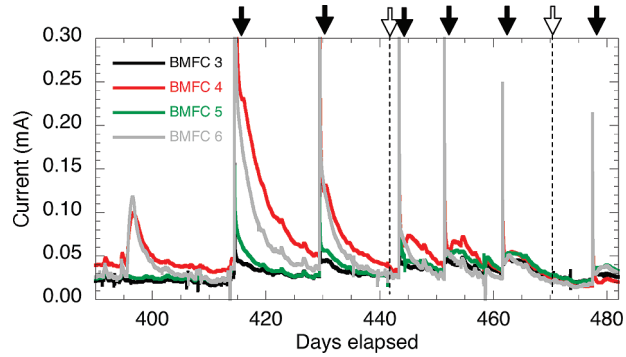

FIGURE 5. Current versus time for all BMFCs during the course of molybdate injections (days 414−470). BMFCs 3 and 5 were injected with molybdate only and BMFCs 4 and 6 were injected with molybdate and lactate. Peaks from days 394−404 in BMFCs 4 and 6 were typical lactate injections and are shown for comparison. Brief periods of zero current production (day 413 and 459) were caused by changing the cathode water in the circulation system and were not related to molybdate injections. Open arrows indicate blank injections of sparged seawater without molybdate.

with the resulting products serving as substrates for bacteria capable of transferring electrons to an anode (*36*).

Effects of Molybdate on BMFC Current. To further investigate the role of sulfur in the unsupplemented BMFCs, we conducted a series of molybdate injections designed to block sulfate reduction. Figure 5 shows the current responses of the four BMFCs that received molybdate injections. All BMFCs showed an initial spike in current production followed by a secondary peak above baseline in response to repeated molybdate injections. Molybdate—which interrupts the synthesis of ATP in SRB thus leading to cell death (*27*)—was hypothesized to produce a decline in current as sulfate reduction and the subsequent oxidation of sulfide ceased. However, these data suggest that some process other than sulfate reduction is responsible for maintaining the nominal current in these BMFCs. This deduction is consistent with the observation of dissimilatory metal reducers and other bacterial groups capable of breaking down complex organic matter (e.g., flavobacteria) in the samples collected from the BMFC 1 anode.

BMFCs 4 and 6, which received regular lactate supplements, showed larger current increases in response to molybdate injections than BMFCs 3 and 5 which had not received any supplements. We hypothesize that SRB populations in BMFCs 4 and 6 reduced molybdate (*37*) and then the reduced form was reoxidized at the anode (*38*). Subsequent molybdate + lactate injections to BMFCs 4 and 6 yielded progressively smaller increases until the results were nearly identical to those of the unsupplemented cells. On day 470, BMFCs 4 and 6 were injected with lactate only and there was no apparent response. The lack of response to lactate indicates that the earlier molybdate injections succeeded in killing off organisms that could use lactate (likely SRB) and the underlying process that supported current generation could not be stimulated by lactate. The microbiological sample from BMFC 2 was collected before any molybdate injections and indicated the presence of sulfur metabolizing bacteria within the *Deltaproteobacteria*. The molybdate injections probably inhibited such bacteria leaving other members of the *Deltaproteobacteria* that do not rely on a sulfur metabolism.

We can only speculate why molybdate injections caused immediate (albeit short-term) current spikes in both the supplemented and unsupplemented BMFCs. Molybdenum within the molybdate anion is fully oxidized as Mo(VI) and therefore could not be oxidized further at the anode surface.

We can also rule out stirring effects because we observed no peaks when injections were made without molybdate on days 441 and 470. It is likely that the molybdate reacted with something in solution that lowered the electrical potential of the anode (*39, 40*). Targeted experiments will be required to assess these possibilities.

Model for Anode Processes. These experiments were designed to determine if supplementing a BMFC with an exogenous electron donor selects for a particular electron transfer process at the anode. The data suggest that exogenous electron donors can influence microbially mediated processes in mixed communities because the supplemented BMFCs responded to additional electron donor and had a larger proportion of phylotypes that metabolize sulfur species (either sulfate reduction and/or sulfur disproportionation). It is also likely that lactate additions stimulated sulfate reduction away from the anode surface resulting in a microbial community that was not necessarily represented by anode samples. After injections of supplemental electron donor, current returned to baseline levels.

Previous BMFC experiments have invoked a sulfur-mediated system in which sulfate reducing bacteria generate sulfide that is subsequently oxidized at the anode surface to produce current (*8, 10, 41*). Results of molybdate injections showed that the BMFCs were able to continue to produce current even when sulfate reduction was inhibited. Fluid in the anode chambers of all the BMFCs had relatively low pH and alkalinity, which is contrary to expectations if the system were mediated solely by sulfur transformations. Coulombic efficiency was greater than can be explained by sulfate reduction and subsequent sulfide oxidation. Sulfur disproportionation could account for additional current, but pH in the cells was outside of the known range for sulfur disproportionators.

A model of current generation coupled to lactate degradation that is consistent with the chemical and electrical data is one in which exogenous lactate stimulates sulfate reduction but is incompletely oxidized to acetate (*26*) which then can be oxidized by a DET process at the anode as represented by the following reactions (*4, 42*):

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + HS^- + H^+ \quad (4)$$

$$2CH_3COO^- + 8H_2O \rightarrow 4HCO_3^- + 18H^+ + 16e^- \quad (5)$$

These coupled reactions can explain the BMFC response to lactate additions while also being consistent with the results of the molybdate block experiment. Acetate oxidation at the anode is also consistent with the low pH and alkalinity that we observed in the BMFC chambers. The Coulombic efficiency of the model proposed by eqs 4 and 5 is 67%, which is in agreement with the data from these BMFCs.

In sum, these experiments demonstrate that current could be generated from sandy sediments for over a year without added electron donors. During the course of these experiments, baseline current appeared to be independent of the sulfur cycle (at least, independent of sulfate reduction). This observation may be specific to sandy or iron-rich sediments and may not apply to highly reducing muddy environments where other BMFCs have been deployed. However, understanding the mechanisms in sandy environments will be important as this technology is further developed since a large proportion of continental margin sediments are sandy and many freshwater sediments are Fe-rich.

Acknowledgments

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Supporting Information Available

Plots of electrode potential and current for each BMFC and detailed methods for chemical and phylogenetic analyses. This information is available free of charge via the Internet at http://pubs.acs.org/.

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