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A miniature microbial fuel cell operating with an aerobic anode chamber

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Abstract

A miniature microbial fuel cell (mini-MFC) is described that utilizes an aerobic culture of *Shewanella oneidensis* DSP10 as the active electrochemical species in the anode chamber. We find that the maximum aerobic mini-MFC power without the addition of exogenous mediators was 0.40 mW, a 33% decrease when compared with an anaerobic DSP10 culture (0.6 mW) operating in the mini-MFC. This decrease is most likely due to the presence of dissolved oxygen in the anode chamber that scavenges electrons to form water, thereby reducing the number of electrons donated to the anode. Aerobic power and current density at maximum power using the true surface area of the anode (611 cm^2) were calculated to be 6.5 mW m^{-2} and 13 mA m^{-2}. The power density rises to 2.0 W m^{-2} and 330 W m^{-3} when calculated using the cross-sectional area and volume of the device (2 cm^2, 1.2 cm^3). The Coulombic efficiency was also reduced from 11 to 5% when using the aerobic versus anaerobic culture. Similar results were found when the external mediator anthraquinone-2,6-disulfonate (AQDS) was added to the aerobic culture, resulting in a maximum power of 0.54 mW, a 37% drop in power when compared to the anaerobic mediated system.
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Keywords: Microbial fuel cell (MFC); Mediated; Aerobic; Anaerobic; Miniature microbial fuel cell (mini-MFC); *Shewanella oneidensis*

1. Introduction

There are many commercial, military, and homeland security applications for sustained underwater surveillance systems (rivers, lakes, littorals). However, there are challenges to creating a distributed autonomous network of sensors in an aquatic environment. The first difficulty is achieving wireless communication either between the sensors or from a sensor to a centralized node. Recent advances in acoustic modems may be used for wireless underwater communication [1,2], but most scenarios for networked sensors depend upon intermittent surfacing, through ballast control or motorized propulsion, for the purpose of radio frequency, rf, communication (poor propagation underwater). Devices that operate throughout the water column, rather than only on the seafloor, could be positioned closer to the surface and require less power by surfacing and submerging over shorter depths. The second challenge is the power source. Batteries may not be an adequate solution due in part to the remote and corrosive environment but also due to the long surveillance periods required (10+ years) [3–5]. Solar power is also a possibility, but the device would require repeated and time-consuming exposure to the air/water interface for recharging [6].

An ideal power source for these applications would be small (stealthy, not dominating the size of the device), function in an aerobic environment (in the water column, close to the surface), provide continuous power (no recharging or lifetime issues), and not require high levels of solar radiation (sub-surface, nighttime, etc.). Due to natural nutrient supplies, microbial fuel cells (MFCs) have been shown to generate continuous power for many years in aquatic environments [7]. However, these demonstrations have been limited to the seafloor and riverbeds in order to maintain a near-anaerobic environment for the anode (subsediment). Therefore, the prospect of achieving rf communication for a sensor network using these types of microbial fuel cells is poor, and at the very best inefficient based on the need to surface and submerge the device to the seafloor.

We have designed a miniature microbial fuel cell (mini-MFC) that utilizes *Shewanella oneidensis* (DSP10 strain), a bacterium that remains active in anaerobic and aerobic environments [8]. Previous studies have shown that anaerobic *and* aerobic DSP10 can reduce Cr(VI) to Cr(III), demonstrating that electrons from the bacteria can be used to reduce metals in the presence of oxygen [9]. Many MFCs utilize strains of bacteria that expire when exposed to aerobic environments for extended periods of time (*Geobacter* sp., *Clostridium* sp., etc.), eliminating their ability to

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donate electrons to the anode in the presence of oxygen [10–13]. Recent studies on *Geobacter sulferreducens* indicate a slight tolerance to oxygen (up to 24 h without growth inhibition), but the ability to utilize the microbe for current production under these conditions was not investigated [14]. In addition, the prospect of boosting output power by using hydrogen is impossible in an aerobic environment because hydrogen is evolved only under anaerobic culture conditions [15]. One macroscopic MFC maintained with anaerobic *Shewanella putrefaciens* reported no power generation when switching the culture to aerobic respiration, but this MFC had such low power density (5 μW m^{-2} per true anode surface area) it is possible that the aerobic MFC produced lower power rather than no power [16]. Other work has shown that higher Coulombic efficiencies can be achieved in H-cell MFCs by utilizing chemical and biological methods to reduce trace levels of oxygen in the anode chamber that crossover from the aerobic cathode [17]. This increased efficiency is attributed to the near-elimination of electron scavenging by oxygen in the anode chamber, thereby allowing more electrons to be donated to the anode.

We believe that aerobic power generation by an MFC is possible by switching from a traditional H-cell design to a more efficient miniature design that relies on high surface area electrodes in a small anode chamber. Fig. 1 demonstrates the potential differences between the mini-MFC design and the more traditional H-cell. Even though there may be 50–10^4 cm^2 of electrode surface area in the anode chamber of a typical H-cell MFC, there is usually a relatively narrow pathway (2–5 cm^2) for protons to traverse across the proton exchange membrane (PEM). Therefore, electron transfer to the anode can be highly efficient while proton throughput across the PEM is highly inefficient. Efficient proton transport to the PEM and electron transport to the anode become even more crucial when discussing an aerobic anode chamber due to the thermodynamically favorable oxygen reaction with protons and electrons to form water:

$$4H^+(aq) + O_2(aq) + 4e^- \rightarrow 2H_2O(l) \quad (1)$$

This scavenging reaction is most likely responsible for the null power output for previous attempts at aerobic MFCs [15].

As shown in Fig. 1, the mini-MFC design reduces electron and proton diffusion lengths by placing a high surface area electrode (611 cm^2) in a small volume (1.2 cm^3) chamber and by positioning the electrodes in direct contact with the PEM. This is in stark contrast to the H-cell design where the electrode is placed in a relatively large volume chamber and is spaced several centimeters away from the PEM. If the rate of acceptance of electrons by the anode in the mini-MFC competes with the removal of electrons via reaction (1), then measurable current should be generated, even in an aerobic environment. In addition, the entire anode chamber in the mini-MFC would then be slightly deficient in electrons (donated to anode), making the rate of reaction (1) slower, increasing the concentration of protons in the chamber and enabling a higher percentage of protons to diffuse to the PEM. This electron deficiency may occur in a traditional H-cell design, but because the anode is spaced far from the PEM, the probability of significant proton diffusion to the PEM is small in an aerobic environment based on the efficiency of reaction 1. Electrons and protons generated inside the anode chamber have a higher probability of diffusing to the anode and PEM, respectively, in the mini-MFC when compared to the H-cell design. We therefore predict that power can be generated in an aerobic MFC, but the output should be reduced by oxygen scavenging when compared to an anaerobic counterpart.

2. Materials and methods

2.1. Bacterial culture conditions

The facultative anaerobe/aerobe *S. oneidensis* strain DSP10 was used for all experiments. Luria–Bertani (LB) broth (Difco Laboratories, Detroit, MI) was inoculated with DSP10 and incubated at room temperature for 5 days with shaking at 100 rpm in air. After assembling the mini-MFC (described below), 50 mL DSP10 culture was transferred to a sterile 100 mL Erlenmeyer flask, which was then capped with a sterile rubber stopper fit-

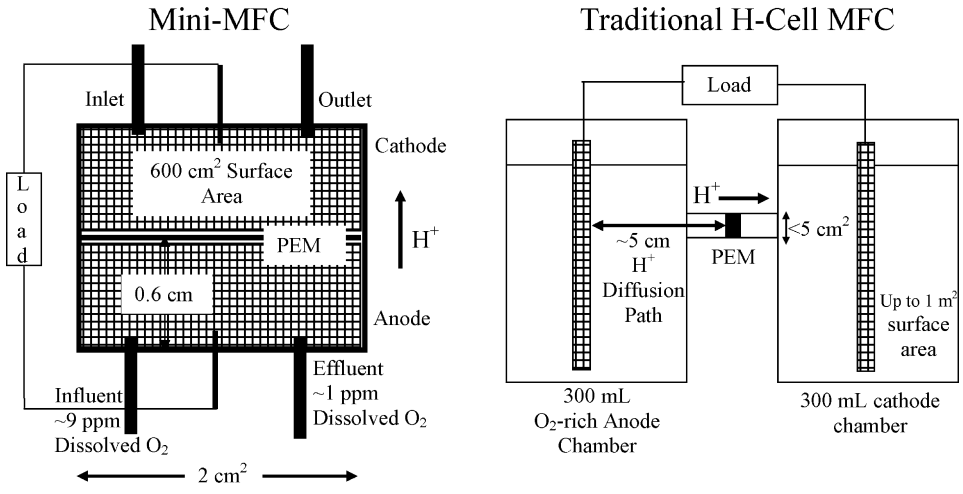

Fig. 1. Schematic of mini-MFC and a traditional H-cell MFC.

ted with a cotton-plugged tube open to air and two glass tubes attached to influent (to the anode chamber) and effluent (from the anode chamber to the reservoir) lines. Sodium lactate as substrate was added to the reservoir culture every 1–3 days to the culture at 10–30 mM. Some bacterial cultures had 100 μM of the soluble electron mediator anthraquinone-2,6-disulfonate (AQDS) added to the anolyte. For experiments where exogenous mediators (AQDS) were not used in the DSP10 culture, it was assumed that DSP10 self-mediated through excretion of soluble quinones into the media. This excretion has previously been shown to occur for various *Shewanella sp.* [18].

Within 10 min of placing the aerobic DSP10 culture in an unstirred environment for the fuel cell experiment, dissolved oxygen measurements (ISO2 probe, WPI Inc., Tampa, FL) for both influent and effluent lines showed that the DSP10 culture had scavenged all available dissolved O_2 (0.1 ± 0.2 ppm). In order to maintain an aerobic culture and an aerobic anode chamber, sterile air was continuously and vigorously bubbled through the anolyte flask. Dissolved oxygen in the anolyte was measured in the influent and effluent by using two dissolved oxygen probes located immediately before and after the anode chamber. Dissolved oxygen was found to be near saturation prior to the anode chamber (8–9 ppm) and at 15% of saturation after the chamber (1 ppm). These measurements show that dissolved oxygen in the anolyte is reduced during its residence time in the chamber, but that at all times during the experiment dissolved oxygen was measurable, ensuring aerobic conditions.

2.2. Mini-MFC assembly and operation

The mini-MFC design (machining performed by Edmonds Engineering, Bowie, MD) with graphite felt electrodes and flow tubing has been described previously, and is shown schematically in Fig. 1 [8]. The fuel cell chambers were made from non-conducting plastic. The cross-section of the working area of the device was 2.0 cm^2 and the anode and cathode chamber volumes were each 1.2 cm^3. Two electrodes were formed from equal sized graphite felt (Electrosynthesis Company, Lancaster, NY; 0.47 $m^2\,g^{-1}$) cut to 0.13 g (true surface area = 611 cm^2; volume = 1.2 cm^3, cross-sectional area = 2.0 cm^2). Thin titanium wire was wound around the electrodes to ensure electrical contact. The wire was then fed through a hole in the mini-MFC chambers to connect with external loads. After 3 weeks exposure to a DSP10 culture, electrodes were examined by environmental scanning electron microscopy (ESEM) to determine if biofilm had developed. After standard preparations, ESEM showed little to no cell attachment or biofilm formation on either electrode type.

A 175 μm thick proton exchange membrane (Nafion® 117, Fuel Cell Store, Boulder, CO) was positioned between the two chambers during fuel cell operation and secured with four stainless steel screws. Both chambers were sealed by o-rings placed between the Nafion® and the outer wall. Two 0.3 cm o.d. Teflon® tubes were attached to each chamber for influent and effluent flow of anolyte and catholyte. Flow rates were set at 1.2 mL min^{-1} using a peristaltic pump (Masterflex, Cole Parmer, Vernon Hills, IL). The total volume of fluid residing in the influent and effluent tubing and the chambers averaged 7.2 mL, making round trip times for fluid cycling 6 min. The distance between the electrodes was held constant at ~175 μm.

Due to its stability, the catholyte in all experiments was unstirred 50 mM ferricyanide solution (Sigma–Aldrich, St. Louis, MO) in 100 mM phosphate buffer (pH 7.4). This catholyte also enables the mini-MFC measurements reported here to be easily compared to other MFC experiments. Previous experiments have been reported where oxygen reduction cathodes were used in the mini-MFC in place of ferricyanide that resulted in an approximately 50% drop in maximum power [19]. The anolyte was an aerobic stationary phase DSP10 culture with cell counts ranging from 1×10^7 to 5×10^7 cells mL^{-1} as determined by plating. The exogenous mediator AQDS was used in some experiments as an electron shuttle to aid in electron transfer by DSP10 to the anode. Previous reports show that the addition of AQDS to the anolyte increases the maximum power output of the mini-MFC by up to 30% [8]. Exogenous mediators enable higher current production by allowing more DSP10 to participate in electron transfer to the anode. Without mediators such as AQDS, the only electron transfer mechanisms are through self-mediation or direct electron transfer to the anode. Both the mini-MFC and reagents were kept at room temperature (19 °C) for all experiments.

2.3. Data acquisition and calculations

Potential difference, V, between the anode and cathode were measured by a Personal DAQ/54 data acquisition system (IOTech, Cleveland, OH) under one of two configurations: (1) open circuit where V_{oc} = electromotive force (EMF) of the mini-MFC, or (2) closed circuit configuration, where current, I, through a load resistance, R, was calculated using Ohm's law: $V = IR$. Output power P was then calculated by $P = IV$. Voltages were recorded every 2 min by a computer with Personal DaqView software (IOTech, Cleveland, OH). Short circuit currents (I_{sc}) were measured with a Fluke 77 multimeter (Fluke Inc., Everett, WA) when the anode and cathode were connected directly through the multimeter. Coulombic efficiency of the aerobic mini-MFC was calculated as described in previous publications [8,20].

3. Results

Fig. 2a and b plots current and power generated over 6 days by the mini-MFC using an aerobic culture of DSP10 without the addition of exogenous mediators. Dissolved oxygen measurements throughout the experiment showed ≥8 ppm of oxygen in the inlet tubing and ≥1 ppm in the outlet tubing. The data shows a slight rise in current and power on day 1, prior to any addition of lactate. This low level of current is most likely due to DSP10 metabolism of remaining nutrients from the original LB broth. After the first day, 4.3 mmol of lactate was then added to the 50 mL DSP10 culture, resulting in a slow and steady rise of output current and power over the course of 3 days, culminating in the highest aerobic output measured at 1 mA and 0.50 mW. After day 4, the current and power drop sharply as the lactate

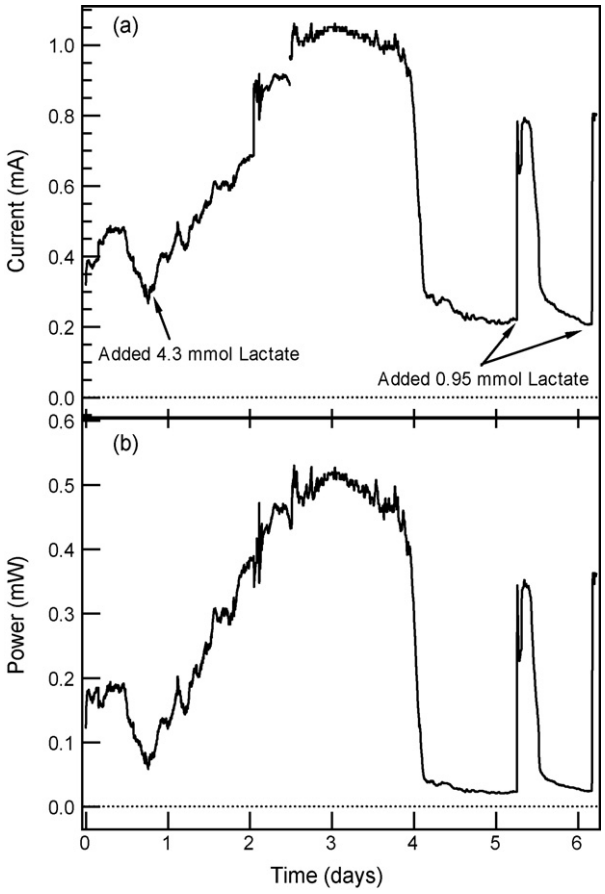

Fig. 2. (a) Current and (b) power vs. time for an aerobic culture of DSP10 operating in the mini-MFC.

is extinguished and rise again as a smaller amount of lactate is added on day 5 and again on day 6.

The slightly lower current and power measured on days 5 and 6 (0.8 mA, 0.36 mW) may be due to a dependence on lactate concentration or slight changes in the concentration of DSP10 in the anolyte. Previous mini-MFC experiments have determined that larger lactate concentrations do promote higher currents and longer periods of high current and power output when compared to lower concentrations [8]. This effect is observed for both anaerobic and aerobic mini-MFCs. Cell counts were taken throughout the experiment via dilution and plating methods, showing relatively consistent concentrations ranging from a maximum of 5×10^7 on day 4 to a minimum of 2×10^7 on days 1 and 6. It is possible that these variations in cell counts are responsible for the lower current and power observed on days 1 and 6. It is also possible that metabolites excreted by the DSP10 culture result in lower cell concentrations and output current over time. Overall this plot demonstrates constant function of the mini-MFC with an aerobic anode chamber over the course of 6 days with sustained output current ($\geq$0.80 mA) and power ($\geq$0.36 mW).

Based on the number of usable electrons present in the added lactate substrate, the Coulombic efficiency of the aerobic mini-MFC can be calculated from the data shown in Fig. 2. Assuming a theoretical maximum yield of four electrons per lactate molecule (metabolized from lactate to acetate) [12], the Coulombic efficiency peaks at $5.5 \pm 0.4\%$ for the addition of 4.3 mmol lactate (days 1–4) and is reduced to $3.1 \pm 0.3\%$ for the 0.95 mmol additions of lactate. This observed reduction in efficiency is possibly a result of lower cell concentration and the need to refresh the anolyte culture. The calculated efficiency for the aerobic mini-MFC is roughly half the efficiency calculated for the anaerobic system. This reduced efficiency is most likely due to dissolved oxygen in the anode chamber that scavenges electrons via reaction (1). This scavenging reaction competes with electron diffusion to the anode, thus reducing the overall Coulombic efficiency of the system. Due to the presence of the PEM between the cathode and anode chambers, oxygen crossover between the chambers is most likely minimal compared to the initial concentration of dissolved oxygen in the anolyte and catholyte (near saturation). Even under aerobic conditions, we find the efficiency for the mini-MFC to be similar to previously published MFCs using a purely anaerobic *Shewanella* culture with no exogenous mediators [15].

A more detailed look at the current production from the aerobic anode reveals a complex environment where oxygen scavenging, electron donation to the electrode, and lactate consumption occur simultaneously. Fig. 3a is a plot of output current versus time as the pumping is stopped (time 0 s), while Fig. 3b is a graph of both estimated lactate consumption and measured dissolved oxygen concentration over the same time period. The rate

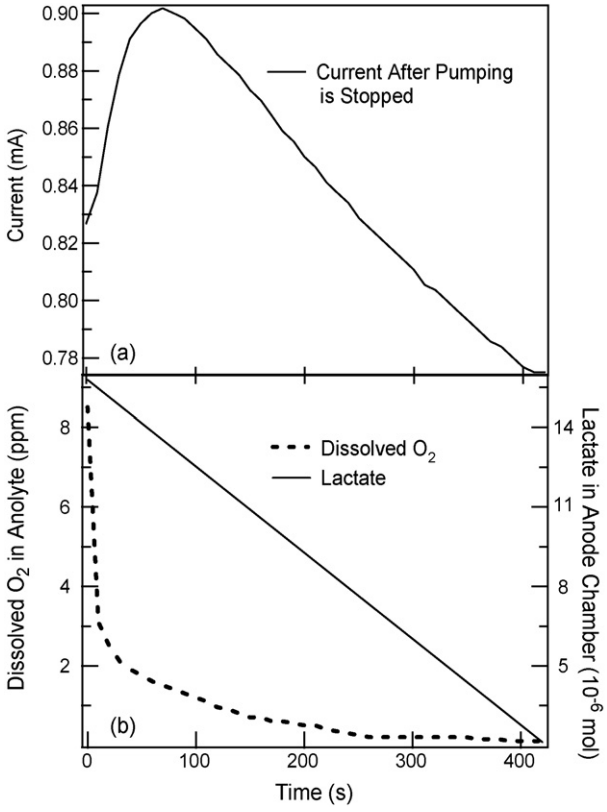

Fig. 3. (a) Current vs. time after pumping is stopped. (b) Dissolved oxygen concentration in the anolyte and amount of lactate in the anode chamber over the same time period.

of lactate loss (2.9×10^{-5} mmol s^{-1}) is calculated from Fig. 1 by dividing the amount of lactate added (0.95 mmol) by the consumption time (9 h). The amount of lactate inside the anode chamber when the pump is stopped is calculated from the initial concentration (16 mM) and the free volume inside the anode chamber (~1 mL). Because the anode and cathode chambers are sealed, fresh input of oxygenated anolyte through pumping is the only condition that maintains an aerobic environment in the anode chamber. Fig. 3b shows that when the pump is stopped, the dissolved oxygen concentration in the anolyte is reduced to below 2 ppm in less than 30 s. The dissolved oxygen is then more slowly reduced to produce anaerobic conditions over the following 400 s. If electrons are actively being scavenged by dissolved oxygen in the anode chamber (i.e., aerobic conditions), the output current should increase when the concentration of oxygen decreases due to the stopped fluid flow (i.e., more electrons can diffuse to the electrode and more protons can diffuse to the PEM before being scavenged via reaction 1). This process is shown by the data in Fig. 3a as we find that the current increases by nearly 10% over the first 80 s after the anolyte flow is stopped. This increase is followed by a decrease over the next 300 s that follows the estimated decrease in available lactate in the anode chamber. This peaking behavior in measured output current is evidence that the anode chamber is under aerobic conditions when 1.2 mL min^{-1} flow is maintained, and quickly becomes anaerobic as the pumping is stopped.

Voltage and power spectra were also measured and compared to previous results obtained using an anaerobic DSP10 culture in the mini-MFC [8]. Fig. 4a and b are plots of voltage and power versus output current for the aerobic (closed circles) and anaerobic (open squares) mini-MFC (*no exogenous mediators added*). Similar open circuit voltages (0.725 V) are found for both systems, but the voltage is sustained at larger output currents for the anaerobic system than for the aerobic. Short circuit current for the aerobic mini-MFC (1.2 mA) was 37% lower than for the anaerobic culture (2.0 mA). Maximum output power (0.40 mW) and current at maximum power (0.80 mA) for the aerobic mini-MFC is 33% lower than the anaerobic system (0.60 mW, 1.2 mA). We therefore find a slight reduction in output, but significant current and power can be generated under completely aerobic conditions.

By using the true surface area of the graphite felt anode (611 cm^2), the current density at maximum power and power density of the aerobic mini-MFC are 13 mA m^{-2} and 6.5 mW m^{-2}, respectively. The current and power density is greatly increased when the cross-sectional area (2 cm^2) and anode chamber volume (1.2 cm^3) are used for the calculation, resulting in 4 A m^{-2}, 0.67 kA m^{-3}, 2.0 W m^{-2}, and

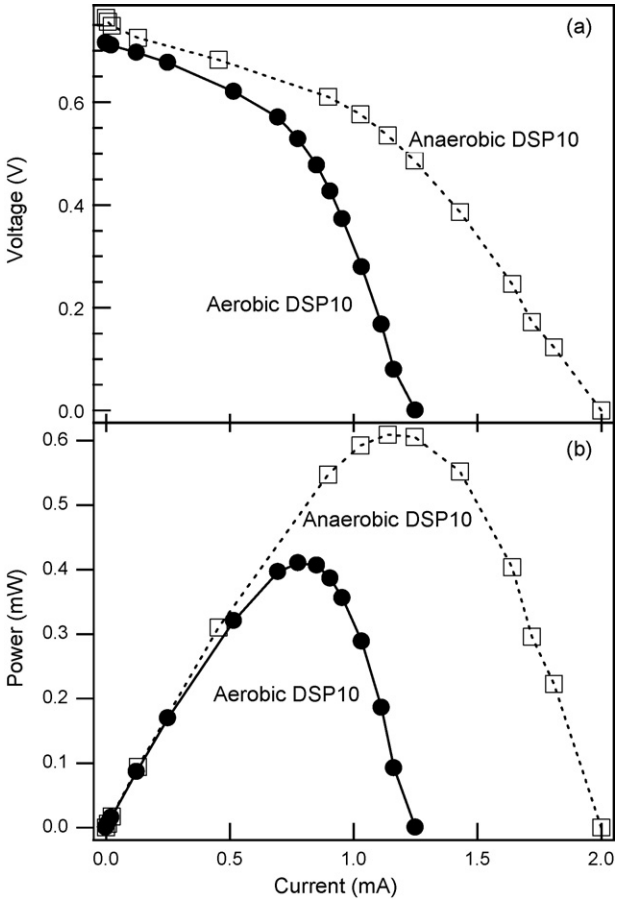

Fig. 4. (a) Voltage and (b) power vs. output current for the aerobic and anaerobic mini-MFC operating with no exogenous mediators.

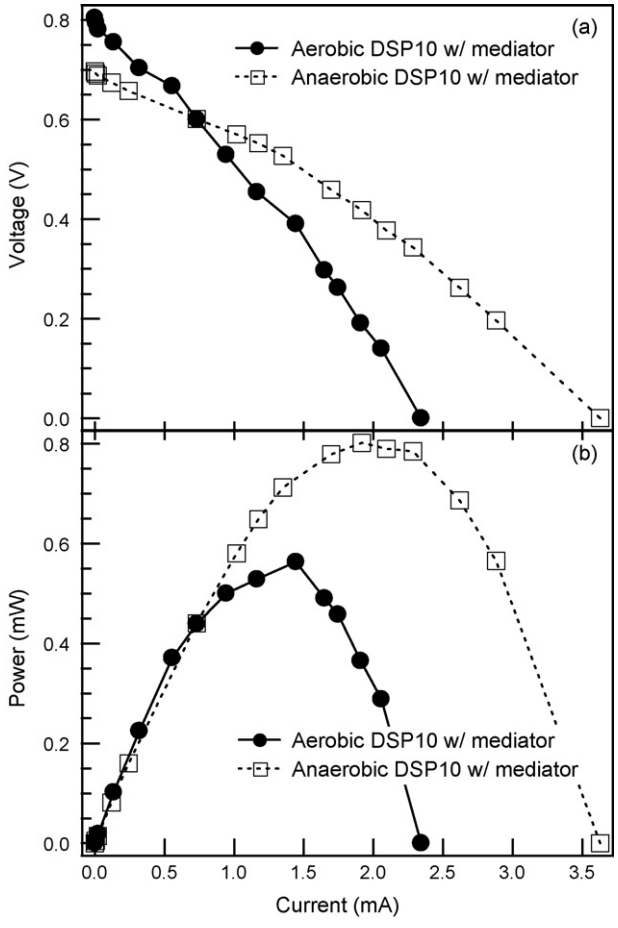

Fig. 5. (a) Voltage and (b) power vs. output current for the aerobic and anaerobic mini-MFC operating with the electron mediator AQDS.

0.33 kW m^{-3}. These current and power densities are a result of the small dimensions of the device and the large surface-area-to-chamber volume ratio (500 cm^{-1}).

Similar current and power data were taken for the mini-MFC operating in aerobic conditions in the presence of the externally added electron mediator AQDS. Fig. 5a and b are plots of voltage and power versus output current for the mediated system. Maximum current (2.4 mA), current at maximum power (1.4 mA) and maximum power (0.54 mW) for the aerobic mediated mini-MFC are each reduced by 33% when compared to the anaerobic system (open squares, dashed line; Fig. 5b). These results are consistent with the aerobic system without the addition of exogenous mediators that also showed a 33% reduction in current and power when compared to the anaerobic system. However, the presence of externally added soluble electron mediators does seem to enhance the performance of the aerobic mini-MFC, increasing the maximum output power by 35%.

4. Discussion

The current and power density for the mini-MFC when operating with an aerobic anode chamber compare favorably to MFCs that operate with anaerobic *Shewanella* cultures. A macroscopic MFC utilizing pure *Shewanella* without exogenous mediators in an H-cell reactor produced no measurable current with an aerobic anode and 8 mA m^{-2} and 0.3 mW m^{-2} with an anaerobic anode (calculated with anode cross-sections). For the same microbe, the measured power density of the mini-MFC was 4 A m^{-2} and 2 W m^{-2} with an aerobic anode and 6 A m^{-2} and 3 W m^{-2} with an anaerobic anode. These results show 750 times higher current density and 10^4 times higher power density for the mini-MFC versus the H-cell reactor under anaerobic conditions, and even larger differences for an aerobic culture. There are possible power density limitations for the mini-MFC design when scaling up several orders of magnitude (longer diffusion paths, etc.), so comparing macroscopic MFC's to the mini-MFC is difficult. In addition, the H-cell MFC utilized an oxygen electrode and the mini-MFC used ferricyanide, which can result in up to eight times higher power density [21]. Even with these considerations, it appears that the efficient design of the mini-MFC enables enhancements over the traditional H-cell design.

Other MFC's have utilized non-traditional designs (reagent flow reactors) or consortiums of mixed bacteria from various sources. The highest power density per volume reported in the literature is for a mixed-culture, macroscopic MFC (102 W m^{-3}) run with flowing wastewater [22]. There is also a tubular design that places the anode and cathode in close proximity and utilizes reactant flow [23]. This approach has resulted in slightly lower power densities (90 W m^{-3}). The highest power per surface area for a macroscopic MFC is reported using solid graphite electrodes and a mixed culture of bacteria from anaerobic sludge (3.6 W m^{-2}) [24]. None of these previous literature examples reported operating MFC's with an aerobic anode. The observed power density for the pure culture mini-MFC (2 W m^{-2}, 330 W m^{-3}), even when functioning with an aerobic anode, compares favorably with each of these MFC's. In fact, the mini-MFC's power density per volume is over three times larger than the highest density reported to date. Based on these results, it is possible that the power density per volume of macroscopic MFCs may be enhanced by using several serially stacked mini-MFCs rather than a single MFC of equal volume.

There is also one example in the literature of a reduced-size MFC. Chiao et al. describes a microfabricated MFC (2D electrodes) that utilized an anode yeast culture, a ferricyanide catholyte, and a large surface area-to-chamber volume ratio [25]. Current and power density per true surface area (0.5 cm^2) was reported to be 30–100 mA m^{-2} and 5×10^{-3} mW m^{-2} with a power per volume of 0.5 W m^{-3}. Very short run times were reported, with significant drop in output current after only 15 min. We find a similar current density per true surface area over a period of 6 days and a much higher total current and power output for a similar cross-section device (mini-MFC: 2 cm^2; microfab. MFC: 1.5 cm^2). The utilization of high surface area 3D electrodes in the mini-MFC increases the power density nearly three orders of magnitude when compared to this 2D microfabricated MFC.

5. Conclusions

Contrary to previously published results, we find that a redesigned MFC can produce measurable current and power when the anode chamber is purposely maintained under aerobic conditions. By using an efficient miniaturized MFC design with *S. oneidensis*, which is capable of both anaerobic and aerobic respiration, we demonstrate that electron donation to the anode and proton diffusion to the PEM can compete with oxygen scavenging in the anode chamber. The aerobic mini-MFC was found to produce over 60% the output power and current of the anaerobic device, while the Coulombic efficiency was halved. These results may enable power generation devices to be engineered that are capable of operation in the oxygen-rich water column rather than previously demonstrated MFCs that function solely in the sub-sediment, near-anaerobic regions of rivers and the ocean.

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