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Short communication

# Anaerobes unleashed: Aerobic fuel cells of *Geobacter sulfurreducens*

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ABSTRACT

One of the limitations of power generation with microbial fuel cells is that the anode must typically be maintained under anaerobic conditions. When oxygen is present in the anode chamber microorganisms oxidize the fuel with the reduction of oxygen rather than electron transfer to the anode. A system in which fuel is provided from within a graphite anode and diffuses out to the outer surface of the anode was designed to overcome these limitations. A biofilm of *Geobacter sulfurreducens* strain KN400, pregrown on the surface of a graphite electrode in a traditional two-chambered system with an anaerobic anode chamber and acetate as an external fuel source, produced current just as well under aerobic conditions when acetate was provided via diffusion from an internal concentrated acetate solution. No acetate was detectable in the external medium. In contrast, aerobic systems in which acetate was provided in the external medium completely failed within 48 h. Internally fed anodes colonized by a strain of KN400 adapted to grow at marine salinities produced current in aerobic seawater as well as an anaerobic anode system. The ability to generate current with an anode under aerobic conditions increases the potential applications and design options for microbial fuel cells.



## 1. Introduction

Although many potential large-scale applications for producing electrical power with microbial fuel cells have been proposed, at the present stage of development the short-term practical deployment of microbial fuel cells appears limited to localized powering of electronic devices in remote locations [1]. For example, sediment fuel cells are simple, effective devices that can extract electrons from organic matter naturally present in aquatic sediments [2–5]. The anode, which is buried in anoxic sediments, and the cathode, which is suspended in the overlying aerobic water, are both exposed to the environment. In contrast to many microbial fuel cell applications, there is no need to enclose the anode in a chamber to promote anaerobic conditions at the anode; or to incorporate ion-selective membranes to limit diffusion of oxygen toward the anode. This is because the anoxic sediment naturally provides anaerobic conditions at the anode surface. The sediment microbial fuel cell is a static system, eliminating the energy inputs associated with pumping and stirring in many other microbial fuel cell designs. Thus, although the current outputs of sediment fuel cells are low, there is a net energy output sufficient to power electronic monitoring devices [6]. In contrast, most laboratory scale microbial fuel cells serving as prototypes for other microbial fuel cell applications have designs that will consume more energy in long-term application than they produce.

The potential that sediment microbial fuel cells may serve as a long-term power source, extracting energy from a constantly renewing source of organic matter in sediments, is very attractive. However, a limitation of sediment microbial fuel cells is the necessity to anchor the anode in anoxic sediments. Microbial fuel cells in which the anode could function in aerobic water would expand the range of aquatic locations in which microbial fuel cells might be used to power electronic devices [7]. Such 'aerobic microbial fuel cells' might have the advantage over traditional batteries in lower costs of materials and presenting a lower explosion hazard during transportation prior to deployment.

Biofilms growing on stainless steel anodes were able to produce current in aerobic seawater that was nearly one-third that of a similar anaerobic system [8]. However, the system was not a true microbial fuel cell because the anode was electronically poised at a negative potential, thus net power was not produced. Furthermore, electronically poising the anode could potentially provide electrons to promote removal of oxygen within the biofilm. Current-producing biofilms were not produced if the anode was not electronically poised [8].

A laboratory microbial fuel cell inoculated with the facultative anaerobe, *Shewanella oneidensis*, continued to produce power when dissolved oxygen was purposely introduced into the anode chamber [7]. In this system a relatively large culture reservoir was maintained under aerobic conditions and continuously

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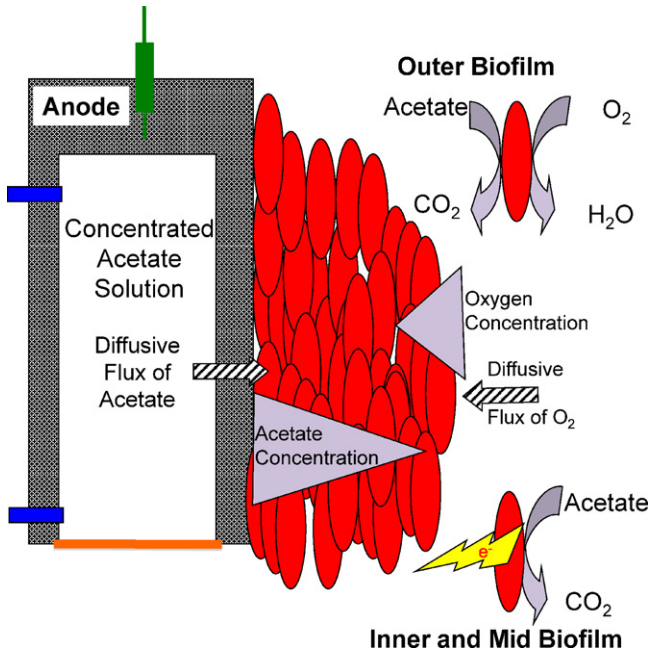


**Fig. 1.** Model for functioning of aerobic microbial fuel cell. The electrical connection, the ports for adding acetate, and the cap over the drilled out chamber are designated in green, blue, and orange, respectively. Red rods designate bacterial cells.

cycled through a small anode chamber. Current was produced (6.5 mW $m^{-2}$ and 13 mA $m^{-2}$) from a lactate fuel source, despite the presence of oxygen in the anode chamber. Cells did not appreciably attach to the anode and electron transfer between *S. oneidensis* and the anode was thought to proceed via electron shuttles [7] which *Shewanella* species have previously been shown to excrete [9–11].

However, the previously described *Shewanella*-based system would not be directly applicable to powering electronics in aerobic water. It was a complex system requiring continuous pumping to recirculate the anolyte between the anode and the large anolyte reservoir. Therefore, it is likely that the power consumption of the system far exceeds the power output at the anode. Instead of reducing oxygen as the electron acceptor, the catholyte was ferric cyanide, which is a non-renewable, toxic electron acceptor that would not be suitable for long-term field deployments. Columbic efficiencies were low (<6%), when calculated based on the incomplete oxidation of lactate to acetate. The accumulation of two-thirds of the electrons available in lactate in the waste product acetate meant that the overall efficiency of conversion of fuel to current was even lower.

There are organisms, such as *Geobacter* species, that can effectively oxidize acetate with electron transfer to electrodes [3,12]. For example, *Geobacter sulfurreducens* converts acetate to current with columbic efficiencies of over 90% [13,14]. Although *G. sulfurreducens* is considered to be an anaerobe, it is capable of withstanding low levels of oxygen and under these conditions may use oxygen as an electron acceptor to support growth [15].

The purpose of the study reported here was to determine whether power could be produced in a *G. sulfurreducens* microbial fuel cell suspended in aerobic seawater. It was hypothesized that if the electron donor was provided from within the anode, diffusing through the porous graphite material, that this would provide a natural anaerobic zone within the *G. sulfurreducens* biofilm, analogous to those observed in aquatic sediments (Fig. 1). The cells on the outer surface of the biofilm, exposed to dissolved oxygen, would consume the oxygen, leaving an anaerobic internal zone in which acetate is consumed with electron transfer to the anode. The results demonstrate that this strategy was successful.

## 2. Methodology

### 2.1. Organism and culture conditions

*G. sulfurreducens* strain KN400 [14], hereafter referred to as KN400, was obtained from our laboratory culture collection. KN400 was routinely cultured in a freshwater anaerobic medium with acetate (10 mM) as electron donor and fumarate (40 mM) as electron acceptor, as previously described [16].

### 2.2. Colonization of anodes with KN400

Biofilms of KN400 were pregrown on anodes in H-type, two-chambered devices, in which the anode and cathode chambers are separated with a Nafion, cation-selective membrane [12,17]. The solid block graphite anodes that are typically employed were replaced with anodes with an interior chamber (Fig. 1). The anodes were Grade G20 graphite blocks (2.54 cm by 7.62 cm by 1.27 cm) or cylinders (7.62 cm by 2.54 cm dia.) (Graphite Engineering and Sales, Greenville, MI). Two evenly space holes were drilled 2 in. into the 1.27 cm by 2.54 cm side of the anode. These 2 holes were connected by drilling a hole 5.08 cm up the 1.27 cm by 7.62 cm side from the end of the anode with the 2 holes. The hole in the side of the anode was sealed using epoxy and a 1 cm by 1 cm piece of glass coverslip. The resulting anode has a U shaped hole in it. Pharmed tubing and stainless steel fittings were used to connect tubing to the anode. Cylindrical anodes had a 6.35 cm deep hole drilled in the end sealed by epoxying a piece of glass over the end. The tubing was run out of the anode chamber of the H-cell through one of the stoppers.

Growth on the anodes was initiated in the anaerobic anode chamber that contained acetate (10 mM)/fumarate (40 mM) medium with a 10% inoculum of acetate/fumarate-grown cells. The anodes were poised at −400 mV (versus Ag/AgCl) with a potentiostat, as previously described [13]. Once an $A_{600nm}$ of 0.2 was reached the medium was completely exchanged with medium that still contained acetate as the electron donor, but with the fumarate omitted. Thus, there was no electron acceptor other than the anode. When current production began fresh medium was continuously added to the anode chamber as previously described [13]. Biofilms were grown until a current of 10 mA was achieved in the poised system. The medium input to the anode chamber was then changed to one in which the acetate was excluded and the internal chamber of the anode was filled with a concentrated (5 M) acetate solution. Current remained steady for 30 days even though acetate became undetectable (<10 μM) in the external medium within 5–10 days.

Once acetate was no longer detectable in the anode chamber of systems with an internally fed anode, the systems were switched to fuel cell mode. The bicarbonate-buffered medium in the cathode chamber was replaced with medium that had the same components, but with Tris buffer (22 mM) substituted for the bicarbonate and the cathode chamber was bubbled with air. The use of Tris buffered-medium was required because the bicarbonate-buffered medium reached an unacceptably high pH (>9) when bubbled with air. The potentiostat was disconnected and a direct connection was made between the anode and the cathode across a 560 Ω resistor. When the anode chamber was maintained under anaerobic conditions the current output of these fuel cells was ca. 0.35 mA.

### 2.3. Aerobic growth

In order to examine the potential for current production under aerobic conditions, the Nafion separating the anode and cathode chambers was removed and the medium in both chambers was replaced with Tris-buffered acetate-free medium. Controls were treated the same, except that the anodes were solid graphite blocks

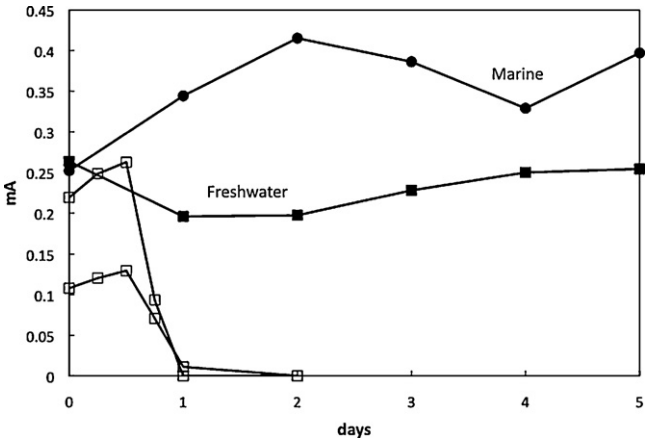


**Fig. 2.** *G. sulfurreducens* strain KN 400 in membrane-less aerobic H-type fuel cells. Filled and open symbols designate internally and externally fed anodes, respectively.

and the anode-chamber received acetate-containing medium throughout.

In order to determine whether a system capable of functioning in seawater could be developed, a strain of KN400 was adapted to grow in a marine salinity medium, which was the standard freshwater medium amended with ($g\,L^{-1}$): NaCl, 18; $MgCl_2{\cdot}6H_2O$, 5.4; $CaCl_2{\cdot}2H_2O$, 0.27. The strain was adapted by successively increasing the salinity in the medium supplied to cells growing on the surface of a graphite electrode poised at −400 mV. Once the current reached 10 mA with freshwater medium, the medium fed into the anode chamber was replaced with one with a salinity 20% that of marine salinity. Current production initially declined, but once it restabilized at 10 mA the salinity was increased by 20% increments until full marine salinity was achieved. Cells of this marine-adapted strain grown on an anode were scraped off the anode with a sterile blunted needle and used to inoculate systems for studies at marine salinities.

Initial studies on internally fed anodes at marine salinity were conducted in H-cell systems as described above. In addition, internally fed anodes producing current under aerobic conditions in H-cells were transferred to 20 L of Tris-buffered marine medium in a fish tank. The anodes were placed within a perforated pipe and connected to an array of 8 stainless steel brushes (2.54 cm dia. by 25.4 cm overall length with 12.7 cm bristle fill) that functioned as cathodes. The medium was continuous bubbled with air. The marine medium was exchanged with artificial seawater (Instant Ocean, United Pet Group, Inc., Cincinnati, OH) and production of current monitored further.

### 2.4. Other analyses

Acetate and other organic acids were determined via HPLC with an Aminex HPX-87H column (Biorad, Hercules, CA) with an eluent of 8 mM $H_2SO_4$ and UV detection at 210 nm. Oxygen was monitored with a DO meter (YSI model 550A, Yellow Springs, OH). In order to image anode biofilms, cells were treated with live/dead BacLight Stain and were imaged with scanning laser confocal microscopy, as previously described [13,17].

## 3. Results and discussion

KN400 fuel cells with rectangular internally fed anodes continued to produce current when switched from a traditional fuel cell with an anaerobic anode chamber to the aerobic system (Fig. 2), at a level comparable to anaerobic fuel cells of the same design [12]. No acetate could be detected in the external medium, suggesting that the KN400 biofilm consumed the acetate as fast as it diffused from the anode interior. It was not possible to reliably estimate how much of the acetate that was oxidized was converted to current because the very high concentrations of acetate within the anode made it impossible to detect a loss of acetate during the time frame of the studies.

After 7 days one of the internally fed electrodes was removed and examined with scanning laser confocal microscopy (Fig. 3A). All of the cells stained green with live/dead stain, suggesting that the cells throughout the biofilm were metabolically active and that the presence of oxygen in the external medium did not adversely affect the biofilm.

In contrast to the steady current production with the anode biofilm supplied acetate from within the anode, current production rapidly declined when acetate was provided externally (Fig. 2). Cells within the biofilm imaged after two days under aerobic conditions stained red, indicating the cells were compromised (Fig. 3B). It is speculated that the death of the biofilm when both acetate and oxygen are provided in the external medium might be attributed to high concentrations of both acetate and oxygen being available at the outer surface of the biofilm, resulting in high rates of metabolism and free radical formation. As the outer surface cells

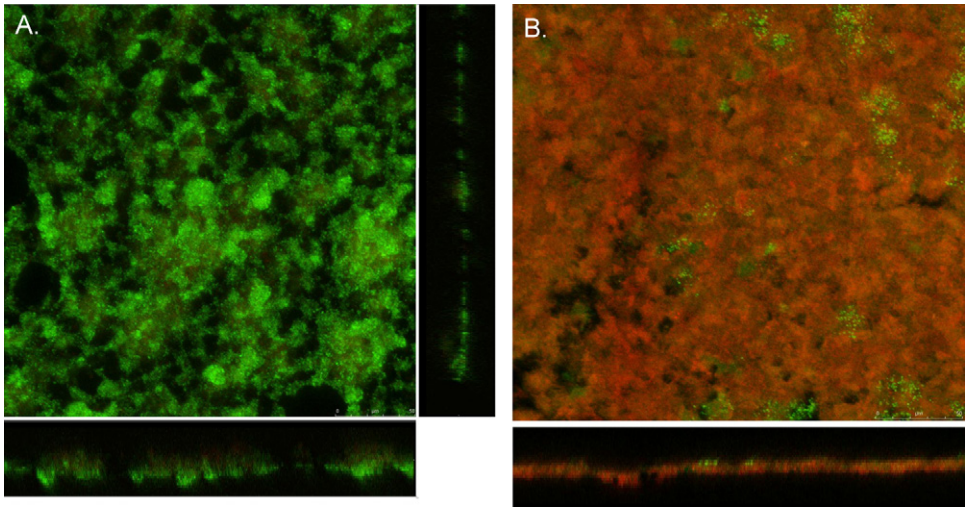


**Fig. 3.** Confocal laser scanning microscopy of biofilms of *G. sulfurreducens* strain KN400. (A) Internally fed biofilms after 5 days in aerobic media. (B) Externally fed biofilms after 2 days in aerobic media.

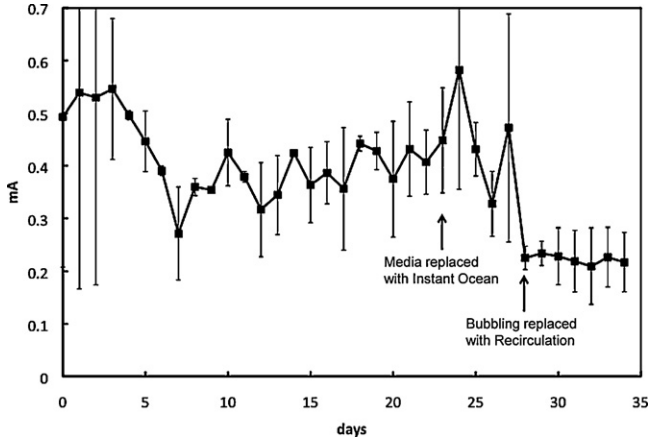


**Fig. 4.** Current average of two internally fed aerobic fuel cells of marine-adapted *G. sulfurreducens* strain KN400. Error bars indicated range.

are killed due to oxidative stress, high acetate and oxygen will be available at successively lower levels of the biofilm, resulting in the eventual death of the entire biofilm. Alternatively, when acetate is provided from within the anode, acetate concentrations, and hence rates of metabolism, are expected to be highest deep within the biofilm, which remains anaerobic. Slower rates of metabolism at the outer surface, due to low acetate availability, may be sufficient to consume oxygen, without overproduction of radicals, reducing the level of oxidative stress. Furthermore, if as previously proposed [17,18], the biofilms of *G. sulfurreducens* are electrically conductive, then electrons released into *G. sulfurreducens* biofilms primarily near the acetate source with internally fed anodes may be conducted not only to the anode, but also to the outer surface of the biofilm where oxygen is available. This would provide a source of electrons for oxygen consumption without the metabolism-dependent production of free radicals by cells in the outer surface.

A strain of KN400 adapted to grow at marine salinities also successfully produced current under aerobic conditions when acetate was provided from within the rectangular anode (Fig. 2). As in the freshwater strain, acetate appeared to be completely consumed within the anode biofilm because it was undetectable in the external medium. When the cylindrical anodes were placed within a fish tank containing marine medium, they continued to produce current (Fig. 4). Current production continued when the marine medium was replaced with artificial seawater (Fig. 4). The concentration of dissolved oxygen at all locations in the tank was steady at slightly less than that expected (5 mg $L^{-1}$) for equilibration with the atmosphere at all locations in the tank. The drop when aeration was replaced with recirculation is likely due to the forces of the water moving, as the dissolved oxygen remained near saturation throughout.

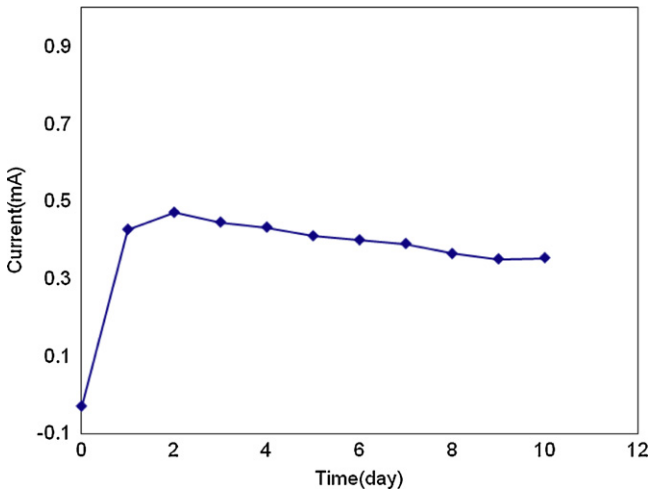


**Fig. 5.** Potentiostat poised internally fed aerobic fuel cells of marine-adapted *G. sulfurreducens* strain KN400. Data is representative of multiple replicates.

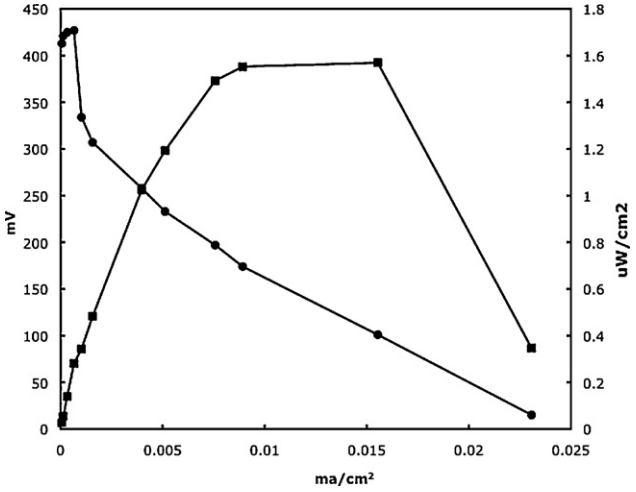


**Fig. 6.** Current–voltage curve of internally fed aerobic fuel cells of marine-adapted *G. sulfurreducens* strain KN400. Circles are voltage. Squares are power density.

In order to determine whether electron transfer at the cathode was limiting the current production, studies were conducted in which the anode potential was maintained with at +300 mV with a potentiostat (Fig. 5). Current output was not significantly improved suggesting that some other factor limited current production. Other factors that could limit current production would include diffusion of protons, availability of electron donor, etc.

Current–voltage studies were performed on the open tank fuel cells with cylindrical anodes, and the results were similar (same traditional curve shape and power response) to those typically obtained in two-chambered anaerobic/aerobic systems [3] (Fig. 6). This provides further evidence that the aerobic conditions of the tank did not adversely affect the function of the biofilm.

## 4. Conclusions

Freeing the anode of the microbial fuel cells from the necessity to be in an anaerobic environment increases the range of their potential applications. This approach does require that a fuel be provided within the anode interior, which could make it inappropriate for some applications. With the current design, it is calculated that the acetate fuel supply will last for 750 days, assuming a 50% columbic efficiency of conversion of acetate to current. Thus, these results suggest that supplying anode biofilms with fuel from within the anode might offer a strategy for long-term current production with inexpensive non-toxic, non-explosive materials.

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