

# A microbial fuel cell operating at low pH using the acidophile *Acidiphilium cryptum*

Abhijeet P. Borole · Hugh O'Neill ·  
Costas Tsouris · Scott Cesar

Received: 13 December 2007 / Revised: 29 February 2008 / Accepted: 3 March 2008 / Published online: 27 March 2008  
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**Abstract** For the first time, a microbial fuel cell has been developed using an acidophile, *Acidiphilium cryptum*, as the anode biocatalyst. Electricity production using its natural electron acceptor, iron, as the electron mediating agent at pH values  $\leq 4.0$  was demonstrated. Accumulation of Fe(III) at the electrode, however, restricted current output. The

combination of nitrilotriacetic acid and Phenosafranin as electron mediators increased the power output to  $12.7 \text{ mW/m}^2$  in a two-chamber air-sparged fuel cell. Direct electron transfer from the microorganisms to the anode was also investigated but was not detected under the conditions studied.

**Keywords** *Acidiphilium cryptum* · Acidophile · Biofuel cell · Electricity · Mediated electron transfer

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A. P. Borole (✉)  
Biosciences Division, Oak Ridge National Laboratory,  
Oak Ridge, TN 37831-6226, USA  
e-mail: borolea@ornl.gov

H. O'Neill  
Center for Structural Molecular Biology, Oak Ridge  
National Laboratory, Oak Ridge, TN 37831-6226, USA

H. O'Neill  
Chemical Sciences Division, Oak Ridge National  
Laboratory, Oak Ridge, TN 37831-6226, USA

C. Tsouris  
Nuclear Science and Technology Division, Oak Ridge  
National Laboratory, Oak Ridge, TN 37831-6226, USA

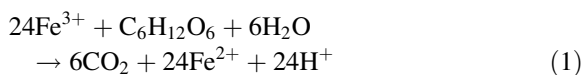
S. Cesar  
Oak Ridge Associated Universities, Oak Ridge,  
TN 37830, USA

## Introduction

Microbial fuel cells (MFCs) have been demonstrated using a variety of organisms, such as those from the taxa Firmicutes,  $\delta$ -,  $\gamma$ -,  $\beta$ -, and  $\alpha$ -Proteobacteria, as anode catalysts (Logan and Regan 2006). The class of organisms populating the electrodes depends on the carbon source as well as the inoculum. Research indicates that several of the organisms originating from anaerobic sediments as the inoculum include iron-reducing organisms (Lovley 2006). These organisms are typically neutrophilic heterotrophs. Use of acidophilic bacteria as anode biocatalysts has not been reported to date. Here, we investigate the use of *Acidiphilium cryptum*, an iron-reducing acidophilic heterotroph, as a biocatalyst in the anode chamber of an MFC with glucose as the electron donor. The organism can tolerate pH from 2.1 to 5.8 under aerobic conditions, with optimum growth at pH 3.2

(Kusel et al. 1999). The pH range for iron respiration is not known, but the maximum rate of respiration was at pH 3.5 (Bilgin et al. 2004, 2005).

The advantages of operating an MFC at low pH are the availability of protons at the cathode and the proton transfer process across the cation-exchange membrane separating the anode and cathode. Because of a higher driving force, the use of low pH at the anode side is expected to result in higher proton transfer rates and can alleviate proton limitations at the cathode. However, this is true only in systems that have a high electron transfer rate (equivalent to a power density of 1 W/m<sup>2</sup> or higher). A system that can operate at low pH and also produce a high electron transfer rate has not been demonstrated. Here we investigate the potential of the acidophile *A. cryptum* as an anode biocatalyst under low pH conditions. The ability of *A. cryptum* to function in a pH range in which Fe(II) is soluble (pH > 4) and to use glucose, a renewable resource, as the electron donor were important reasons for choosing this organism. Iron reduction coupled with glucose oxidation can be described by the following reaction (Bilgin et al. 2005):



The reduced iron is oxidized at the electrode, giving Fe(III) as follows:



The electrons are received at the cathode by oxygen, producing water:



Acidophilic organisms and systems capable of transferring electrons at a high rate to the electrodes may have significant potential for generation of electricity via microbial fuel cells.

## Materials and methods

### Bacterium and media

*Acidiphilium cryptum* ATCC 33463 was grown on Lhet2 medium containing (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (2 g), KCl (0.1 g), K<sub>2</sub>HPO<sub>4</sub> (0.5 g), MgSO<sub>4</sub> · 7H<sub>2</sub>O (0.5 g), tryptic soy broth without dextrose (0.1 g) and glucose

(1 g) in 1 l H<sub>2</sub>O (Bilgin et al. 2005). The pH was adjusted to 4 using 1 M H<sub>2</sub>SO<sub>4</sub>. The cells were grown in a 1 l stirred fermentor and transferred to the fuel cell apparatus at OD<sub>600 nm</sub> 0.5–1.

### Microbial fuel cell

The fuel cell used in this study was a two-chamber bottle-type design (Oh et al. 2004), with anode and cathode volumes of 600 ml each. A cation-exchange membrane (Nafion-115), 5 cm diameter, separated the two chambers and was held in place between two O-rings in the arm. The anode was composed of a 2.5 × 7.5 × 0.63 cm graphite felt connected to a 6 mm diameter graphite rod. A 5 × 5 cm platinum-deposited carbon cloth was used as the cathode. The distance between the electrodes was approximately 17.5 cm. The bacteria were added to the anode side of the MFC while 100 mM potassium phosphate buffer (pH 4) was used as the catholyte. The MFC was operated at 30°C ± 1°C. During operation, the anode side was sparged with nitrogen while the cathode was sparged with air.

### Analytical

Glucose analysis was done using a YSI analyzer, Model 2300 (Yellow Springs, Ohio). Total Fe analysis was performed using the FerroVer method. Analysis of Fe(II) was with the 1,10-phenanthroline method. Voltage output was measured using a multimeter. The data were continuously collected using a four-port DATAQ DI-158 USB data acquisition device, which was interfaced via USB cable with a computer running the WinDaq data acquisition software. A variable-load resistor (0–5,000 Ω) was used to generate power density curves.

## Results

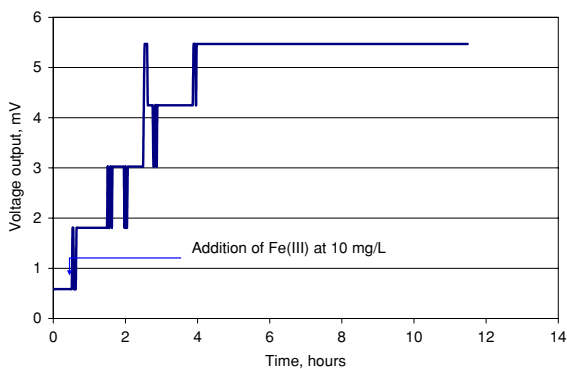
### Fuel cell design

The experiments were carried out in a two-chamber bottle design (Oh et al. 2004). Preliminary experiments highlighted several issues with microbial fuel cells operating at low pH. Firstly, electrode materials and electrical connectors were found to require non-metallic components, because copper or stainless

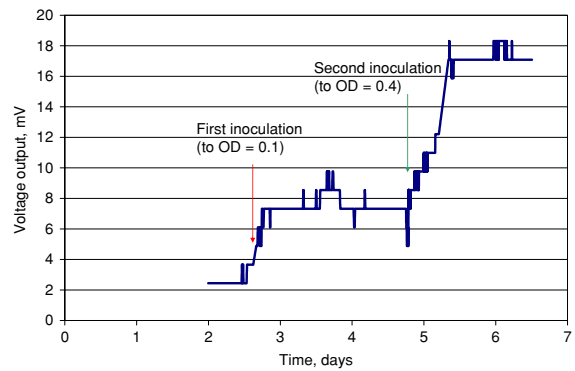
steel contributed to electricity production due to increased metal oxidation at low pH. Thus, all electrodes and connectors were made using carbon, gold, or platinum. Secondly, ferricyanide was found to be unsuitable as a catholyte due to the formation of a bluish-black precipitate on the electrode surface that inhibited further reduction. The precipitation was caused by the formation of insoluble Prussian blue, a complex of ferri- and ferro-cyanide, under low pH conditions.

### Properties of Fe as an electron donor

Initial experiments were conducted to investigate the role of Fe as an electron mediator in the MFC. *A. cryptum* is a facultative organism and has been shown to be capable of iron reduction both in the presence and the absence of oxygen (Bilgin et al. 2005). Addition of aerobically grown *A. cryptum* to the nitrogen-sparged anode chamber led to establishment of anaerobic conditions. The bacteria partitioned into the carbon felt electrode within 4–6 h. No current output was observed until FeCl<sub>3</sub> (10 mg/l) was added to the MFC (Fig. 1). Also, addition of FeCl<sub>3</sub> (25 mg/ml) prior to the *A. cryptum* culture did not produce any current output (Fig. 2). These experiments demonstrated that *A. cryptum* was capable of catalyzing electricity production with Fe(III) as an electron mediator. Concentrations of Fe(III) greater than 25 mg/l did not significantly increase the current output of the MFC (data not shown). However, increasing the initial cell density



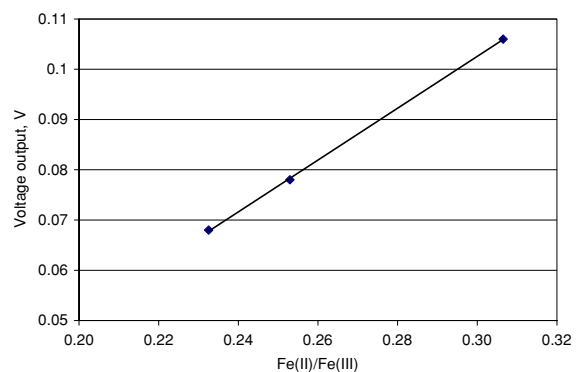
**Fig. 1** Voltage output from the *A. cryptum* MFC at 500  $\Omega$  load, demonstrating electricity production after addition of Fe(III) as the electron carrier. The initial OD<sub>600</sub> of the cells in the anode chamber was 0.03



**Fig. 2** The effect of cell density on voltage output from the *A. cryptum* MFC at 500  $\Omega$  load

of the inoculum did increase the power output (Fig. 2). The maximum open circuit voltage of an MFC with FeCl<sub>3</sub> as the mediator was 0.295 V. Current output was determined to be a function of the Fe(II)/Fe(III) ratio in the bulk anode solution (Fig. 3), indicating that the current output can be increased by targeting higher Fe(II)/Fe(III) ratios.

The potential for direct transfer to the electrode by *A. cryptum* was investigated by promoting biofilm formation on the anode electrode. An MFC was designed using a flow-through carbon felt electrode. The current output from this MFC, which contained negligible iron, was close to zero. The open circuit voltage of this MFC was similar to that of the bottle-type design containing *A. cryptum* (0.3 V), indicating that the microorganism was unable to conduct electrons directly to the electrode.

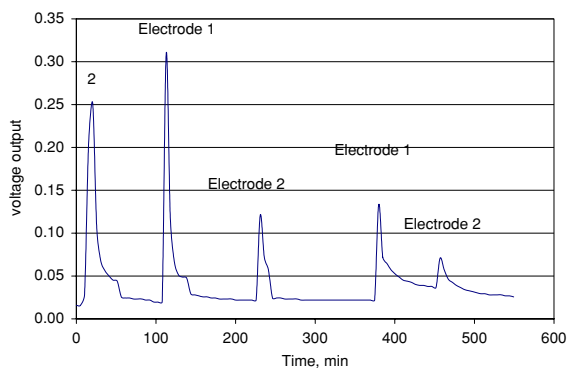


**Fig. 3** Voltage output as a function of Fe(II)/Fe(III) ratio. The cell density in the MFC anode compartment used in this study was OD<sub>600 nm</sub> = 1.0

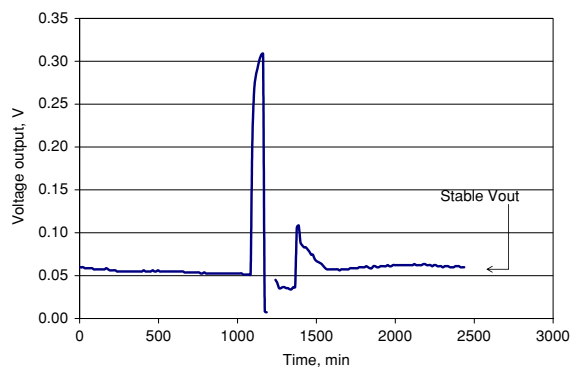
## Regeneration of anode

Application of an external load to a fuel cell usually results in an initial voltage drop to a value corresponding to the resistance applied to the cell (Logan et al. 2006). However, a different behavior was observed in the case of the *A. cryptum* fuel cell. A 500  $\Omega$  external load resulted in an initial voltage drop to 0.25 V, which gradually decreased over 40–60 min to an equilibrium value of 0.032 V (equivalent to a power output of 0.002 mW).

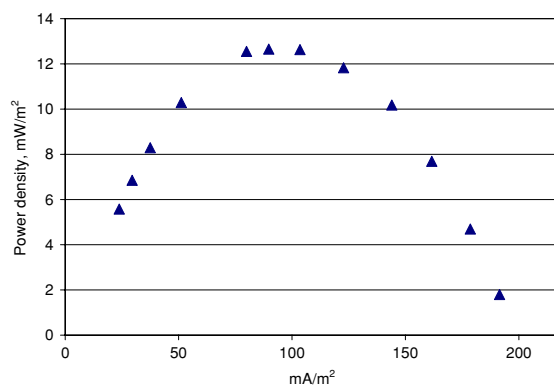
To investigate the gradual decrease in voltage output observed after application of a 500  $\Omega$  external load, two electrodes were placed in the anode chamber and included in the circuit alternately at 2 h intervals. The electrode not connected in the circuit was left undisturbed in the anode solution during that period. The results from the cycling of the two anode electrodes are shown in Fig. 4. As one electrode was used as the anode, the other was regenerated. This behavior suggests that the electrode was passivated, perhaps by formation of a layer of  $\text{Fe}(\text{OH})_3$  on the surface. In the absence of a load, the reducing potential of the anodic solution was sufficient to reduce the  $\text{Fe}(\text{III})$  to  $\text{Fe}(\text{II})$ . An alternative explanation is that the  $\text{Fe}(\text{III})$  accumulated near the electrode surface because of its higher valence, thus screening the surface charge and providing a mass-transfer resistance for  $\text{Fe}(\text{II})$  transferring from the bulk solution to the electrode surface. A reduction in the pH of the anode media to 2.5 did not have a significant effect on the steady-state power output of the *A. cryptum* fuel cell.



**Fig. 4** Voltage output from two anode electrodes during cycling, demonstrating that the accumulation of ferric species on the anode can be partially reversed



**Fig. 5** Voltage output from a mediator-based (NTA-Fe) Phenosafranin *A. cryptum* MFC



**Fig. 6** Power density of an *A. cryptum* microbial fuel cell in a two-chamber configuration with NTA-Fe and phenosafranin as electron mediators

## Alternative electron mediators

Because the inactivation appeared to be due to  $\text{Fe}(\text{III})$  accumulation at the electrode surface, the effect of  $\text{Fe}$ -chelating agents was studied. Replacement of  $\text{FeCl}_3$  with NTA-Fe did not change the steady-state power output (Fig. 5). However, the combination of the organic electron mediator phenosafranin and NTA-Fe resulted in higher steady-state voltage output from the *A. cryptum* MFC (0.07 V at 500  $\Omega$  load, see Fig. 6), which was equivalent to 0.01 mW for the cell output and a power density of 5  $\text{mW}/\text{m}^2$ .

## Discussion

This study was designed to develop a microbial fuel cell that can operate in acidic environments. Acidic

pH has two advantages: firstly, it results in a higher proton concentration, and, secondly, it improves the solubility of ferrous iron. A higher proton concentration can result in improved proton transport across the membrane, while higher solubility of the reduced iron species has the potential to improve the electron mediation to the electrode. Tanaka et al. (1983) reported use of iron chelate compounds as mediators with *E. coli* as the anode biocatalyst in microbial fuel cells at neutral pH. The decay of the voltage output with time reported by Tanaka et al. (1983) was similar to that observed in Fig. 3, although the time scale in the earlier experiment was longer. The open circuit voltage of the cell dropped (from 0.295 to 0.032 V), while the anode half-cell potential increased (from 0.06 to 0.29 V) within an hour after application of the load (500  $\Omega$ ). Upon removal of the load, the voltages reversed and became close to the original values over a 2 h period. These changes may be a result of the changes in the Fe(II)/Fe(III) ratio at the electrode surface. The bulk Fe(II)/Fe(III) ratio measured during the course of the experiment did not change significantly. Thus, the drop in voltage output may be due to precipitation of iron on the electrode surface after its oxidation, creating a barrier against further electron transfer to the electrode. The electrode exchange experiment reported in Fig. 3 suggests that this form of iron can at least be partially converted back into a reduced form by the microorganisms, albeit at a slower rate.

The maximum power density obtained for the *A. cryptum*—based MFC was 12.6 mW/m<sup>2</sup> in the presence of NTA-Fe and phenosafranin (Fig. 6). Comparison of the power density with others reported in the literature shows that the output of the *A. cryptum* system is on the same order of magnitude as MFCs with similar configurations (Table 1). Electrode spacing is known to affect power density significantly (Bilgin et al. 2005; Kim et al. 2005), which may explain the comparatively lower power density for the *A. cryptum* system. Secondly, the maximum voltage output obtained in this study was 0.105 V. As mentioned previously, increase in the voltage output via use of higher biocatalyst densities or organisms capable of operating at lower redox potential may lead to higher power densities, even for the two-chamber configuration. Preliminary results from our laboratory indicate the existence of electrogenic organisms capable of operation under low pH conditions. Further work is in progress to determine the overall and individual resistances within the MFCs operating at low and neutral pH conditions.

Recent studies have reported MFCs with power densities on the order of 1,000 mW/m<sup>2</sup> or higher (Logan et al. 2007). These values were achieved by using novel MFC engineering designs that targeted reduction of the internal resistance by decreasing the separation of the anode and cathode and increasing the electrode surface area. Similar improvements in power output could likely be realized with the

**Table 1** Range of power densities reported for the two-chamber MFC

Anode electrolyte and electrodes	Spacing between electrodes	Anode biocatalyst	Power density, mW/m <sup>2</sup>	References
Nutrient buffer solution pH 7.0, Toray carbon paper electrode	~10 cm	Anaerobic sewage sludge	8	Kim et al. (2005)
Lhet2 medium, pH 4.0, with NTA-Fe and Phenosafranin as mediators, 2.54 × 7.62 cm graphite felt	17.5 cm	<i>Acidiphilium cryptum</i>	12.7	This work
Mineral media, pH 7.0, 2.54 × 7.62 cm graphite		<i>Geobacter sulfurreducens</i>	14.7	Bond and Lovley (2003)
Wastewater diluted with 100 mM NaCl, pH 7.0, 5 × 5 cm graphite electrodes		Wastewater	20	Gil et al. (2003)
Mineral media, pH 7.0, 2.54 × 7.62 cm graphite		<i>Rhodospirillum rubrum</i>	33	Chaudhuri and Lovley (2003)
50 mM potassium phosphate, pH 7.0 with mediator, HNQ and graphite felt		<i>P. vulgaris</i>	32	Allen and Bennetto (1993)

*A. cryptum* microbial system in an MFC with minimized electrode spacing and higher-surface-area anode material.

Research has suggested that MFCs with iron-reducing bacteria as anode biocatalysts may transfer electrons to the electrode directly via biological nanowires (Gorby et al. 2006) and other mechanisms involved in metal reduction (Bretschger et al. 2007). While *A. cryptum* may be capable of electron transfer to solid iron minerals (Bilgin et al. 2004; Kusel et al. 2002), direct electron transfer to the anode was not observed under the conditions investigated in this study.

## Conclusions

The use of an acidophile, *Acidiphilium cryptum*, as an anode biocatalyst in a microbial fuel cell was demonstrated. Electricity production in the presence of iron as a mediator was demonstrated at low pH. The power output gradually decreased over time, potentially due to increased Fe(III) concentration at the electrode surface and formation of a mass-transfer-limiting Fe(III) layer. Use of NTA as a chelator and phenosafranin as the secondary electron mediating agent increased power output, resulting in a maximum of 12.7 mW/m<sup>2</sup>. This power output was obtained at a cell density equivalent to OD<sub>600</sub> of 1 in the anode chamber. Further improvements are possible using higher cell densities or organisms capable of iron reduction at lower redox potential.

**Acknowledgments** This research was sponsored by the Laboratory Directed Research and Development Program of Oak Ridge National Laboratory (ORNL), managed by UT-Battelle, LLC, for the US Department of Energy under Contract No. DE AC05-00OR22725. The authors would like to thank Choo Hamilton for technical assistance with *A. cryptum* and Barbara Evans and Babu Raman for reviewing the manuscript. S. C. would like to acknowledge partial financial support from Oak Ridge Associated Universities.

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