# Oxygen-Reducing Biocathodes Operating with Passive Oxygen Transfer in Microbial Fuel Cells

**ARTICLE** *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · JANUARY 2013

Impact Factor: 5.33 · DOI: 10.1021/es3027659 · Source: PubMed



**6 AUTHORS**, INCLUDING:

Fang Zhang
Pennsylvania State University
**30** PUBLICATIONS **640** CITATIONS
SEE PROFILE

Peng Liang
Tsinghua University
**88** PUBLICATIONS **1,679** CITATIONS
SEE PROFILE

Xia Huang
Tsinghua University
**242** PUBLICATIONS **5,235** CITATIONS
SEE PROFILE

Bruce E Logan
Pennsylvania State University
**458** PUBLICATIONS **33,160** CITATIONS
SEE PROFILE

Environmental Science & Technology

Article

pubs.acs.org/est

# Oxygen-Reducing Biocathodes Operating with Passive Oxygen Transfer in Microbial Fuel Cells

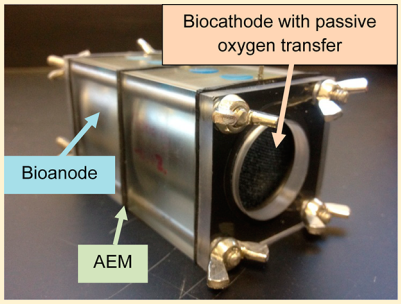
Xue Xia,[†] Justin C. Tokash,[‡] Fang Zhang,[‡] Peng Liang,[†] Xia Huang,*[,†] and Bruce E. Logan*[,†,‡]

[†]State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, P.R. China

[‡]Department of Civil and Environmental Engineering, Penn State University, 231Q Sackett Building, University Park, Pennsylvania 16802, United States

Supporting Information

**ABSTRACT:** Oxygen-reducing biocathodes previously developed for microbial fuel cells (MFCs) have required energy-intensive aeration of the catholyte. To avoid the need for aeration, the ability of biocathodes to function with passive oxygen transfer was examined here using air cathode MFCs. Two-chamber, air cathode MFCs with biocathodes produced a maximum power density of 554 ± 0 mW/m$^2$, which was comparable to that obtained with a Pt cathode (576 ± 16 mW/m$^2$), and 38 times higher than that produced without a catalyst (14 ± 3 mW/m$^2$). The maximum current density with biocathodes in this air-cathode MFC was 1.0 A/m$^2$, compared to 0.49 A/m$^2$ originally produced in a two-chamber MFC with an aqueous cathode (with cathode chamber aeration). Single-chamber, air-cathode MFCs with the same biocathodes initially produced higher voltages than those with Pt cathodes, but after several cycles the catalytic activity of the biocathodes was lost. This change in cathode performance resulted from direct exposure of the cathodes to solutions containing high concentrations of organic matter in the single-chamber configuration. Biocathode performance was not impaired in two-chamber designs where the cathode was kept separated from the anode solution. These results demonstrate that direct-air biocathodes can work very well, but only under conditions that minimize heterotrophic growth of microorganisms on the cathodes.




## INTRODUCTION

Microbial fuel cells (MFCs) are devices that can directly extract electricity from diverse organic wastes through bioelectrochemical reactions using bacteria.[1] MFCs hold great promise for applications in wastewater treatment, electricity production, pollutant removal, and as biosensors.[2−4] However, scale up of MFCs is hindered by electrode costs and the performance of the cathode.[5] A variety of substances can be used as the cathode electron acceptors,[6,7] but oxygen is the most practical one because of its availability and high redox potential.[8] The most commonly used catalyst for oxygen reduction in MFCs is Pt, but its high cost and sensitivity to poisoning limit widespread applications in larger-scale systems.[9,10] To make more cost-effective cathodes, various non-noble metal catalysts have been developed to replace Pt, such as transition metal macrocyclic complexes[11] and metal oxides,[12] as well as carbon materials.[13] While these materials can work well, their performance can be degraded over time.[14]

Biocathodes are very appealing alternatives to cathodes with inorganic catalysts as they use self-replenishing microorganisms to reduce the overpotential for oxygen reduction. Microorganisms effective for oxygen reduction can be enriched from natural environments,[15] and their continued growth on a cathode could enable their long-term use without degradation in performance. In spite of these potential advantages, there are still some aspects of biocathodes that need to be improved. While most chemical catalysts function in gas diffusion electrodes, with passive transport of oxygen in air,[7] oxygen-reducing biocathodes have so far only been proven to be effective in two-chamber, aqueous cathode MFCs that require aeration of the catholyte solution.[15,16] Aeration requires a high energy input, and thus this process eliminates the benefit of energy production using the bacteria. In addition, ion exchange membranes (IEMs) are used in these systems to keep the anode and cathode chambers separate,[15,16] even though it is well-known that the use of these membranes increases the internal resistance and results in pH gradients, which can reduce power production.[17] Thus, developing oxygen-reducing biocathodes with passive oxygen transfer is critical for practical applications of biocathode MFCs.

In this study, we developed an oxygen-reducing biocathode and systematically compared its performance with a Pt cathode under several different operating conditions, using two-chamber and single-chamber MFC configurations. The effect of biodegradable organic matter on the biocathode was evaluated by comparing performance of the biocathode exposed to air in a two-chamber MFC with that obtained in a single-chamber

Received: July 10, 2012
Revised: January 18, 2013
Accepted: January 28, 2013
Published: January 29, 2013

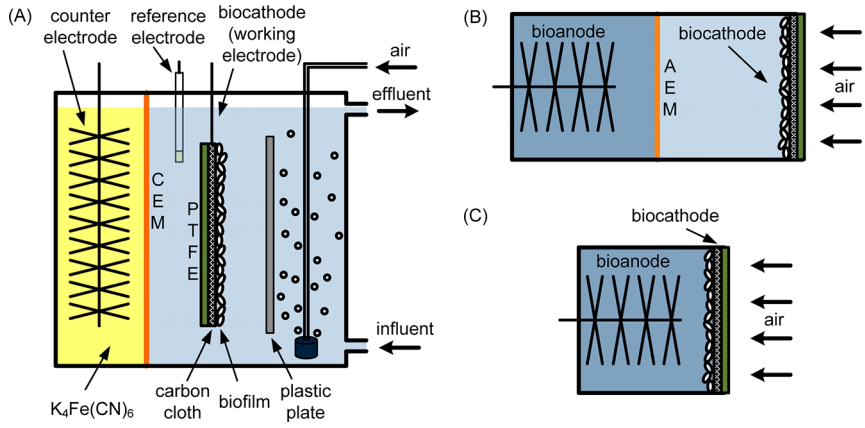



**Figure 1.** Schematics of the three types of reactors. (A) Two-chamber, aqueous cathode; (B) two-chamber, air-cathode; (C) single-chamber, air-cathode configuration.

MFC. To demonstrate an optimal potential range for biocathodes in these systems, the electrochemical activities of the biocathodes were tested at several different potentials using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

## MATERIALS AND METHODS

**Reactor Configuration.** Three different types of MFCs were used in these studies: two-chamber, aqueous cathode; two-chamber, air-cathode; and single-chamber, air-cathode (Figure 1). A two-chamber, aqueous cathode reactor[18] was used to start up the biocathodes and test their initial electrochemical performance with solution aeration (Figure 1A). The cathode chamber (945 mL) and anode chamber (420 mL) were separated by a cation exchange membrane (CEM, projected area of 105 $cm^2$; CMI-7000, Membranes International, USA). Cathodes made of carbon cloth (5 cm ×10 cm; 30% wet proofing, CCWP4030, Fuel Cell Earth, USA) were painted with 4 polytetrafluoroethylene (PTFE) diffusion layers to one side as previously described[19] and connected to the external circuit through a titanium wire. Therefore, bacteria could only grow using current from the cathode on the side of the cathode without PTFE, although both sides were immersed in the catholyte. An Ag/AgCl reference electrode (RE-5B, BASi, +211 mV vs. standard hydrogen electrode, SHE) was also placed in the catholyte. All potentials are reported here versus SHE. A carbon fiber brush (6 cm in diameter and 7 cm long; ~7730 $cm^2$ surface area based on fibers) made by twisting carbon fibers between two titanium wires was used as the counter electrode.

After start-up, biocathodes were cut into two pieces (each with a 3.8 cm diameter), and then placed in two-chamber, air-cathode MFCs (Figure 1B) to test the feasibility of biocathodes with passive oxygen transfer. Anode and cathode chambers, separated by an anion exchange membrane (AEM, projected area of 7 $cm^2$; AMI-7001, Membranes International, USA), were formed from 4 cm long cubic blocks with an inner cylindrical chamber 3 cm in diameter. The anode was a 450 °C heat-treated carbon brush (2.5 cm in diameter and 2.5 cm long; 1150 $cm^2$ surface area based on fibers)[20] that was placed in the center of the cylindrical chamber, with the end of the brush 1 cm from the AEM. The cathode was placed at the end of the cathode chamber with the diffusion layer facing air and the catalyst layer facing the solution. The liquid volumes were 25 mL for the anode chamber, and 28 mL for the cathode chamber. Cathodes were also made with and without a Pt catalyst as controls. All of these cathodes were made using 30% wet proofed carbon cloth, and painted with 4 PTFE diffusion layers on the air-facing side. For the cathodes with Pt as catalyst, 5 mg/$cm^2$ 10% Pt on Vulcan XC-72 was applied on the side facing the solution.[19]

Another two new biocathodes that were acclimated in the two chamber aqueous cathode reactor were also examined for performance in single-chamber MFCs, with the cathode directly exposed to the anode solution. These MFCs had one chamber 4 cm long and 3 cm in diameter, and no separator (Figure 1C).[21]

**Reactor Operation.** To develop a biocathode in a short time and with good catalytic activity, a two-chamber, aqueous cathode reactor was used (Figure 1A). The cathode potential was set at 311 mV vs. SHE using a potentiostat (WMPG 1000, WonATech, Korea) to acclimate the biocathode under optimal conditions, as previously described.[18,22,23] The reference electrode was checked and recalibrated as needed every 7−10 days to ensure its potential drift was less than 10 mV. The inoculum for the biocathode was 50 mL of activated sludge from the Penn State wastewater treatment plant. After inoculation, the catholyte contained sodium bicarbonate (1.0 g/L) and a 50 mM phosphate buffer solution (PBS) containing $Na_2HPO_4$, 4.58 g/L; $NaH_2PHO_4{\cdot}H_2O$, 2.45 g/L; $NH_4Cl$, 0.31 g/L; KCl, 0.13 g/L; trace minerals (12.5 mL/L); and vitamins (5 mL/L). During startup, the anode chamber was filled with 50 mM $K_4Fe(CN)_6$ and 50 mM PBS. Air was sparged into the cathode chamber to provide dissolved oxygen. Fresh catholyte was continuously fed into the cathode chamber at 0.3 mL/min (~ 2 d hydraulic retention time), and the anolyte was replaced every 2 days. After 50 days operation, the biocathodes produced a stable negative current, and they were then removed and used in the air-cathode MFCs (Figure 1B and C).

For two-chamber, air-cathode MFCs, anodes were inoculated with the effluent of an MFC operated for over one year. The anolyte contained 1 g/L acetate as the fuel, 50 mM PBS, trace minerals (12.5 mL/L), and vitamins (5 mL/L). The catholyte was the same as described in the two-chamber, aqueous cathode MFC. All the reactors were operated in fed-batch mode with a 1000 Ω external resistor at 30 °C. When the cell voltage was lower than 50 mV, the anolyte and catholyte were replaced at the same time by pouring all the medium out and then refilling the reactors with fresh medium.


dx.doi.org/10.1021/es3027659 | Environ. Sci. Technol. 2013, 47, 2085−2091

The medium used in the single-chamber, air-cathode MFCs (Figure 1C) contained both acetate (1.0 g/L) and sodium bicarbonate (1.0 g/L); 50 mM PBS; trace minerals and vitamins. Reactors were operated in fed-batch mode with a 1000 Ω resistor. Medium was also replaced by emptying the reactor and refilling it with fresh medium when the cell voltage was lower than 50 mV.

**Measurements and Calculations.** Voltages across an external resistor (1000 Ω) were automatically recorded at 20-min intervals using a data acquisition system (2700, Keithley Instrument, Cleveland, OH). Polarization and power density curves were obtained by varying external resistance in a decreasing order with 20-min intervals at each resistance (single-cycle method). Current density was calculated from $I = U/RA$, where $U$ is the cell voltage (V), $R$ is the external resistance (Ω), and $A$ is the projected surface area of cathode (7 $cm^2$). Power density was calculated using $P = IU/A$.

Half-cell CVs were conducted to examine the electrochemical behavior of biocathodes in two-chamber, air-cathode MFCs. All experiments were conducted in duplicate, and all reference electrodes were verified to have a potential of +211 mV vs SHE prior to each test. Before tests, the reactors were emptied, refilled with fresh medium, and left with an open circuit for 2 h. The CV was conducted with the cathode as the working electrode, a platinum plate (1 cm × 1 cm; inserted into the anode chamber when replacing the medium) as the counter electrode, and an Ag/AgCl electrode located in the cathode chamber as the reference electrode. Tests were performed at 1 mV/s over a range of 50 to 650 mV (vs. SHE). Three cycles were performed, with the last two cycles showing good agreement (lines overlapped), so only the last cycle was reported. To further evaluate the electrochemical activity of biocathodes at different potentials, first derivative CV (DCV) plots were prepared from the CV data by calculating the slope of each data using the central difference quotient,[24,25] and plotting the slope against the potential.

The internal resistances of biocathodes and Pt cathodes in two-chamber, air-cathode MFCs were determined using EIS conducted with a potentiostat (VMP3, BioLogic, USA). The tests were carried out in the same three electrode arrangement used for CV tests. Two biocathodes were used to carry out each test (duplicate experiments). A small alternating current signal with amplitude of 10 mV and a frequency range from 100 kHz to 1 mHz was applied. The impedance spectra of the biocathodes were collected at 619, 500, 410, and 300 mV vs. SHE. The choice of these potentials for the biocathodes was based on the following: the biocathodes had an OCP of 619 mV; the highest potential during each fed batch cycle with these cathodes was ~410 mV; the maximum activity in DCVs occurred at ~500 mV, which indicated that this was the midpoint potential of the irreversible electrochemical reaction; and the onset of the limiting current density occurred at ~300 mV, which indicated that this was the lowest potential where meaningful EIS results could be obtained for this system. The impedance measurements for the Pt cathodes were conducted at its OCP (493 mV), 410, 300, and 170 mV vs. SHE, as 300 mV was the highest potential of Pt cathodes in each fed batch cycle, and 170 mV was the cathode potential when MFCs achieved the maximum power density in polarization tests. Data fitting was accomplished using EC-Lab software (V10.19). Spectra were fitted into an equivalent circuit in terms of the solution resistance, $R_s$, diffusion resistance, $W$, and charge transfer resistance, $R_{ct}$, in parallel with a double layer capacitance, $Q$ (Figure S1 in the Supporting Information).

## RESULTS AND DISCUSSION

**Electrochemical Performance of Biocathodes with Passive Oxygen Transfer in Two-Chamber Configuration.** Biocathodes developed in two-chamber aqueous cathode MFCs were transferred into two-chamber, air-cathode MFCs. After one cycle of operation, the MFCs produced consistent and reproducible voltages over the subsequent cycles, with good agreement between duplicate reactors (Figure 2). The two-chamber air-cathode MFCs produced a maximum voltage up to 604 mV (1000 Ω external resistance), which was 50 mV higher than that produced with Pt cathodes (550 mV). The MFCs lacking a cathode catalyst generated only 90 mV. This startup procedure based on initial acclimation in the two-chamber, aqueous cathode MFC was critical to successful development of the air-cathode biocathode. Tests to directly develop biocathodes in air-cathode MFCs (no preincubation in aqueous cathode $MFC_S$) at the previously determined optimum set potential of 311 mV[18,22,23] failed to produce current within 25 days (data not shown). Other initial set potentials were not explored as the main focus here was to examine the performance of these successfully developed biocathodes under different conditions.

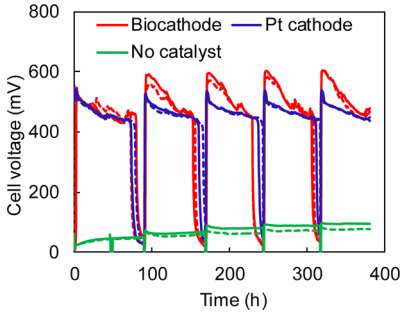



**Figure 2.** Cell voltages produced in two-chamber, air-cathode MFCs (1000 Ω) with biocathodes or Pt cathodes used in an air cathode configuration (replicates shown with dashed lines).

The maximum power density of the two-chamber, air cathode MFCs with biocathodes was 554 ± 0 $mW/m^2$, which was comparable with that of the Pt cathode MFCs (576 ± 16 $mW/m^2$) and 38 times higher than that of the MFCs without cathode catalyst (14 ± 3 $mW/m^2$) (Figure 3A). The open circuit voltage (OCV) of biocathode MFCs was 908 ± 1 mV, compared with the 751 ± 1 mV for Pt cathode MFCs and 575 ± 18 mV for MFCs without cathode catalyst. The open circuit potential (OCP) of the biocathodes (623 ± 1 mV vs. SHE) was much higher than that of Pt cathodes (470 ± 5 mV), indicating that biocathodes produced a potential much closer to the thermodynamic equilibrium potential for oxygen reduction to water, compared to Pt.

Anode potentials were essentially the same in both the biocathode and Pt cathode MFCs, demonstrating that the cathode was responsible for the different performances of the MFCs (Figure 3B). The measured potentials for the biocathodes were about 150 mV more positive than those of the Pt catalyzed cathodes at current densities <0.9 $A/m^2$. However, the cathode potential dropped sharply at ~1 $A/m^2$, and there was little further increase in current. In contrast, the Pt cathode showed a typical increase in current with a gradual


dx.doi.org/10.1021/es3027659 | Environ. Sci. Technol. 2013, 47, 2085−2091

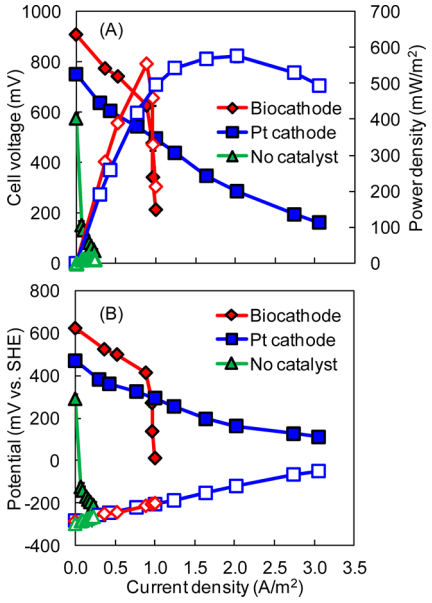



**Figure 3.** (A) Cell voltages (filled symbols) and power densities (open symbols), and (B) electrode potentials (cathode, filled symbols; anode, open symbols) as a function of current density for two-chamber, air-cathode MFCs with biocathodes, Pt cathodes, or without cathode catalysts. (Error bars based on duplicate reactors are smaller than the symbol sizes).

drop in voltage, demonstrating that current densities could be higher than 3 A/m$^2$ with a different catalyst. This indicated that oxygen transfer to the cathode was sufficient to sustain a current density higher than 3 A/m$^2$ for either the Pt catalyst or a biocathode, as the cathodes are otherwise identical. The sudden drop in the potential of the biocathode at ~400 mV vs. SHE is therefore due to the microorganisms.

The performance of the biocathode was improved through the use of the two-chamber, air-cathode configuration. The maximum current density of the biocathode MFCs with an air-cathode was 1.0 A/m$^2$, compared to 0.49 A/m$^2$ when they were originally acclimated as aqueous cathodes in two-chamber MFCs (Figure S2). Since the same biocathode was used in the two different configurations, this enhancement in current was due to the improved performance of this cathode with exposure to oxygen in air, compared to dissolved oxygen. These results demonstrated that properly acclimated biocathodes can be successfully applied in air-cathode MFCs, and that they can achieve performance similar to that produced by Pt cathodes, and better performance than aqueous cathodes, as long as the cathode potential remains favorable (> 400 mV). The use of the air cathode configuration not only eliminated the energy that would be needed for aeration of aqueous biocathodes, but it also improved electrochemical performance. It has been shown in previous studies that the electrochemical activities of oxygen-reducing biocathodes (with catholyte aeration) were much higher than that of biocathodes using nitrate[3] and perchlorate (lower than 110 mV vs. SHE).[26] Thus, it appears that air biocathodes will have higher potentials than biocathodes that use other electron acceptors.

**Electrochemical Activity Analysis.** CV scans were conducted on the catholyte solution following a fed batch test (after the substrate was removed) in order to better understand the performance of the biocathodes. There were no redox peaks in these solutions (data not shown). A lack of such peaks, and the fact that mediators in solution would be removed every time the catholyte was replaced, suggests that mediators were not used for electron transfer from the cathode to the microbes. While no current was observed for bare cathodes, the absolute value of the reduction current density on biocathodes increased along with the decrease of electrode potential in the range of 600−350 mV vs. SHE, and achieved a maximum value of 1.2 A/m$^2$ at lower potentials (Figure 4A),

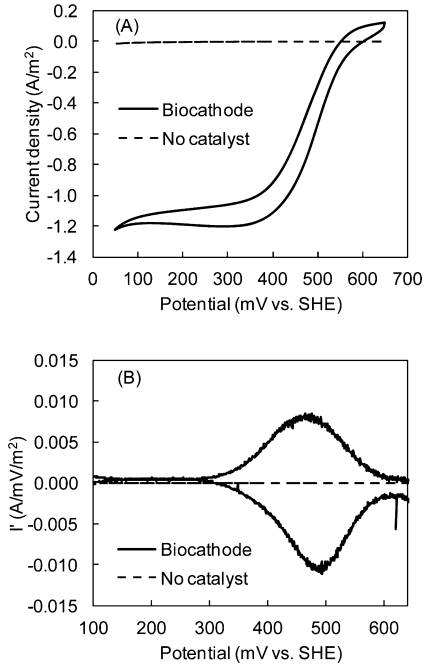



**Figure 4.** (A) Cyclic voltammogram and (B) first derivative cyclic voltammogram of the biocathodes and cathodes without a catalyst in two-chamber, air-cathode MFCs.

consistent with the polarization results (Figure 3B). Based on DCV analysis of biocathodes, thermodynamically driven reductive activity was clearly seen over the potential range of 600 to 300 mV, with little change at potentials <300 mV (Figure 4B) due to inherent kinetic limitations in the system. The standard redox midpoint potential of the irreversible electrochemical reaction occurring on the biocathodes appeared at ~500 mV. IR compensation was not carried out since it had a minor effect on potentials reported for these tests (Figure S3). These electrochemical results provided direct supporting evidence to polarization data that the biocathode could not sustain increased current densities below potentials of ~300−400 mV vs. SHE. This lack of increased activity at these lower potentials explains the failure of the MFC to produce current densities higher than ~1−1.2 A/m$^2$.

The charge transfer resistances of the biocathodes were further quantified by EIS at various potentials to evaluate electrochemical activity. The solution resistances, obtained from the high frequency data (where the impedance crossed the real axis), were identical at all tested potentials (22 ± 1 Ω). A finite diffusion resistance was apparent at low frequencies (Figure S4C, D). Nyquist plots of the biocathodes exhibited two semicircles at high frequencies (Figure S4C, D), suggesting either a porous biocathode structure[27] or a complex oxygen reduction mechanism (an indirect four-electron reaction or a two-electron reaction followed by a disproportionation reaction).[28,29] The occurrence of multiple semicircles on a


dx.doi.org/10.1021/es3027659 | Environ. Sci. Technol. 2013, 47, 2085−2091

single electrode was also detected in several previous studies with each semicircle representing one separate charge transfer resistance.[27−29] Therefore, two charge transfer elements ($R_{ct1}$, $R_{ct2}$) were used in the equivalent circuit (Figure S1) to account for the total charge transfer resistance ($R_{ct} = R_{ct1} + R_{ct2}$). The charge transfer resistance ranged from $R_{ct}$ = 1070 ± 2 Ω at the OCP (619 mV vs. SHE) to 114 ± 8 Ω at 500 mV and 273 ± 73 Ω at 410 mV (Figure 5). The highest electrochemical activity

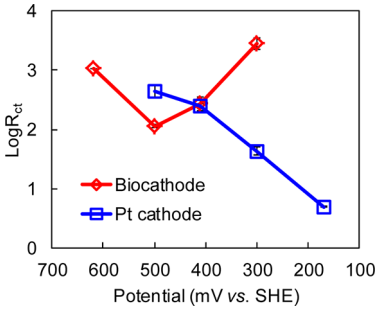



**Figure 5.** Charge transfer resistances of the biocathodes and Pt cathodes at different potentials. The biocathode has an open circuit potential (OCP) of 619 mV, a maximum activity at ~500 mV, and no activity at <300 mV in DCV curves. The highest potential of the biocathode in each fed batch cycle was ~410 mV, compared to 300 mV for the Pt cathode. EIS tests for Pt cathodes were conducted at its OCP (~ 493 mV), 410, 300, and 170 mV.

was obtained in DCV curves at 500 mV, consistent with the minimum charge transfer resistance based on Nyquist plots at these different potentials. At potentials lower than 500 mV, the activity declined and the charge transfer resistance increased. When the voltage was set to 300 mV, which is outside the optimum range identified using polarization and DCV results, the charge transfer resistance was very high (2824 ± 618 Ω). This further confirmed that the current saturation phenomenon in biocathodes was caused by their limited activity at lower potentials. The results of $R_{ct1}$ and $R_{ct2}$ were not analyzed separately since it cannot be determined at this time what each of these resistances individually represent (see Figure S4E for the calculated individual resistances).

An EIS analysis was also conducted on the Pt cathodes, but only at potentials of 493 mV (the OCP) or less (Figure S4B). The charge transfer resistance of Pt cathodes decreased with the electrode potential (Figure 5). At 493 mV, the charge transfer resistance of Pt cathodes (450 ± 94 Ω) was larger than that of biocathodes (114 ± 8 Ω) at about the same potential (500 mV). When the potential was decreased to 410 mV, the charge transfer resistance of the Pt cathodes (250 ± 24) was the same as that of the biocathodes (273 ± 73 Ω) (Figure 3). However, it decreased to 43 ± 7 Ω at 300 mV for the Pt cathodes, which allowed for increased current. In contrast, the biocathodes had a much increased resistance at this potential (2824 Ω). Therefore, Pt cathodes could sustain higher current densities than biocathodes.

**Electrochemical Performance of Biocathodes in a Single-Chamber MFC.** When the acclimated biocathodes were put into single-chamber reactors, the performance initially exceeded that of the Pt air-cathode, consistent with two-chamber comparisons. Over the first four cycles, the maximum cell voltage of the biocathode MFCs was 609 ± 42 mV (1000 Ω external resistor), compared to 557 ± 1 mV for the Pt cathode, and only 84 ± 1 mV in the absence of a catalyst (Figure 6). However, from the fifth cycle on, the maximum cell voltages produced using the biocathodes steadily decreased,

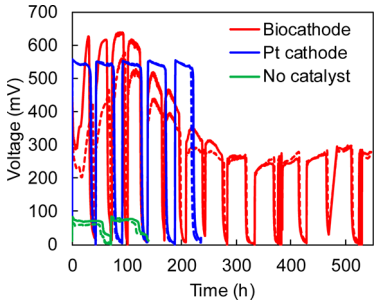



**Figure 6.** Cell voltages of single-chamber, air-cathode MFCs (1000 Ω). Replicates are shown with dashed and solid lines.

and finally stabilized at around 297 ± 0 mV after the ninth cycle (~ 270 h).

Polarization curves were obtained after the cell voltage of biocathode MFCs stabilized at this lower voltage. The maximum power density of biocathode MFCs (199 ± 0 mW/m$^2$) was much lower than that produced by Pt cathode MFCs (1253 ± 45 mW/m$^2$), although it was still 17 × higher than the MFCs lacking a cathode catalyst (12 mW/m$^2$) (Figure S5A). Even though the power density of the biocathode MFCs was very low, it still had a higher OCP (520 ± 4 mV vs. SHE) than the MFC with a Pt cathode (442 ± 5 mV).

In contrast with our results, it was claimed in one study using air cathodes that development of a biofilm on the noncatalyzed cathode surface produced power densities only slightly less than those obtained with Pt catalyzed MFCs.[30] However, there were some unusual conditions which made those findings inconclusive. First, the performance of the Pt-cathode MFCs improved over time, whereas it is well-known from many other studies that cathode performance degrades over time.[9,10] This improvement may have been due to a feeding strategy (adding acetate, but not replacing the medium) that increased solution conductivity and allowed for accumulation of mediators.[31] Second, the open circuit potential (OCP) of the cathodes (~ 96 mV vs. SHE) did not show any increase over time, which should have occurred with development of a biocathode. Third, the cathode potentials were much lower than those normally obtained with biocathodes (400−600 mV vs. SHE).[16,32] While biofilm clearly developed on the cathodes over time, the presence of cathode biofilm alone is not a proof of catalytic activity, as biofilms commonly develop on cathodes in single-chamber MFCs. The study by Santoro et al.[30] also did not provide electrochemical evidence of oxygen reduction under conditions outside MFC experiments, for example using linear sweep voltammetry and abiotic anodes. Combined, these observations do not support their claims of direct air biocathode activity in single-chamber MFCs.

## ■ OUTLOOK

The results with the single-chamber air cathode in this study provide needed insight into the conditions required to successfully develop and maintain air biocathodes in MFCs. Biocathodes performed as well as, or better than, Pt cathodes in two-chamber, air-cathode MFCs at higher resistances and current densities <1 A/m$^2$, and they initially performed better than Pt cathodes in single chamber systems at a fixed resistance of 1000 Ω. However, after only a few cycles in the single-


dx.doi.org/10.1021/es3027659 | Environ. Sci. Technol. 2013, 47, 2085−2091

chamber MFC, their performance declined, resulting in much less voltage and power densities than those obtained with Pt. The reason for this loss of performance in the single chamber configuration is likely the overgrowth by heterotrophs of the autotrophic biocathode community. Sodium acetate (1 g/L) in the medium was directly exposed to the cathode in the single-chamber configuration, but not in the two-chamber configuration. As a result, biofilms readily form on the cathodes that are primarily composed of heterotrophs that grow using sodium acetate as a carbon and energy source. It is likely that these heterotrophic bacteria out-competed the cathodic autotrophic bacteria for oxygen. Alternatively, electrotrophic microorganisms may have switched from using electrons from the cathode to acetate, since more energy can be obtained with sodium acetate than with the electrode. Both of these factors would have led to the decrease in the cell voltage of the single-chamber biocathode MFCs after several cycles. Therefore, in order for biocathodes to function well as air cathodes, microbes on the biocathode must be kept separated from direct contact with solutions having high concentrations of organic substances. This will allow the sustained growth of biofilms capable of reducing cathode overpotentials for oxygen reduction, and improved performance of MFCs without the need for precious metal catalysts.

## ■ ASSOCIATED CONTENT

### Supporting Information

Supplementary figures. This information is available free of charge via the Internet at http://pubs.acs.org/.

## ■ AUTHOR INFORMATION


### Corresponding Author

*Tel: +86 10 62772324; e-mail: xhuang@tsinghua.edu.cn (X.H.). Tel: +1 814 863 7908; e-mail: blogan@psu.edu (B.L.).


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We thank Dan Sun for her assistance with some of the experiments. This research was supported by funding through the King Abdullah University of Science and Technology (KAUST) (Award KUS-I1-003-13), and the National High Technology Research and Development Program of China (863 Program) (2011AA060907).

## ■ REFERENCES


(1) Logan, B. E.; Hamelers, B.; Rozendal, R. A.; Schrorder, U.; Keller, J.; Freguia, S.; Aelterman, P.; Verstraete, W.; Rabaey, K. Microbial fuel cells: Methodology and technology. *Environ. Sci. Technol.* **2006**, *40* (17), 5181−5192.

(2) Cheng, S. A.; Logan, B. E. Increasing power generation for scaling up single-chamber air cathode microbial fuel cells. *Bioresour. Technol.* **2011**, *102* (6), 4468−4473.

(3) Xie, S.; Liang, P.; Chen, Y.; Xia, X.; Huang, X. Simultaneous carbon and nitrogen removal using an oxic/anoxic-biocathode microbial fuel cells coupled system. *Bioresour. Technol.* **2011**, *102* (1), 348−354.

(4) Cao, X. X.; Huang, X.; Zhang, X. Y.; Liang, P.; Fan, M. Z. A mini-microbial fuel cell for voltage testing of exoelectrogenic bacteria. *Front. Environ. Sci. Eng. China* **2009**, *3* (3), 307−312.

(5) Rozendal, R. A.; Hamelers, H. V. M; Rabaey, K.; Keller, J.; Buisman, C. J. N. Towards practical implementation of bioelectrochemical wastewater treatment. *Trends Biotechnol.* **2008**, *26* (8), 450−459.

(6) Clauwaert, P.; Rabaey, K.; Aelterman, P.; De Schamphelaire, L.; Ham, T. H.; Boeckx, P.; Boon, N.; Verstraete, W. Biological denitrification in microbial fuel cells. *Environ. Sci. Technol.* **2007**, *41* (9), 3354−3360.

(7) Liu, H.; Logan, B. E. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. *Environ. Sci. Technol.* **2004**, *38* (14), 4040−4046.

(8) Zhao, F.; Harnisch, F.; Schrorder, U.; Scholz, F.; Bogdanoff, P.; Herrmann, I. Challenges and constraints of using oxygen cathodes in microbial fuel cells. *Environ. Sci. Technol.* **2006**, *40* (17), 5193−5199.

(9) Kiely, P. D.; Rader, G.; Regan, J. M.; Logan, B. E. Long-term cathode performance and the microbial communities that develop in microbial fuel cells fed different fermentation endproducts. *Bioresour. Technol.* **2011**, *102* (1), 361−366.

(10) Saito, T.; Merrill, M. D.; Watson, V. J.; Logan, B. E.; Hickner, M. A. Investigation of ionic polymer cathode binders for microbial fuel cells. *Electrochim. Acta* **2010**, *55* (9), 3398−3403.

(11) Zhao, F.; Harnisch, F.; Schroder, U.; Scholz, F.; Bogdanoff, P.; Herrmann, I. Application of pyrolysed iron(II) phthalocyanine and CoTMPP based oxygen reduction catalysts as cathode materials in microbial fuel cells. *Electrochem. Commun.* **2005**, *7* (12), 1405−1410.

(12) Zhang, L. X.; Liu, C. S.; Zhuang, L.; Li, W. S.; Zhou, S. G.; Zhang, J. T. Manganese dioxide as an alternative cathodic catalyst to platinum in microbial fuel cells. *Biosens. Bioelectron.* **2009**, *24* (9), 2825−2829.

(13) Zhang, F.; Cheng, S. A.; Pant, D.; Van Bogaert, G.; Logan, B. E. Power generation using an activated carbon and metal mesh cathode in a microbial fuel cell. *Electrochem. Commun.* **2009**, *11* (11), 2177−2179.

(14) Zhang, F.; Pant, D.; Logan, B. E. Long-term performance of activated carbon air cathodes with different diffusion layer porosities in microbial fuel cells. *Biosens. Bioelectron.* **2011**, *30* (1), 49−55.

(15) Clauwaert, P.; Van der Ha, D.; Boon, N.; Verbeken, K.; Verhaege, M.; Rabaey, K.; Verstraete, W. Open air biocathode enables effective electricity generation with microbial fuel cells. *Environ. Sci. Technol.* **2007**, *41* (21), 7564−7569.

(16) Wei, J. C.; Liang, P.; Cao, X. X.; Huang, X. Use of inexpensive semicoke and activated carbon as biocathode in microbial fuel cells. *Bioresour. Technol.* **2011**, *102* (22), 10431−10435.

(17) Zhang, X. Y.; Cheng, S. A.; Wang, X.; Huang, X.; Logan, B. E. Separator characteristics for increasing performance of microbial fuel cells. *Environ. Sci. Technol.* **2009**, *43* (21), 8456−8461.

(18) Xia, X.; Sun, Y. M.; Liang, P.; Huang, X. Long-term effect of set potential on biocathodes in microbial fuel cells: Electrochemical and phylogenetic characterization. *Bioresour. Technol.* **2012**, *120*, 26−33.

(19) Cheng, S.; Liu, H.; Logan, B. E. Increased performance of single-chamber microbial fuel cells using an improved cathode structure. *Electrochem. Commun.* **2006**, *8* (3), 489−494.

(20) Feng, Y. J.; Yang, Q.; Wang, X.; Logan, B. E. Treatment of carbon fiber brush anodes for improving power generation in air-cathode microbial fuel cells. *J. Power Sources* **2010**, *195* (7), 1841−1844.

(21) Logan, B.; Cheng, S.; Watson, V.; Estadt, G. Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. *Environ. Sci. Technol.* **2007**, *41* (9), 3341−3346.

(22) Liang, P.; Fan, M. Z.; Cao, X. X.; Huang, X. Evaluation of applied cathode potential to enhance biocathode in microbial fuel cells. *J. Chem. Technol. Biotechnol.* **2009**, *84* (5), 794−799.

(23) Ter Heijne, A.; Strik, D.; Hamelers, H. V. M; Buisman, C. J. N. Cathode potential and mass transfer determine performance of oxygen reducing biocathodes in microbial fuel cells. *Environ. Sci. Technol.* **2010**, *44* (18), 7151−7156.

(24) Fricke, K.; Harnisch, F.; Schroder, U. On the use of cyclic voltammetry for the study of anodic electron transfer in microbial fuel cells. *Energy Environ. Sci.* **2008**, *1* (1), 144−147.

(25) Hong, Y. Y.; Call, D. F.; Werner, C. M.; Logan, B. E. Adaptation to high current using low external resistances eliminates power

overshoot in microbial fuel cells. *Biosens. Bioelectron.* **2011**, *28* (1), 71−76.
(26) Butler, C. S.; Clauwaert, P.; Green, S. J.; Verstraete, W.; Nerenberg, R. Bioelectrochemical Perchlorate Reduction in a Microbial Fuel Cell. *Environ. Sci. Technol.* **2010**, *44* (12), 4685−4691.
(27) Lasia, A. Nature of the two semi-circles observed on the complex plane plots on porous electrodes in the presence of a concentration gradient. *J. Electroanal. Chem.* **2001**, *500* (1−2), 30−35.
(28) Itagaki, M.; Hasegawa, H.; Watanabe, K.; Hachiya, T. Electroreduction mechanism of oxygen investigated by electrochemical impedance spectroscopy. *J. Electroanal. Chem.* **2003**, *557*, 59−73.
(29) Tokash, J.; Engelhardt, G.; Macdonald, D. D. On the development of a general electrochemical impedance model. *ECS Trans.* **2009**, *19* (20), 13−29.
(30) Santoro, C.; Lei, Y.; Li, B.; Cristiani, P. Power generation from wastewater using single chamber microbial fuel cells (MFCs) with platinum-free cathodes and pre-colonized anodes. *Biochem. Eng. J.* **2012**, *62*, 8−16.
(31) Rabaey, K.; Boon, N.; Hofte, M.; Verstraete, W. Microbial phenazine production enhances electron transfer in biofuel cells. *Environ. Sci. Technol.* **2005**, *39* (9), 3401−3408.
(32) You, S. J.; Ren, N. Q.; Zhao, Q. L.; Wang, J. Y.; Yang, F. L. Power generation and electrochemical analysis of biocathode microbial fuel cell using graphite fibre brush as cathode material. *Fuel Cells* **2009**, *9* (5), 588−596.