A MICROFLUIDIC MICROBIAL FUEL CELL ARRAY FOR ELECTROCHEMICALLY-ACTIVE MICROBE SCREENING AND ANALYSIS

Huijie Hou1, Cemile Umrzan Ceylan1, Lei Li2, Paul de Figueiredo2,3, and Arum Han1,4,*

1Department of Electrical and Computer Engineering, 2Department of Plant Pathology and Microbiology, 3Department of Veterinary Pathobiology, 4Department of Biomedical Engineering.

Texas A&M University, College Station, TX, USA

ABSTRACT

Microbial fuel cells (MFCs) are devices that convert chemical energy directly to electrical energy through metabolic activities of microorganisms. Here, we present an MFC array for screening electrochemically active microbes (“electricigens”) and performing high-throughput MFC studies. The MFC array is equipped with controlled fluidic circulation systems that enable substrate/catholyte replenishment during operation. Shewanella oneidensis MR-1 loaded microfluidic MFC units maintained their power outputs for more than 1000 min with catholyte replenishment, compared to the 234% drop in power without replenishment. The microfluidic MFC array will prove useful for the long-term parallel analysis of electricigens.

KEYWORDS: Microbial Fuel Cell (MFC), Microbial Fuel Cell Array, High Throughput Screening, Electricity Generating Microbes, Microfluidics

INTRODUCTION

Microbial fuel cells (MFCs) are “green energy” devices that generate electricity from organic compounds through microbial metabolism [1]. Research into MFCs has generated significant excitement because of their potential use in wastewater treatment and electricity generation from biomass [2]. However, the power generation efficiencies of current MFCs are significantly lower than conventional fuel cells. Microbial mechanisms mediating electron transfer in MFCs are also largely unknown. Currently, only a few groups of electricigens have been uncovered, with the most intensively studied microbes being Geobacter sulfurreducens and Shewanella oneidensis MR-1 (S. oneidensis MR-1). Most current MFC devices are not suitable for characterizing large numbers of microbial species in parallel due to the lack of high-throughput capacity. We have previously reported a 24-well MFC array that is capable of high-throughput screening to find environmental electricigens with enhanced power generating abilities [3]. The device demonstrated a 24-fold increase in screening throughput, but was less suitable for long term studies (e.g. several weeks) due to miniature chamber sizes (less than 1 ml) that limited the amount of catholyte and carbon substrates that were available to the interrogated microbes. We have recently developed an air-cathode MFC array that uses oxygen from air as the electron acceptor, making long-term studies possible [4]. However, compared with an MFC array that uses ferricyanide, the air-cathode array generated lower power due to the limited electron acceptor concentration. In addition, the reproducibility of findings obtained in the air-cathode MFC array was lower than the ferricyanide-based MFC array, making it less suitable for parallel comparison studies. The 24-well microfluidic MFC array (MMFCA) presented here is based on the previously reported ferricyanide MFC array configuration. However, microfluidically controllable anode and cathode chambers have been introduced. These innovations allow periodic or continuous replenishing of catholytes and anode substrates. The microfluidic cathode chamber configuration also allows long-term operation by replenishing the electron acceptors (ferricyanide), where the microfluidic anode chamber configuration allows replenishing of microbial nutrients, and hence supports long-term, parallel studies.

EXPERIMENTAL

Twenty-four well microfluidic array (MMFCA) design and microfabrication

Figure 1 shows a schematic illustration of the microfluidic MFC array (MMFCA). The array consists of five functional layers: an anode electrode layer, an anode chamber layer, a proton exchange membrane (PEM) layer, a cathode chamber layer, and a cathode electrode layer (Figure 1A). The anode electrode layer (50 mm x 75 mm) consists of 24 addressable gold electrode pads (diameter: 8 mm), with each corresponding to one of the 24 anode chambers. Four sample inlets on the anode chamber layer, each connecting 6 anode chambers (diameter: 7 mm), allow 4 different carbon substrates to be replenished for long-term operation or parallel characterization. Waste from all anode chambers is collected through the common waste port. The cathode chamber layer consists of a microfluidic cathode channel layer, a pneumatic control channel layer, and a PDMS membrane sandwiched between (Figure 1B). The microfluidic channel connects all cathode chambers so that catholyte can be continuously or periodically replenished using a single fluidic inlet. The pneumatic valves block these channels during power output measurement to isolate each cathode chamber from the neighboring chambers. Pt-loaded carbon paper (diameter: 7 mm) (EC-10-05-7, Electrochem Inc., Woburn, MA), pasted on an electrode substrate same as anode with silver paste, is used as cathode electrode. All layers are fabricated using soft-lithography and standard microfabrication techniques as described before [3].

Media and growth conditions

S. oneidensis MR-1 was used as a reference strain for the MMFCA characterization. Cells stored in tryptic soy broth (TSB) supplemented with 15% glycerol at -80°C were streaked onto a TSB agar plate. The resultant colonies was inocu-
lated into 5 ml of TSB liquid medium, and then cultured for 48 h at 30°C with agitation (150 rpm). Cells (OD$_{600}$ = 1.7) were then ready to be used. Detailed information were described in our previous work [3].

**Figure 1:** Schematic illustrations of the 24-chamber microfluidic MFC array (MMFCA). (A) The MMFCA consists of five layers: (1) an anode electrode layer, (2) a microfluidic anode chamber layer, (3) a PEM layer, (4) a microfluidic cathode chamber layer, and (5) a cathode electrode layer. (B) Microfluidic cathode chamber layer with a catholyte circulation channel and a pneumatic control channel.

**MMFCA characterization and data acquisition**

*S. oneidensis* MR-1 was loaded into each anode chamber and ferricyanide (100 mM) was used as catholyte. Voltages of all MMFCA chambers were continuously recorded with a digital multimeter through a switch box controlled by a LabView™ (National Instruments, Austin, TX) interface. After about 1000 min running at open circuit, load resistors were individually connected to all MFC array chambers to characterize the effect of periodic catholyte replenishment on the MMFCA power output. For each catholyte replenishment, 100 mM ferricyanide was fed into the chambers at a flow rate of 1.2 ml/min for 2.5 min. The channels were then blocked by the pneumatically controlled valve layer to isolate all 24 chambers. To obtain a power density-current density (P-I) curve, power outputs of all MMFCA units were measured at various load resistances (1 kΩ to 2 MΩ).

**RESULTS AND DISCUSSION**

An MMFCA device with two microfluidic channels (channel 1 & 2) on the cathode chamber layer controlled by two separate pneumatic controllers, each connecting 12 cathode chambers, was used with the 24 anode chambers loaded with *S. oneidensis* MR-1. After running about 1110 min at open circuit, all MMFCA units were then connected to 200 kΩ load resistors (Figure 2), which caused a sharp drop in voltage (at 1110 min). Catholyte chambers connected through channel 1 were replenished at 1160 min and a sharp increase in voltage was observed only from the chambers controlled by channel 1. Chambers without replenishment (controlled by channel 2) were not affected (i.e. stable voltage output). Catholyte chambers connected through channel 2 were then replenished at 1290 and 1480 min. As can be seen in Figure 2, catholyte replenishment resulted in voltage increases in the MMFCA units with catholyte replenished without influencing the units without catholyte replenishment.

**Figure 2:** Voltage output of the MMFCA during periodic catholyte replenishment. Catholyte (100 mM ferricyanide) was replenished to cathode chambers connected through channel 1 at 1160 min after cell loading. Cathode chambers connected through channel 2 was replenished with catholyte at 1290 and 1480 min after cell loading. N = 10 ~ 12 for each channel.
Power densities of S. oneidensis MR-1 with and without catholyte replenishment were then characterized (Figure 3). TSB media was used as the negative control. At 200 min after cell loading, only channel 1 controlled catholyte chambers were replenished and power densities of both channel 1 and channel 2 controlled MMFCA units were measured (Figure 3A). The MMFCA units with and without catholyte replenishments showed no obvious difference (14.8 mW/m² and 13.8 mW/m² respectively) (Figure 3A). However, after 1085 min of operation, power densities of the MMFCA units without catholyte replenishment (channel 2 controlled) (5.9 mW/m²) dropped by 234% compared to the power output at 200 min (13.8 mW/m²), while the units with replenishment (channel 1 controlled) showed no significant difference in power output (15.9 mW/m²) compared with that at 200 min (14.8 mW/m²) (Figure 3B). This power output from the MMFCA units with catholyte replenishment represents a 270% higher power output than the MMFCA units without replenishment measured at the same time. The result shows that catholyte depletion (i.e. depletion of electron acceptor) is one of the key factors that limit the power generation of miniature MMFCA over time.

These results clearly demonstrate that the developed MMFCA can solve the power drop problem caused by catholyte depletion in long-term MFC array operation. The device can also be potentially used for monitoring the catholyte depletion status. Identical power densities of both replenished chambers (channel 1 controlled) and chambers without replenishment (channel 2 controlled) at 200 min showed that catholyte solution was not limiting the power generation of the MFC array during the first 200 min of operation. Also, identical power densities measured at 200 min and 1085 min from the replenished MMFCA units showed that catholyte replenishment could compensate power degradation caused by catholyte limitation. The developed MMFCA could be potentially used for parallel MFC array studies that require long-term operation.

![Figure 3: Power generations of S. oneidensis MR-1 with and without catholyte replenishment. TSB media was used as negative control. Catholyte was replenished only for the 12 miniature MMFCA units connected through channel 1, but not for the units connected through channel 2. (A) Power densities of the MMFCA units controlled by channel 1 and 2 at 200 min after cell loading. (B) Power densities of the MMFCA units controlled by channel 1 and 2 at 1085 min after cell loading. N = 7 ~ 8 for each channel.](image)

CONCLUSION
We have demonstrated that the presented microfluidic MFC array (MMFCA) supports the analysis of 24 individual MFC experiments in parallel. The integrated substrate/catholyte replenishing system overcomes substrate/catholyte-limited operation status. Therefore, this MMFCA is ideal for long-term parallel MFC studies.

ACKNOWLEDGEMENTS
The work is being supported by National Science Foundation (NSF #0854684).

REFERENCES

CONTACT
*Arum Han, tel: +1-979-9686; arum.han@ece.tamu.edu; http://biomems.tamu.edu/*