**ABSTRACT**

This study demonstrates two microfluidic microbial fuel cells (µMFCs) for rapid screening of electroactive microorganisms. The µMFCs with Y-shaped channel determined the electroactivity by measuring the open circuit voltage (OCV) generated from different anolytes and the results indicate that electroactivity of microbes contributed 40 mV while the composition difference between streams contributed 70 mV. The second type of µMFCs applied conductive particles as carriers of microbes to perform long-term examination. Microparticles loaded with microbes generated much higher OCV than unloaded microparticles in a 6-hr test, showing that conductive microparticles have great potential in serving as anode in µMFCs.

**KEYWORDS**

Microfluidic microbial fuel cell, Electroactive microorganisms. Rapid detection, membraneless

**INTRODUCTION**

MFC is a device which uses biological materials such as microorganisms and enzymes as catalysts to generate electricity. Conventional macroscale MFCs are consisted of complex components and take days to identify the electroactivity of interested microorganisms [1]. Furthermore, the requirement of proton exchange membrane (PEM) may raise issues of high cost and easy fouling. µMFCs have high surface to volume ratio, which enables their greatly higher energy densities than their macroscale counterparts. Therefore, they have higher sensitivity in electroactivity and are ideal for the screening of electroactive microorganisms. However, existing µMFCs require similar setups to macroscale ones or relatively complicated processes to fabricate electrodes with high efficiency and biocompatibility [2]. Therefore, we propose µMFCs with simple structure and straightforward fabrication to resolve the abovementioned issues. Moreover, the proposed µMFCs are easy to operate and low-cost, making them equally ideal to serve as small-scale power sources for portable electronics and implantable devices [3].

**EXPERIMENT**

The channels for both µMFCs were fabricated by standard soft lithography [4]. Microelectrodes were formed by E-beam evaporator and wet etching. All reagents were infused into the device by syringe pumps. The mixed culture microorganisms were obtained from the seacoast of Taiwan and have been proven to have electroactivity in a lab-scale H-type MFC. In Y-shaped µMFC, catholyte was phosphate buffer prepared by adding 1.0712 g K$_2$HPO$_4$ and 1.0712 g KH$_2$PO$_4$ into 1L of deionized water and anolyte was either fresh medium, inactive microbes, or active microbes. Microbes were rendered inactive by autoclaving at 120 °C for 30 min. The conductive microparticles were composed of poly Diallyldimethylammonium chloride (pDADMAC). The batch operation was performed by collecting the microparticles in a 250 μl centrifuge tube and 200 μl of fresh medium was infused into the tube. Two platinum electrodes were inserted in the sediment and supernatant, respectively, to measure the open circuit voltage.

The Y-shaped µMFC, which is similar to microfluidic chemical fuel cells, with two inlets for buffer (catholyte) and microbial solution (anolyte) is shown in Figure 1. Due to the merit of laminar flow in microchannel; the µMFC has well separated catholytic and anolytic streams without applying PEM. Two L-shaped gold films without modification lied at the edge of the channel to serve as electrodes. Figure 2 shows the open circuit voltage (OCV) obtained from Y-shaped µMFCs with different anolytes, including active microbes, inactive microbes, and medium. The OCV produced by active microorganisms was higher than others. Open circuit voltages from the Y-shaped µMFC were mainly produced from two sources: the electroactivity of microbes in the anolyte and the differences of contents between the two streams. In this study, the electroactivity of microbes contributed about 40 mV (the difference between active and inactive microbes) and other anolytic contents such as metabolic products contributed about 70 mV (the difference between inactive microbes and fresh medium). The dead time (time required for the voltage to reach steady state) for the detection was shorter than 10 minutes. Although the Y-shaped µMFC successfully identified the electroactivity of active microorganism, its accuracy was susceptible to impurities in the anolyte (inactive microbes also produced higher OCV than medium). Therefore, this device is more suitable for analyzing
samples with known ingredients.

In order to reduce the effects of impurity on the detection accuracy, an innovative μMFC is designed to monitor the electricity generation from microorganisms encapsulated in microparticles made of conductive polymers (Fig. 3). Microdroplets containing monomers and photo-initiator were generated at the T-junction (Fig. 4a) and transported to the downstream for UV exposure ($t_{exposure}$=7 sec, Fig. 4b) to form microparticles (Fig. 4c and d). We first examine the electricity generated from these particles using a batch-type operation. Fig. 5(b) shows the time course of OCV generated by conductive particles containing microbes. Microparticles containing microbes generated 8 mV in the first hour and the OCV gradually decreased to 0 mV in 6 hours, indicating the depletion of substrate. Particles without microbes had relatively stable OCV around 1 mV throughout the operation except for the first 10 min. These results show that the conductive microparticles were suitable for serving as the carrier of microbes for electricity generation. We are currently working on integrating the particle generation and continuous-type μMFC in series. After microparticles were generated, they would then be packed in an enlarged section to perform electroactivity detections. Fresh medium is infused constantly into this tubular packed bed μMFC and electroactivity is determined by the increase of electricity along cultivation time. The change of OCV depends on the microbe population/status instead of the difference between cathode and anode; therefore, it can truly reflect the electroactivity of tested microorganisms. Furthermore, conductive microparticles provide enormous surface area for electron transfer and hopefully the internal resistance can be reduced greatly.

**Figure 1.** The schematics of Y-shaped μMFC. Two inlets infused buffer solution and microbial solution, separately, to form laminar flows. The OCV is monitored by a multi-meter connected to gold microelectrodes.

**Figure 2.** The OCV generated by active microbes, inactive microbes and medium.

**Figure 3.** The schematics of integrated microparticle fabrication and tubular packed bed μMFC.
Figure 4. The generation of microdroplets and microparticles. (a), (b), and (c) were taken at positions A, B, and C, respectively, in Figure 3. (a) formation of droplets at T-junction. (b) exposure under UV-light in channel turn no. 30 to 35. (c) microparticles at the exit of the fabrication section. (d) the swelled microparticles in the medium.

Figure 5. (a) The setup for batch-type operation. (b) The time course of open circuit voltage generated by conductive particles in a batch-type operation.

REFERENCES:

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