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A microliter-scale microbial fuel cell array for bacterial electrogenic screening☆

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ABSTRACT

We have developed an array of six MEMS (micro-electro-mechanical systems) microbial fuel cells (MFCs), a compact and reliable platform for rapid screening of electrochemically active bacteria. The MFC array contains vertically stacked 1.5 μL anode/cathode chambers separated by a proton exchange membrane (PEM), and represents the smallest MEMS MFC array currently available. Each layer, except for the PEM, was micro-patterned by using laser micromachining and was precisely aligned. Within just 5 h, we successfully determined the electricity generation capacity of two known bacterial electrogens (wild-type *S. oneidensis* and *P. aeruginosa*) and another metabolically more voracious organism with 4 isogenic mutants constructed with the hypothesis that such mutations could alter their electrogenic properties. Genetically engineered genes in *P. aeruginosa* including *nirS* (nitrite reductase), *lasI* (*N*-(3-oxododecanoyl)-L-homoserine lactone synthase), *bdlA* (biofiilm dispersion locus) and *pilT* (controls the number of Type IV pili on the poles of the bacteria) sensitively showed different efficiencies of extracellular electron transfer to the anode in the significantly reduced micro-chambers. In addition, the percent deviation of all six MFC units was less than 1.4% from their open circuit voltages recorded, which is far less than that of mL-sized MFC arrays (25%) and even MEMS MFC arrays (>8%). This analytical platform would provide the practical tools for fundamental study and characterization of the behavior and physiology of microorganisms and their interaction with MFCs with a greater level of insight and productivity.

1. Introduction

One next-generation energy technology will likely originate from fundamental microbial metabolism. Microbial fuel cells (MFCs) are rapidly gaining acceptance as an alternative "green" energy technology, as they generate sustainable electric power from biodegradable organic compounds through microbial metabolism [1]. However, MFC technology has not yet been applied in practical settings as their power generation is insufficiently low compared with other fuel cell technologies. Important strategies for enhancing MFC performance include genetically engineering microbes, optimizing formation of surface-attached, electrically conductive microbial communities known as biofilms, maximize coulombic efficiency and improving cultivation practices [2]. To date, however, a surprisingly small number of bacterial strains and their optimal growth conditions have been investigated for use in MFCs, revealing a crucial lack of fundamental knowledge as to which bacteria species, consortia or specific genes may be best suited for generating power in MFCs [3]. This deficiency is caused by limitations in current screening methods based on larger scale two-bottle MFCs that require long start-up times (from days to weeks), significant space and materials (hundreds of milliliters to liters), and labor-intensive control for MFC experiments, either in series or in parallel circuits [4]. This limitation has motivated efforts to miniaturize MFC arrays, such that the effective chamber volumes are reduced to the microliter scale in a well-controlled manner. However, efforts to miniaturize MFC arrays using microfabrication technologies have been pursued by only a very small number of research groups [3,5]. The first micro-sized MFC array was presented by Biffinger et al. in 2009 [3]. The multi-anode/common cathode MFC array had nine 1 mL pipette tips as MFC chambers, with an anode chamber volume of 500 μL. The array allowed for efficient monitoring of bacterial growth cycles and comparison of carbon source utilization of *S. oneidensis* MR-1 species. The

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second micro-sized MFC array was developed by Hou et al. to have 24 wells by using microfabrication [2]. Each well had an anode chamber volume of about 600 μL and functioned as an independent MFC. Using the array, they succeeded in isolating a bacterial strain that displayed 2.3-fold higher power output relative to wild-type *S. oneidensis* MR-1. Subsequent to that discovery, the same group added an anolyte/catholyte replenishable microfluidic access to their MFC array for more long-term and efficient analysis [6]. Despite the fact that the reduced anode chamber volume drastically decreased the start-up periods, all the aforementioned prior art seemed to be overlooked as potential candidates for a general screening tool due to (1) unreliable and irreproducible properties stemming from the large deviation (>8%) of each MFC unit in the array, and (2) lack of fluidic access and/or non-independent fluidic access to each MFC unit hampering long-term analytical abilities and/or causing contamination from chamber to chamber. Here, we presented a 6-well MFC array with an extremely small anodic/cathodic volume (1.5 μL), leading to short start-up (<2 h), small deviation (1.4%) from unit to unit, and independent/reliable results from 6 spatially distinct fluidic compartments while prior arts had large size of the anodic chamber volume (>400 μL) directly related to its underlying long start-up periods (2–3 days) and large variation (>6%). Finally, we applied this technique for studies using selected genetics mutants in a hypothesis-driven fashion using several isogenic mutants of the metabolically voracious bacterium, *P. aeruginosa*. These efforts demonstrated that spatially distinct wells of the MFC array displayed highly comparable performance characteristics and identified which of the hypothesis-driven, selected genes in *P. aeruginosa* triggered higher power density.

2. Experimental

2.1. Operating principle of microbial fuel cells

Typically, an MFC comprises an anode chamber and cathode chamber separated by a proton exchange membrane (PEM), permitting only H+ or other cations to pass through from the anodic chamber to the cathode chamber (Fig. 1). The two electrodes are connected via a conductive load to complete the external circuit. In the anode, bacteria oxidize organic matters and then complete respiration by transferring the electrons to the anode via extracellular electron transfer. During the process, chemical energy is captured throughout the electron transport chain. Nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide dehydrogenase (NADH) function as the coenzymes for the reactions are repeatedly oxidized and reduced to prompt the synthesis of the biological energy unit, adenosine triphosphate (ATP).

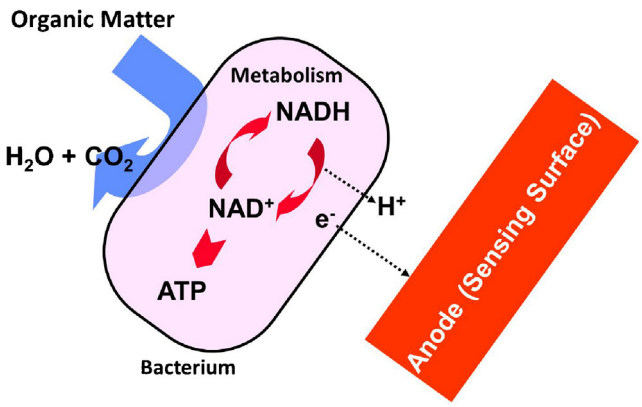

Fig. 1. Principles of operation of a MFC.

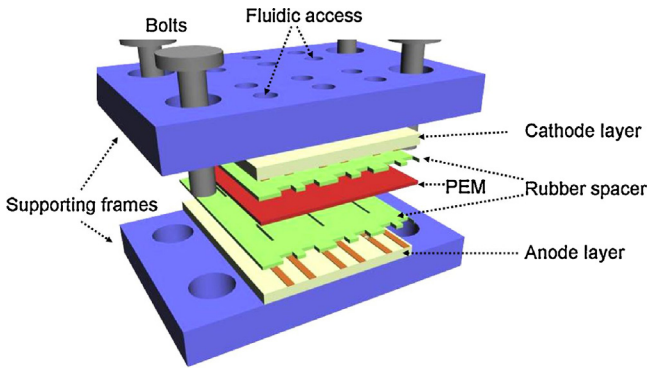

Fig. 2. Schematic of the 6-well MEMS MFC array system. The MFC array consists of five functional layers; an anode electrode layer, an anode chamber layer, a PEM, a cathode chamber layer, and a cathode electrode layer. The array is assembled by the two supporting frames with bolts and nuts.

2.2. Device fabrication and assembly

Fig. 2 shows the schematic illustration of the MFC array [7]. The MFC array consisted of five functional layers; an anode electrode layer (Cr/Au on PMMA (Polymethyl methacrylate)), an anode chamber layer (gasket), a PEM (proton exchange membrane), a cathode chamber layer (gasket), and a cathode electrode layer (Cr/Au on PMMA). The 1.5 μL anode/cathode chamber was defined by a 254 μm-thick gasket; the exposed electrode area per cell is 6 mm^2 (Fig. 3a) The array was assembled by the two supporting frames with bolts and nuts (Fig. 3b). Each layer, except for the PEM, was micro-patterned by using laser micromachining. First, we cut two 5 cm × 5 cm PMMA chips with the laser and drilled 12 holes on each chip for inlets/outlets. The chips were then coated with Cr/Au (20 nm/200 nm) by an electron-beam evaporator and patterned to define the electrodes using a lift-off process. The 254 μm-thick PTEE (polytetrafluoroethylene) rubber gaskets were micro-patterned to define the anode/cathode chambers. We used Nafion 117 as a PEM to permit only cation transport for maintaining electroneutrality between anode and cathode chambers. The Nafion membrane was pretreated by sequentially boiling in H_2O_2 and water, followed by soaking in 0.5 M H_2SO_4 and then water, each for 1 h. The membrane was cut into 5 cm × 5 cm and the exposed surface area of the PEM was 6 mm × 1 mm. The thick supporting frames were cut into 8 cm × 5 cm and drilled 16 holes on each frame: six inlets, sit outlets, and four bolts. Before we assembled the MFC array, the anode/cathode chips were first sterilized with 70% ethanol and then blown dry with nitrogen. All the layers were manually stacked in sequence while carefully aligning the tubing holes for the microfluidic channels. For fluidic access, 24 sterile PEEK (Polyetheretherketone) polymer tubings were plugged into the holes to form twelve independent routes for anolyte/catholyte access (Fig. 4a). The individual microfluidic access path is very important to provide independent and reliable results for each MFC unit.

2.3. Measurement assembly

We measured the potentials between the anodes and cathodes using a data acquisition system (CompactRIO System, National Instrument) and recorded them every 10 min via LabVIEW (Fig. 4b). An external resistor (1 kΩ), connected between the electrodes of each MFC unit, closed the circuit. We calculated current through the resistor via Ohm's law. Current density was normalized to the anode area (6 mm^2) or anode chamber volume (1.5 μL). We droved anolyte and catholyte to fill the anode/cathode chambers