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Bacteria-Powered Battery on Paper

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Paper-based devices have recently emerged as simple and low-cost paradigm for fluid manipulation and analytical/clinical testing. However, there are significant challenges in developing paper-based devices at the system level that contain integrated paper-based power sources. Here, we report a microfabricated paper-based bacteria-powered battery that is capable of generating power from microbial metabolism. The battery on paper showed a very short start-up time relative to conventional microbial fuel cells (MFCs); paper substrates eliminated the time traditional MFCs required to accumulate and acclimate bacteria on the anode. Only four batteries connected in series provided desired values of current and potential to power an LED for more than 30 minutes. The battery featured (i) a low-cost paper-based proton exchange membrane directly patterned on commercially available parchment paper and (ii) paper reservoirs for holding the anolyte and the catholyte for extended period of time. Based on this concept, we also demonstrate the use of paper-based test platforms for rapid characterization of electricity-generating bacteria. This paper-based microbial screening tool did not require external pumps/tubings and represents the most rapid test platform (<50 min) compared with the time needed by using traditional screening tools (up to 103 days) and even recently proposed MEMS arrays (< 2 days).

Introduction

A well-designed, paper-based power source is indispensable to creating an all paper-based system that can work independently and self-sustainably.[1] Paper attracts significant attention for its potential integration in simple, low-cost, portable, and disposable analytic/diagnostic devices that are suitable for resource-limited and remote regions.[2] Designing an integrable, paper-based power source is crucial for powering such on-chip paper electronics.[3,4] Paper-based diagnostic tools are suited to one-time point-of-use and point-of-care tests that can measure the quantity of analytes of interest without the need for obtaining time-consuming and expensive laboratory evidence.[5]

Ideally, paper-based power sources must also be inexpensive, simple, disposable, and accessible to resource-limited settings. Microwatt-level power sources are more attractive for meeting the short operation lifetime of those disposable diagnostic devices than commonly-used longer-operating batteries, which may be wasteful for single-use low-power systems.[6] Therefore, simple, low-cost, easily operable, disposable, on-demand paper micro-batteries are demanded for powering paper-based sensing devices with similar characteristics. These efficient paper-based electronics can never be realized as an independently working system without integrating such paper micro-batteries, because they will always need additional read-out equipment with an appended power source, or rely on conventional power-free colorimetric techniques that only provide minimal "yes/no" or semi-quantitative analysis.[7] To date, several types of paper-based batteries or energy storage devices have been developed for various applications, including an electrochemical fuel cell in paper-based microfluidic devices for on-chip fluorescence assay,[8] a urine-activated paper battery for biomedical devices,[6] a supercapacitor integrated into photoelectrochemical lab-on-paper device,[9] a lithium-ion paper battery with high energy density,[10] and an enzymatic paper-based biofuel cell for potentially powering paper diagnostic devices.[11] In particular, enzymatic fuel cells (EFCs) and microbial fuel cells (MFCs) are two categories of biofuel cells that offer the highest potential for their implementation on paper substrates. In EFCs isolated enzymes are used to catalyze the biochemical processes whereas whole organisms are used in MFCs, this major difference in the principle of operation results in different inherent characteristics between these two technologies. For instance, the short lifetime of enzymes severely limits the operation of EFCs. However, this limitation is not evident in MFCs since the microorganisms can continuously produce the required enzymes during operation. Recently, we summarized the full scope of paper-based batteries and energy storage devices in a review article.[12]

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In this work, we created a paper-based bacteria-powered battery that can generate power with one drop of bacteria-containing liquid which makes on-board energy delivery possible for the next generation of paper-based devices. A bacteria-powered battery or microbial fuel cell (MFC) generates electricity through bacterial metabolism under milder pH and temperature conditions than conventional fuel cells. Moreover, the fuel used can be any type of biodegradable organic substrate, including wastewater, urine, or soiled water in a puddle. River, ocean and pond environments' water generally host various microorganisms that can transfer electrons produced via metabolism across the cell membrane to an external electrode. MFCs typically have a simple, two-chamber structure: anodic and cathodic chambers separated by a proton exchange membrane (PEM) that only H^+ or other cations can pass through.[13,14] Affordable materials and fabrication processes are used to build these devices which renders them more cost effective than other paper-based power sources. However, using a paper substrate anode/cathode chamber or reservoir instead of the usual rigid materials (glass, plastic, and silicon in MFC anode or cathode chambers)[15, 16] allows for rapid adsorption of bacteria-containing liquid. This adsorption immediately promotes bacteria cell attachment to the electrode, where bacterial respiration can then transfer electrons from the organic liquid to the electrode. A bacteria-powered battery on paper can therefore show a very short start-up time relative to conventional MFCs; paper substrates eliminate the time traditional MFCs require to accumulate and acclimate bacteria on the anode.[15]

To realize a bacteria-powered battery on paper as a portable power source, one with substantially upgraded power density and reduced cost, we leveraged techniques recently demonstrated for our conceptual, paper-based microbial fuel cell (MFC)[17] and multi-anode paper MFC.[18] By exploiting this paper's unique feature for bacteria, we also introduced a paper-based microbial array as a high-throughput, rapid screening tool for microbial electricity generation studies. In both microbiology and energy technology research, high-throughput and rapid characterization of genetically-engineered bacteria's electricity-generating capacity has recently been identified as an outstanding challenge of great importance.[19,20] Limitations motivated efforts to develop a miniaturized MFC array as a parallel analysis platform, effectively reducing the chamber/channel volumes to the microliter scale in a well-controlled manner.[21-24] Despite great excitement about these miniaturized formats, however, the sensing platform still lacks two key aspects: parallelization and rapid power assessment. Currently, no technology can even conceptually provide independent access to more than 24 spatially distinct microbial sensing units with dramatic reductions in a screening speed.[21,25] This is because of (i) complex MFC configurations with microfluidic tubings/channels and their operation with external pumps, and (ii) long start-up times required for bacterial accumulation and acclimation on the sensing electrodes. Our paper-based screening platform can therefore be a fundamental device breakthrough that can provide high-throughput with rapid screening capabilities. In this work, we conceptually developed a six-well, paper-based MFC array that allows for the rapid characterization of microbial electricity-generating capabilities.

Results and discussion

MFC operating principle

MFCs are typically comprised of anodic and cathodic chambers separated by a proton exchange membrane (PEM) so that only H^+ or other cations can pass from the anode to the cathode. A conductive load connects the two electrodes to complete the external circuit. Microorganisms oxidize organic matter in the anodic chamber, completing respiration by transferring electrons to the anode. During this process, chemical energy is captured throughout the electron transport chain. Nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide dehydrogenase (NADH) function as coenzymes for the reactions, repeatedly oxidizing and reducing to synthesize adenosine triphosphate (ATP), the biological energy unit. Electrons that are transferred to the anode flow to the cathode through the external resistor. The redox couple is completed when captured electrons reduce ferricyanide, $[Fe(CN)6]^{3-}$ at the cathode (eqn (1)).

$$[Fe(CN)_6]^{3-} + e^- \rightarrow [Fe(CN)_6]^{4-} \quad (1)$$

Bacteria-powered battery on paper

Figure 1 shows a schematic of the paper-based bacteria-powered battery. The MFC utilized flexible carbon cloth anodes for bacterial attachment and paper reservoirs for holding the anolyte and catholyte for an extended period of time. To reduce device cost, we employed commercially-available hydrophobic parchment or wax papers as a PEM, minimizing the anolyte and catholyte transfer while allowing protons to pass through efficiently. Most chemical fuel cells or MFCs use expensive commercial Nafion 117 as a PEM,[17, 18] which leads to low conductivity at low humidity and significant volumetric size change with increasing humidity levels. These qualities make it difficult to integrate them in the bacteria-powered battery with other paper layers.[26] Nafion membranes are also incompatible with microfabrication processes.[15] Even our first version of the paper-based MFC, with its chemically treated paper-based PEM considerably lowering the cost of the device, could not provide an operating voltage and current high enough to power other practical devices. This was due to permeation issues in the paper PEM.[17]

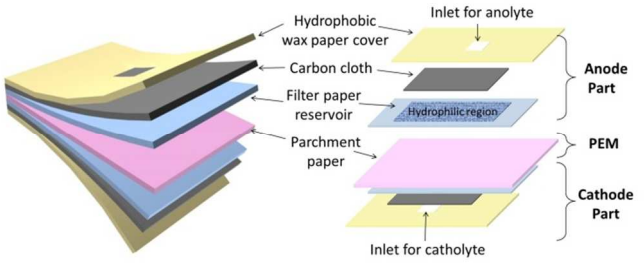

Figure 2. Schematic diagram of the paper-based bacteria-powered battery

In this study, we tested four different papers as the PEM: laser-treated wax paper, non-treated wax paper, laser-treated parchment paper, and non-treated parchment paper. The laser treatment made selective surface modifications using a computer-controlled, CO_2 laser cutting and engraving system.[27] The purpose was to convert hydrophobic areas to hydrophilic ones, the hypothesis being that hydrophilicity might increase the proton exchange rate across the paper-based PEM. The rationale for using wax/parchment papers as a PEM is very clear: (i) the papers are inexpensive, thin, lightweight, and easy to handle; (ii) the paper is hydrophobic enough to physically separate the anode and cathode chambers; (iii) the paper's intrinsically rough and porous structure benefits electron manipulation and ion transport across the entire paper; and (iv) the hydrophilic region can be readily patterned on the paper with laser machining.[27] To evaluate the performance of the paper-based battery with paper-based PEMs, we first measured the open-circuit potential between the anode and cathode by a data acquisition system. Results were recorded every minute via customized LabView interface (Figure 2a). Then, an external resistor (1.5 kΩ) was connected between the anode and cathode electrodes to monitor current generation (Figure 2b). Current through the resistor was calculated via Ohm's law, and the output power via $P = V \times I$. Current and power densities were normalized to the cathode area (2 x 3cm). All the experiments were repeated three times and compared to performances where the paper-based MFC used Nafion 117 and whatman #1 filter paper as the PEM.

Wild-type *Shewanella oneidensis* was grown in L-broth medium as the anolyte, and a phosphate-buffered ferricyanide (50mM, pH 7.0) was used as the catholyte. Inoculum (anolyte) and catholyte were introduced onto the paper by pipettes containing 0.1 mL of each and the MFCs were operated at 30 ºC. The solutions wicked through the carbon cloth and remained in the paper reservoirs. Non-treated paper-based batteries produced higher open circuit voltages than laser-treated ones and were comparable to commercial Nafion membranes. This is mainly due to minimal anolyte and catholyte permeation through the papers, even though a more efficient proton travel rate was expected with laser-treated papers.

Instant operation must be noted as a major advantage of the paper-based bacteria-powered batteries: current generation started immediately upon connection of the external resistor

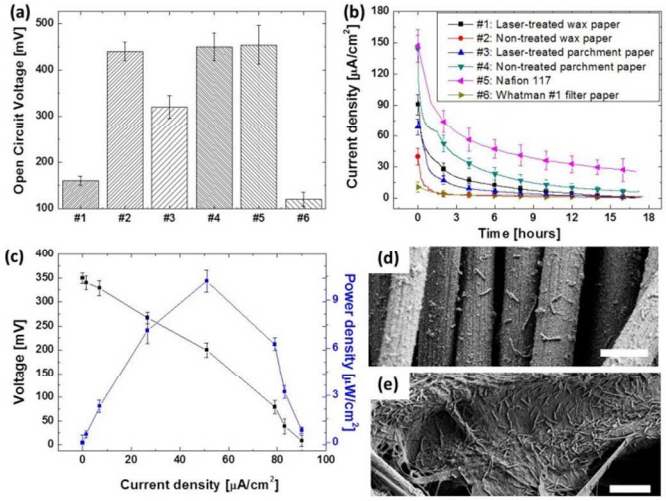

Figure 1. (a) Open circuit voltages and (b) output currents produced from six paper-based batteries with different PEMs ((#1: laser-treated wax paper, #2: non-treated wax paper, #3: laser-treated parchment paper, #4: non-treated parchment paper, #5: Nafion 117, and #6: Whatman #1 filter paper), (c) polarization curve (black circle) and power output (blue square) of the non-treated parchment paper-based battery, and SEM images of (d) the carbon cloth and (e) paper reservoir (scale bar is 10 μm)

between the anode and cathode. This can be attributed to the use of paper reservoirs, which immediately absorb the anolyte and allow for the attachment of a larger number of bacteria cells to the anode. Non-treated wax paper generated significantly lower current than others, even with higher open-circuit voltages, and this is probably due to low proton travel rate through the paper. The initial current generation of non-treated parchment paper was the highest. It was slightly lower than that of the Nafion 117-based battery, though the currents from the Nafion-based MFC provided power for a longer time than paper-based PEM MFCs. This reason is that a slight permeation of solutions through papers is unavoidable. Permeation of the anolyte and catholyte reduces the open circuit potential of chemicals in each chamber, and this can decrease power/current generation in the MFCs. The non-treated parchment paper battery provided the best performance among paper-based PEMs, generating a maximum power of 10 $\mu W/cm^2$ at a current density of 50 $\mu A/cm^2$ (Figure 2c).

This battery produced high power and current densities and operated for more than 18 hours before it dropped to zero current, which is 72 times longer than our previous conceptual MFC on paper.[15] Although further studies need to be done for proton transfer mechanisms through these paper PEMs, non-treated parchment paper can be a low-cost and high performance PEM candidate for paper-based fuel cell devices. Figures 2d and 2e show the SEM images from the carbon cloth and anode paper reservoir, respectively. The paper reservoir (Figure 2e) contained many bacterial cells on paper, while a small number of bacterial cells attached to the carbon fibers (Figure 2d). The cells are not embedded within a full matrix of biofilm on both cases. SEM images indicate that most of the bacteria stay on paper rather than on the carbon cloth due to the strong wicking force of the paper.

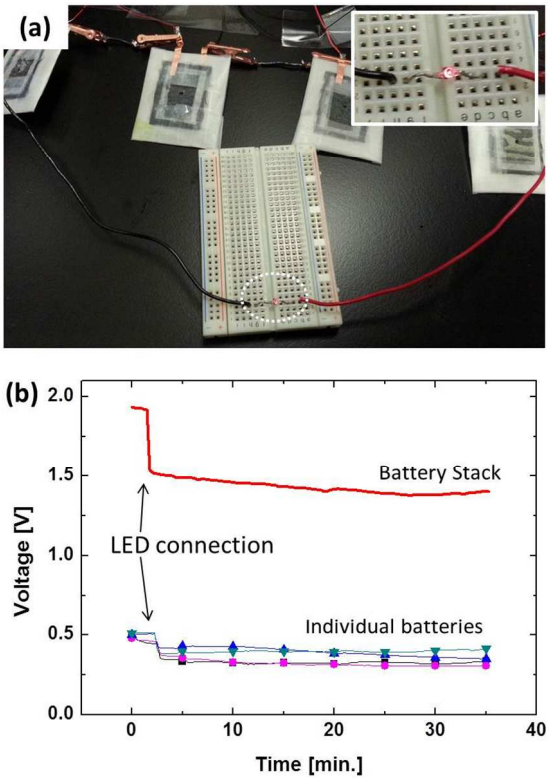

Figure 3. (a) Lighting a red LED with the four batteries connected in series (battery stack) and (b) output voltages of the battery stack (solid line) and individual ones when an LED was connected across the four batteries in series.

Wild-type *Shewanella oneidensis* can theoretically conduct extracelluar electron transfer via three mechanisms; (i) direct electron transfer, where the cells adhere physically to the anode surface and transfer electrons to the solid anode, (ii) shuttle transfer, where the electrons are transferred to the anode via electron mediators, and (iii) nanowire transfer, where a solid conductive wire is biosynthesized and used for electron transfer.[28] Immediate power generation via direct contact is therefore highly feasible; all shuttling compounds were removed by centrifugation and the inoculum was prepared in new medium. Producing conductive wires is expected to require a long period of time.

After confirming that the parchment paper PEM can supply the desirable performance from the paper-based bacteria-powered battery, we connected four batteries in series to produce a targeted power output (Figure 3). The battery stack provided the desired values of current and potential for powering a red LED (HLMP-P156, Digikey) for over 30 minutes without power management interface circuits. While the LED was being illuminated by the battery stack, we recorded the voltage across the individual batteries, and the series array for the duration of the operation. All voltage levels were consistent with each other. Voltage reversal has been reported as one of the main challenges for bacteria-powered battery arrays,[29,30] and interestingly, no voltage reversal was observed in any of the four batteries. The convergent behavior of individual batteries may be explained by hydrophobic parchment paper minimized the crossing of the anolyte and catholyte across the PEM to the opposite chambers, which in turn resulted in a longer retention of the fuel and oxidant.

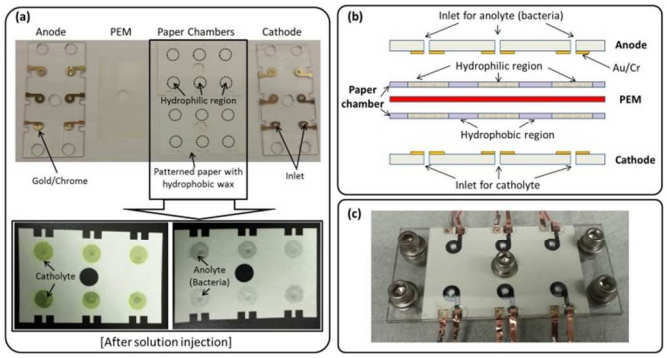

Figure 4. (a) Individual layers of the array and paper chambers after loading the inoculum and catholyte. Hydrophobic wax boundaries were made by heat pressing wax paper onto filter paper, (b) schematic and (c) photo-image of the paper-based microbial sensor array. The array consisted of five functional layers; an anode layer, a paper reservoir layer for anolyte, a proton exchange membrane, a paper reservoir layer for catholyte and a cathode layer. Copper tapes were attached to the gold pads for electrical contacts.

MFC array on paper

We also demonstrated the use of paper-based test platforms for rapidly characterizing electricity-generating bacteria. The presented device (Figure 4) contains vertically stacked anode/cathode paper chambers (or reservoirs) separated by a PEM, and gold anode/cathode interface pads with through-holes in the center to introduce anolyte/catholyte. Paper reservoirs featuring a hydrophilic chamber with hydrophobic wax boundaries were made by heat pressing commercially available wax paper (Reynolds CutRite) onto Whatman #1 filter paper. The paper-based sensor exploits the paper's ability to quickly wick fluid and promote bacterial attachment to the gold anode pads, resulting in instant current generation upon loading the bacterial inoculum and catholyte. Six microorganisms were tested: *Shewanella oneidensis* MR-1, *Pseudomonas aeruginosa* wild-type PAO1, and another, metabolically more voracious organism. This organism has four isogenic *pmpR*, *rpoS*, *lasR rhlR* and *filC pilA* mutants: *pmpR* is a *Pseudomonas* quinolone signal (PQS) regulator; *rpoS* is a positive transcription regulator of single-species biofilm formation; *lasR* and *rhlR* both are important for quorum sensing in *P. aeruginosa* PAO1; and *filC* (flagella) and *pilA* (type IV pilin) are important for bacteria movement, playing a role in cell motility, intracellular trafficking, secretion and vesicular transport. *P. aeruginosa* PAO1 mutants were generated using classical allelic replacement techniques with sucrose counter-selection as described by Hoang et al.[31] All the cell numbers were controlled by using an optical density at 600 nm. The catholyte for the six MFC units was 50 mM ferricyanide in a 100 mM phosphate buffer. After the anolyte and the catholyte were injected, using 100 µL pipettes, into corresponding inlets for each paper reservoir, the inlets were sealed with tape to prevent solution depletion through evaporation (Figure 5a). The voltage curves with and without load are shown in Figure 5b. Before closing the MFC circuits with 1 kΩ resistors, the open

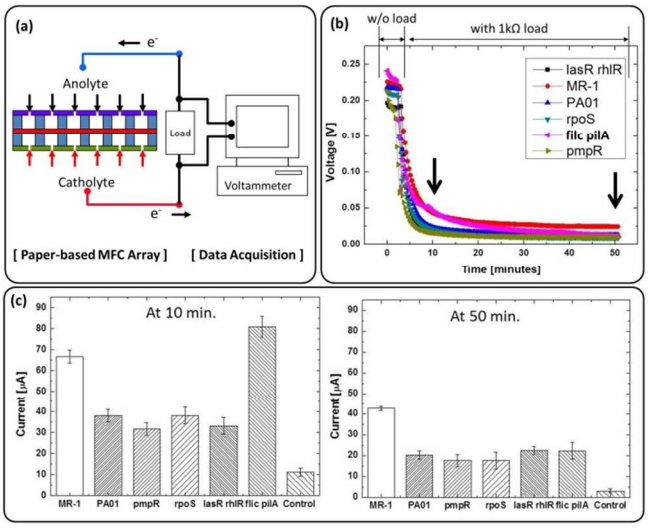

Figure 5. (a) Measurement setup for testing the paper-based MFC array. The array requires only drops of bacterial inoculum and catholyte onto the electrodes for power generation, (b) voltages measured from the device with different bacterial species. The open-circuit voltages were measured for the first three minutes and then all MFC cells were connected to 1 kΩ external loads, and (c) currents calculated from Fig. 4 in 10 min. and 50 min. At 10 min., the *filC pilA* mutant has superior current generation followed by *Shewanella sp.* (MR-1). At 50 min., however, the *filC pilA* performance showed a significant decrease while *Shewanella sp.* continued to have comparable performances.

circuit voltages were recorded for 3 min (Figure 5b). Measured voltages varied between the different MFCs, clearly indicating performance variations in the bacterial species injected into each chamber. The open-circuit voltage values ranged from 0.18 to 0.25 V. Open-circuit voltages are the cell's potential differences; given this, that indicates the difference between the potential under equilibrium conditions and the thermodynamic losses. That the *pmpR* mutant's value is substantially lower than the others clearly shows a large energy loss occurring at the anode. After operating the MFCs under no-load conditions for approximately three minutes, load resistors were connected to enable current generation. Voltage differences under load were then recorded until their values reached zero from solution depletion. This took approximately 50 minutes. Current comparison was made two times, the first after 10 min of operation and the second at 50 min. All experiments were repeated six times and displayed with error bars.

After 10 min, the operation showed significant differences between the various species used in terms of current generation. The array proved useful for bacterial screening and characterization despite the relatively short operation time compared to previous arrays, because the paper reservoirs' ability to rapidly wick the solutions through capillary action allowed for a faster bacterial acclimation and accumulation at the anode surfaces. After 10 min, the current values showed that the *filC pilA* mutant has superior current generation, followed by *Shewanella sp.* (MR-1). This indicates that these two species can quickly acclimate to the anode electrode and start their metabolic and extracellular electron transfer processes. The *pmpR* mutant generated the lowest current, which implies both low metabolism and poor electron transfer capabilities for this particular mutant. After 50 min, calculated operating currents show that the *filc pilA* performance suffered a considerable decrease, probably because of substrate depletion in the corresponding MFC, which was expedited by its high performance at the beginning of the operation. By contrast, *Shewanella sp.* showed a relatively lower decrease in performance and exhibited the highest current generation among all tested species. The other species continued to have comparable performances in paper-based MFCs, but all showed decreased current levels compared to their 10 min current levels.

Conclusion

In summary, we created a bacteria-powered battery on paper readily operated by one-drop of bacteria-containing liquid; it was developed as an alternative power source for paper-based analytical/diagnostic devices targeted for use in resource-limited regions of the world. A series-connected array of four such batteries generated enough power to operate a practical electronic device for more than 30 minutes. We also developed a six-well, paper-based MFC array that allows for the rapid characterization of microbial electricity-generating capabilities. Using paper considerably decreased operating time, and within 50 minutes, current generation abilities for two known bacterial electrogens and four more isogenic mutants were successfully determined. This paper-based microbial screening tool does not require external pumps/tubings and represents the most rapid test platform when compared to traditional screening tools and even recently-proposed MEMS arrays. This array is expected, by virtue of its rapid response, to have widespread applications in the electrogen screening and characterization, and significant cost savings from requiring no expensive pumps to operate, and only small amounts of inoculum and catholyte.

Experimental procedure

The paper-based battery was manually assembled by sandwiching four functional layers; (i) carbon cloth anode/cathode layers, (ii) paper anode/cathode reservoirs (Whatman #1 filter paper), (iii) a paper-based PEM, and (iv) hydrophobic paper covers with holes for sample input. Copper tape (3M^{TM} copper conductive tape) was used to provide electrical contact to the anode and cathode electrodes. Since carbon cloth is hydrophobic, the carbon cloth layers were exposed to oxygen plasma for 1 min for hydrophilization.

The paper-based MFC array comprised five functional layers; (i) an anode layer (Au/Cr on PMMA), (ii) a paper anode reservoir layer, (iii) a PEM, (iv) a paper cathode reservoir layer, and (v) a cathode layer (Au/Cr on PMMA). Except for the PEM, each layer was first micro-patterned using laser micromachining. The 100 nm gold electrodes were deposited on PMMA substrates using e-beam evaporation with chromium as the adhesion layer. Copper tape was attached to the contact pads with silver conductive paint (PELCO® Colloidal Silver). All layers were mechanically held together using bolts and knots.

All cultures were grown in standard L-broth medium for 24 hours at 30 °C before inoculation of the paper-based MFCs. The L-broth media consisted of 10.0 g triptone, 5.0 g yeast extract, and 5.0 g NaCl per liter. To remove biomass, bacterial cells were harvested by centrifugation and resuspended in new L-broth medium. The catholyte was 50 mM ferricyanide in a 100 mM phosphate buffer in which pH was adjusted at 7.5 ± 0.2 with 0.1 M NaOH.

For bacterial fixation and SEM imaging, the devices were disassembled and rinsed; adherent bacteria on each anode were immediately fixed in 2% glutaraldehyde solution overnight at 4°C. Samples were then dehydrated by serial 5 min transfers through 50, 70, 80, 90, 95, and 100% ethanol. Fixed samples were examined using a FESEM (Field Emission SEM) (Supra 55 VP, Zeiss).

Acknowledgments

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