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A Disposable, Papertronic Three-Electrode Potentiostat for Monitoring Bacterial Electrochemical Activity

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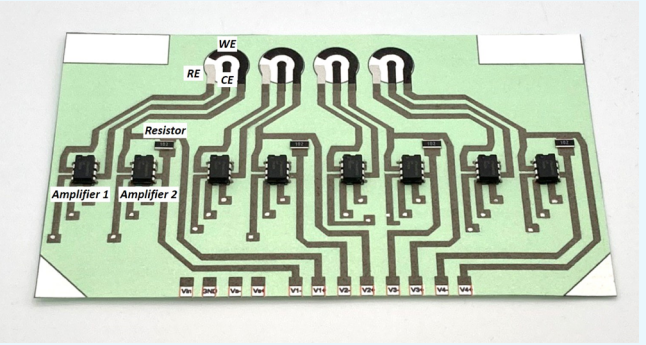
ABSTRACT: Bacterial electrochemical activities can promote sustainable energy and environmental engineering applications. Characterizing their ability is critical for effectively adopting these technologies. Conventional studies of the electroactive bacteria are limited to insensitive, time-consuming, and labor-intensive two-electrode microbial fuel cell (MFC) techniques. Even the latest miniaturized MFC array is limited by irreproducibility and uncontrollability. In this work, we created a 4-well electrochemical sensing array with an integrated, custom-made three-electrode potentiostat to provide a controllable analytic capability without unwanted perturbations. A simple potentiostat circuit used two operational amplifiers and one resistor, allowing chronoamperometric and staircase voltammetric analyses of three well-known electroactive bacteria species: *Shewanella oneidensis* MR1, *Pseudomonas aeruginosa* PAO1, and *Bacillus subtilis*. Portability and disposability were emphasized by integrating all the functions into a paper substrate, which makes analyses possible at the point-of-use and in resource-limited settings without a bulky and expensive benchtop potentiostat. After use, the papertronic system was disposed of safely by incineration without posing any bacterial cytotoxic risks. This novel sensing platform creates an inexpensive, scalable, time-saving, high-performance, and user-friendly platform that facilitates the study of fundamental electrocatalytic activities of bacteria.



1. INTRODUCTION

Bacterial electrocatalysis, which refers to the bacteria's ability to electrochemically exchange electrons with conductive electrode surfaces, has attracted significant scientific interest for more than 100 years.[1,2] There are potentially widespread uses in bioremedation, bioproduction, and sustainable energy generation with various emerging bioelectrochemical systems, including microbial fuel cells (MFCs), microbial electrolysis cells (MECs) and microbial electrosynthesis (MES).[3−5] Many diverse electrochemically active bacteria have been discovered or genetically engineered to revolutionize bacterial electrocatalysis and bring the techniques from the benchtop to practical real-world applications.[6−9] To augment the considerable effort and expense in developing these systems and the microbial biotechnology, a sensing technique that can rapidly, simultaneously, and sensitively characterize electroactive bacteria is in great demand. The novel sensing method can provide fast and reliable information to select the best bacterial species or mutants for the systems. Many emerging sensing techniques using electrochemical colorimetric assay and cell polarizability have received less attention as a standardized screening tool because their capability is limited to specific bacteria having a direct extracellular electron mechanism through *c*-type cytochromes.[10,11] More standardized sensing approaches rely on the most accurate and reliable MFC technique that evaluates all bacterial electrocatalytic activities by directly measuring bacterial electrons transferred from the cell. More recent versions of the MFC tool, however, used energy-intensive and large-scale fluidic feeding systems that require cumbersome experimental operations with long testing times.[12,13] Evolving microfluidics and microelectromechanical systems (MEMS) technologies have shown great promise in MFC-based sensing applications by miniaturizing the MFC units and leveraging the fluidic manipulation.[14−17] The recent technique of fusing the MFC technology with the emerging field of paper-based electronics, known as papertronics, revolutionized the scalability of the MFC as an array and the operation without using complicated microfluidic channels and external pumps.[18] Simple wax printing allowed the batch fabrication of the large-scale, high-throughput MFC array on paper while the intrinsic capillary force of the paper enabled a rapid adsorption and a relatively long-term storage of the

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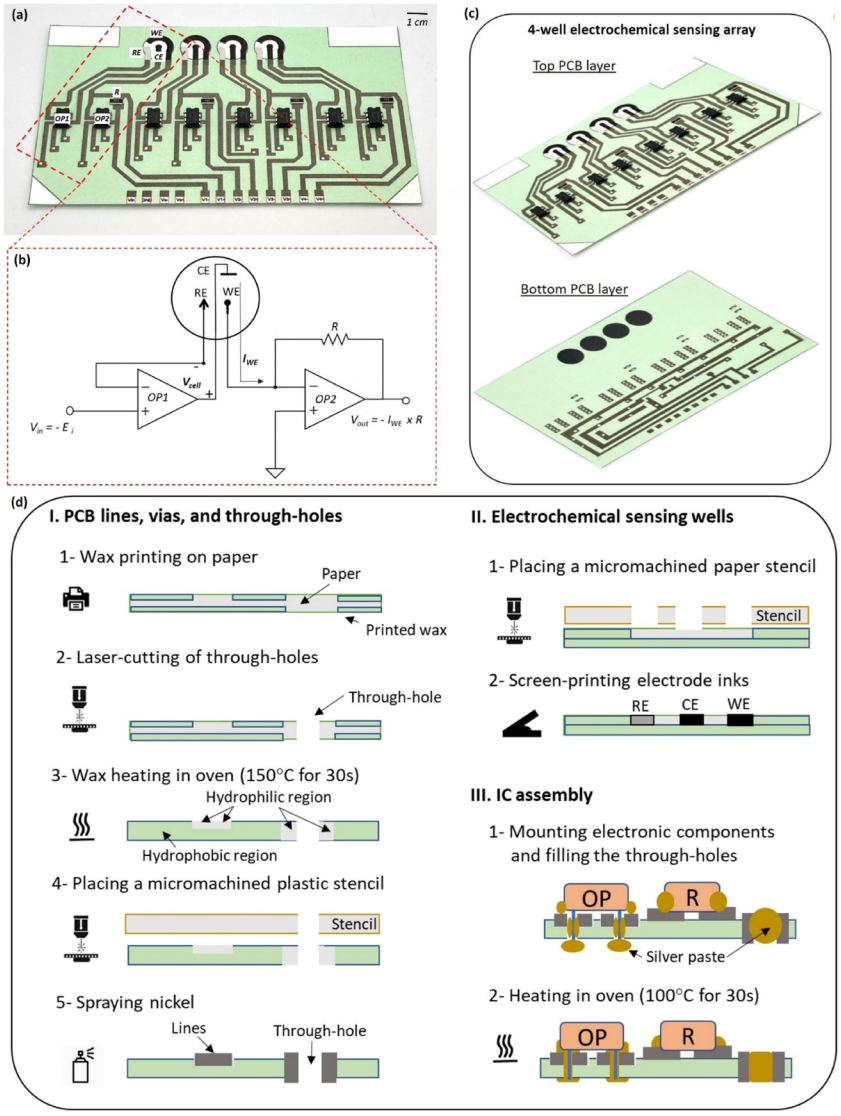

Figure 1. (a) Photo of electrochemical sensing array integrating a custom-made three-electrode potentiostat and (b) circuit diagram of the potentiostat. (c) Photo images of the top and bottom of the paper PCB. (d) Fabrication processes for papertronic PCBs and sensing units.

aqueous bacterial sample without external pumping and fluidic valving manipulation. Furthermore, the paper-based MFC array can be safely incinerated without posing a health risk of bacterial infection. Previously, 4-, 8-, 16-, 48-, 64-, and 96-well MFC arrays were innovatively developed on paper, demonstrating a successful high-throughput characterization of bacterial electrochemical activities without external pumps and bulky electrical measurement systems, and they are suitable for analysis at the point-of-use and in resource-limited settings.[19−23] However, the MFCs in the array used a two-electrode configuration (i.e., anode and cathode) for the current generation assessment, where bacterial electroactivity was controlled by changing the external resistance.[24] The two-electrode MFC platform partially measures the electrochemical behaviors in the anode and reflects the overall efficiency of the MFC, including all the activities occurring in other components (i.e., proton exchange membrane and cathode). Therefore, it is difficult to have an in-depth understanding only for anodic bacteria. Moreover, the two-electrode mode is rarely reproducible because of changes in the bacterial growth and metabolism, and it can be limited by the electrode materials and electrolytes that are not electrochemically inert. In addition, recent studies demonstrated that bacteria use different electron transfer systems with different anodic potentials, and their electrocatalytic capacity can be determined by sweeping anodic potentials, which cannot be done by using the two-electrode configuration.[25−27] To avoid unwanted perturbations to the system and control studies for electroactive bacteria, a three-electrode configuration consisting of a working, a counter, and a reference electrode is a more appropriate sensing tool. In a single chamber with all three electrodes, bacterial samples are introduced to start characterizing the electrocatalytic activities. By applying an anodic potential to the working electrode with respect to the reference electrode, reliable and accurate measurements of electrochemical activity of bacteria can occur by monitoring the current flowing from the counter electrode to the working electrode. While we secure all the advantages of the previous papertronic MFC arrays including low-cost, easy-to-use, but powerful sensing properties, it is quite challenging to integrate three electrodes in a single-well and incorporate a dedicated electric reader in the paper device. Normally, three-electrode

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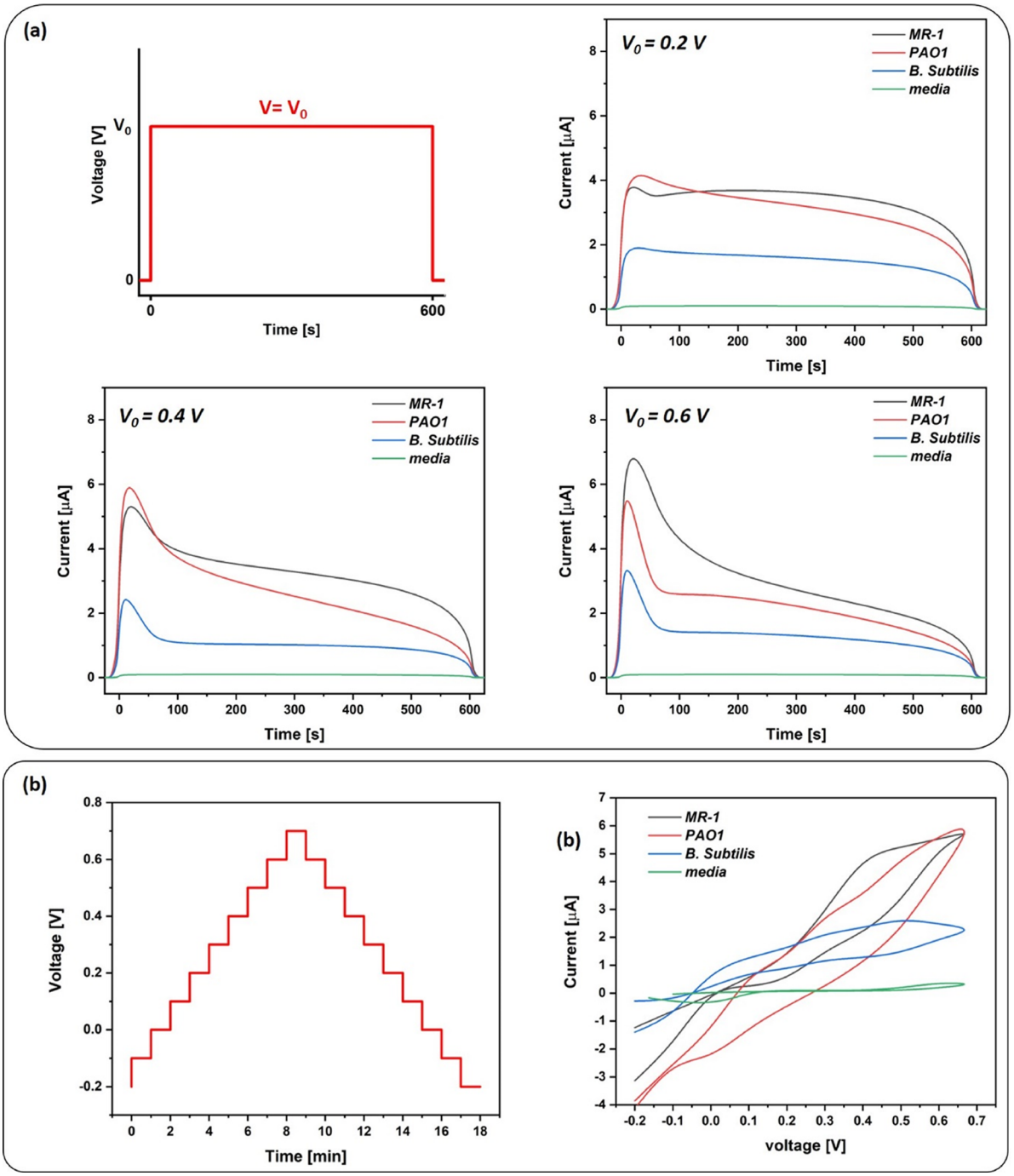

Figure 2. (a) Chronoamperometry: input potential and chronoamperograms at 0.2, 0.4, and 0.6 V. (b) Cyclic staircase voltammetry: input potential steps and *I*−*V* cyclic voltammograms of *S. oneidensis* MR1, *P. aeruginosa* PAO1, *B. subtilis*, and media (control).

electrochemical sensors require a bulky, heavy, and expensive benchtop potentiostat as the reader, which is widely used to perform the electrochemical measurement.

In this work, we created a papertronic 4-well electrochemical sensing array integrated with a custom-made three-electrode potentiostat (Figure 1a−c). Each well was well-defined by hydrophobic wax boundaries on paper and included the working and counter electrodes prepared by a graphite ink and the reference electrode screen-printed by Ag/AgCl ink (Figure 1d). The electric reader was simply formed by two operational amplifiers for a potential control and by a resistor for a current measurement, which were all mounted onto a paper printed circuit board (PCB) patterned with conductive nickel (Figure 1d). Upon the bacterial sample introduction into the wells, a potential with respect to the reference electrode was applied to the working electrode by simply using a widely available and an inexpensive power supply (Figure 1b). With a different working potential, even a small current can be sensitively monitored by calculating the voltage drop across the resistor. Two well-known Gram-negative exoelectrogens, *Shewanella oneidensis* MR1 and *Pseudomonas aeruginosa* PAO1, and one Gram-positive exoelectrogen, *Bacillus subtilis*, were selected as test species while the media without bacteria was used as a control. *S. oneidensis* can perform the extracellular electron

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transfer via the direct or indirect electron transfer mechanism, while *P. aeruginosa* use the indirect route through a soluble redox-active electron shuttle.[6,7] *B. subtilis* are reported as weak electroactive microorganisms.[28] This work makes it possible to create an inexpensive, scalable, time-saving, high-performance, and user-friendly platform that facilitates studies of electrocatalytic bacteria.

2. RESULTS AND DISCUSSION

2.1. Bacterial Electrochemical Activity as a Function of Potential. An OD_{600} of 2.5 was used for all bacterial samples because it was sufficient to saturate the sensing well and maximize the electrochemical activities (Figure S1). Our previous studies demonstrate that a same-sized sensing well was densely packed with the bacterial culture having the OD_{600} of 2.5, generating the maximum current.[21,22] *S. oneidensis* MR1, *P. aeruginosa* PAO1, and *B. subtilis*, were introduced on separately to the three sensing wells while the LB media without bacteria was used in the fourth well as a control. Optimized electrode potential enables bacteria to oxidize the electron donor media and to liberate the electrons more effectively, as the best potential allows the bacteria to create near theoretical levels of energy.[25−27] Furthermore, bacteria could use different electron transfer pathways with different potentials and achieve the significant enhancement of the bacterial electrochemical activity by optimizing the potential.[25−27] To investigate the influences of the electrode potential on the electroactivity of bacteria, we used chronoamperometry to monitor the current generation as an electrical output. Chronoamperometry can be the most suitable evaluation tool for this study because it involves a potential application to the working electrode, at which the bacterial electrochemical activity is performed for a certain time, during which the current flow from the counter electrode to the working electrode is monitored. Chronoamperometry has been widely used to characterize electroactive bacteria and to assess their growth and metabolism.[29] In this work, we realized a simple, disposable chronoamperometry on paper by integrating two operational amplifiers and one resistor (Figure 1a−c). Chronoamperometric experiments were conducted by applying potentials of 0.2, 0.4, and 0.6 V constantly for 600 s to monitor the optimized and stabilized currents in the system (Figure S2 and Figure 2a). Initially, the current significantly increased within 50 s and reached the maximum level, demonstrating the sudden bacterial electrochemical activity by applying the potential with a greater amount of Gibbs free energy.[30] However, it decreased with time afterward because of the depletion of the electron donors. The control without the bacteria did not produce any current, indicating that the electricity was generated from the bacterial electrochemical activities. Throughout all experiments with different potentials, Gram-positive *B. subtilis* demonstrated much lower current generation than the two Gram-negative bacterial samples. Gram-positive bacteria generally have thick nonconductive cell membranes and exhibit weak electrochemical activities.[28] For all samples, the most stable current generation with the fewest current drops from the peak were observed with a potential of 0.2 V at the working electrode. This is because the electron donors were slowly depleted with the lower electrochemical activities of bacteria, generating a more stable current for a longer term operation. With the increase in the electrode potential to 0.4 and 0.6 V, however, a much steeper current increase and then sudden decrease profile was observed from the samples over the first 100 s of the operation. Furthermore, the two Gram-negative bacteria did not exhibit stabilized current outputs with 0.4 and 0.6 V for the next 500 s. This indicates that more electroactive bacteria rapidly depleted the media within the limited volume of the sensing well. With the potential of 0.2 and 0.4 V, *P. aeruginosa* PAO1 showed a greater current output than *S. oneidensis* MR1 for the first 100 s but decreased below the level of *S. oneidensis* MR1 for a longer-term operation. At 0.6 V, *S. oneidensis* MR1 generated more current than the others throughout 600 s. This data indicates that *S. oneidensis* MR1 have the higher electrochemical activity at 0.6 V while *P. aeruginosa* PAO1 become more electroactive at 0.2 and 0.4 V.

Figure 2b shows the cyclic staircase voltammetry with a series of cyclic staircase steps (0.1 V) and a 1 min step period. The cyclic staircase voltammetry is more complex but sensitive compared to the linear scan cyclic voltammetry.[31] A series of cyclic potential step profile is applied to the electrochemical sensing unit and its response is measured at the end of each step. Therefore, the staircase voltammetry allows measuring only the faradaic current directly generated from bacterial electrochemical activities while it removes the capacitive current arising from the double layer charging at the beginning of the potential step. With various potential steps and their period at which current measurement is performed, more quantitative analysis can be performed by minimizing the capacitive current. In this work, to quickly demonstrate this functional capability, a simple potential waveform was made up of 0.1 V discrete steps with a 1 min period sweeping between −0.2 and 0.7 V. The current outputs as a function of the input potential showed the cyclic voltammograms of three bacterial samples. Much higher current generation was observed from *S. oneidensis* MR1 and *P. aeruginosa* PAO1 compared to *B. subtilis*. The control exhibited a negligible current output throughout the potential sweeping. Overall, three sensing arrays displayed a comparable repeatability having less than 3% variation while the individual sensors in one array exhibited 2.3% variation.

2.2. Disposability. The most critical attribute that will allow the widespread use of biosensors to detect biomolecules is whether they can be disposed of safely after use. This is important especially when the biosensors assess bacterial cells because of the risks of infections and contagions. While many conventional biosensors are made from rigid nondisposable materials, paper-based device platforms feature an inherent disposable nature by simple incineration. As shown in Figure 3, our papertronic sensing system required only 8 s to completely disappear by burning. In particular, our papertronic device was significantly cost-effective enough to be used in single-use applications. Total material cost of our device was less than $1, including $0.2 for the paper, $1.0 per g for the Ag/AgCl ink, $2.0 per g for the silver paste, $0.36 per g for the graphite ink, $0.07 per g of nickel, $0.3 for each amplifier, $0.1 for the resistor, and $0.5 per g of wax.

2.3. Future Direction. To more accurately and reliably characterize electroactive bacteria, our potentiostat sensing system should have two main functions: controlling the potential difference between the working and the reference electrodes and measuring the current flow from the counter to the working electrode. To simplify the circuitry on the limited space of the paper PCB, we used the simplest potentiostat topology for a grounded working electrode configuration that uses a transimpedance amplifier and needs only two operational amplifiers and one resistor. Although this circuit

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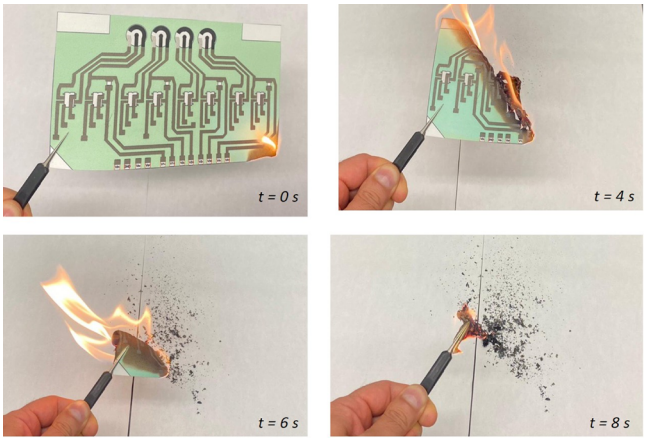

Figure 3. Disposal of the paper-based sensing system by incineration. The whole process took about 8 s.

topology is simple and low cost enough to be used for one-time use, it can be vulnerable to the environmental noise and interference at fast frequencies, have some possibility of instability and oscillation in the potential-control loop, and be limited by a single supply voltage operation.[32] Therefore, further improvement of the circuit topology will be required to provide more reliable electrochemical measurement functions while keeping the cost-effective feature for the disposable application by reducing the components and simplify the circuit at the same time.

3. CONCLUSIONS

In this work, a papertronic potentiostat, suitable for a single use in cost-effective electrochemical sensors, was constructed and characterized by monitoring electroactive bacteria. A three-electrode configuration consisting of working, counter, and reference electrodes was integrated into the papertronic system as a sensing unit, allowing a reliable study of the bacterial electrocatalysis in a controllable manner. The potentiostat was realized by using two operational amplifiers and one resistor, providing a reliable working potential with the respect to the reference electrode and measuring a current generation from the bacterial electrochemical activities. By using the papertronic potentiostat, chronoamperometry and cyclic staircase voltammetry were performed for three bacteria, *S. oneidensis* MR1, *P. aeruginosa* PAO1, and *B. subtilis*. Different current levels were harvested by applying different potentials while cyclic voltammograms were readily obtained without a bulky, heavy, and expensive benchtop potentiostat. After use, the paper-based system was completely incinerated without posing a risk of bacterial cytotoxic infection and contamination. Our portable papertronic potentiostat enabled a simple and rapid but reliable electroanalytical technique to effectively regulate the electroactive bacteria in a system.

4. EXPERIMENTAL SECTION

4.1. Materials, Chemicals, and Equipment. Ag/AgCl ink (NC1114936) and carbon/graphite ink (NC1176443) were purchased from Fisher Scientific Co., LLC. Conductive silver paste was purchased from Ted Pella, Inc. A nickel conductive spray was received from MG Chemicals. Clear scratch- and UV-resistant acrylic sheets (1/16 in.) were obtained from McMaster-Carr. Grade 3MM chromatography papers (20 × 20 cm) were purchased from VWR International, LLC. The plastic-based stencils and the paper substrates were micromachined by laser cutting (Universal Laser System, VLS 3.5). Other electronic components, including LM741 operational amplifiers (LM741CNNS/NOPB-ND) and 1 kΩ resistors (RCL12251K00JNEG) were purchased from Digi-Key Electronics. The LM741 is a DC-coupled high-gain electronic voltage amplifier having one internal operational amplifier. A Xerox ColorQube 8570 wax printer was used for creating hydrophobic and hydrophilic patterns on paper. An air oven (VWR Forced Air Oven) was used to melt the wax and control the boundaries of the wells.

4.2. Fabricating Three-Electrode Sensing Wells and Potentiostat Reader Circuit on Paper. Asymmetric wax printing was first performed on both sides of the paper using computer aided design software (AutoCAD) (Figure 1c). Then, heat treatment at 150° for 30 s enabled penetration of the wax through the paper, defining hydrophilic regions for 4 sensing wells (Figure 1d). Furthermore, the hydrophobically patterned wax regions effectively defined the flow path of the fluidic conductive inks to form conductive lines and through-holes. Through the well-micromachined acrylic stencils, nickel was sprayed on both sides of the patterned paper, filling the hydrophilic lines and through-holes. On the sensing well, the working and the counter electrodes were prepared by screen-printing the graphite ink through the well-micromachined paper stencil, followed by screen-printing the Ag/AgCl ink for the reference electrode. Even with the repeated mechanical bending by 90°, no noticeable change in the resistance of the three electrodes was seen (Figure S3). After the electronic components were mounted through the through-holes, the silver paste anchored them to the PCB and provided a conductive connection. Other through-holes were filled with the silver paste to connect the front and back sides of the PCB lines. The detailed PCB designs and dimensions are shown in Figure S4.

4.3. Circuit Configuration. Each three-electrode sensing unit was operated with two operational amplifiers and one resistor (Figure 1b).[29,33] The grounded working electrode was connected to the second amplifier (OP2), allowing a reliable potential application with respect to the reference electrode. The first amplifier (OP1) controlled the cell current I_{WE} so that the cell application potential V_{cell} with respect to the reference electrode was maintained at its desired present potential E_i. The current I_{WE} generated from bacterial electrocatalysis flowed from the counter electrode (CE) to the working electrode (WE), and the current can be monitored by measuring the voltage drop across the resistor (R).

4.4. Preparation of Bacterial Inoculum. To demonstrate the papertronic three-electrode sensing platform as a characterizing tool for bacterial electrochemical activity, two well-known Gram-negative exoelectrogens, *S. oneidensis* MR1 and *P. aeruginosa* PAO1, and one Gram-positive exoelectrogen, *B. subtilis*, were selected as test species. All bacterial samples were inoculated in Luria Broth (LB) media (1% w/v tryptone; 0.5% w/v yeast extract; and 0.5% w/v NaCl, pH 7.0) with a gentle shaking for 24 h at 35 °C. The bacteria concentration was observed by maintaining the optical density at 600 nm.

■ ASSOCIATED CONTENT

Supporting Information

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SEM images, photos of the experimental setup, sheet resistance of the PCB lines, and design of the papertronic sensing system (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Schröder, U. Discover the possibilities: microbial bioelectrochemical systems and the revival of a 100-year−old discovery. *J. Solid State Electrochem.* **2011**, *15*, 1481−1486.

(2) Gao, Y.; Mohammadifar, M.; Choi, S. From microbial fuel cells to Biobatteries: Moving toward on-demand micro-power generation for Small-scale Single-Use Applications. *Adv. Mater. Technol.* **2019**, *4*, 1970039.

(3) Lovley, D. R. Electromicrobiology. *Annu. Rev. Microbiol.* **2012**, *66*, 391−409.

(4) Borole, A. P.; Reguera, G.; Ringeisen, B.; Wang, Z.-W.; Feng, Y.; Kim, B. H. Electroactive biofilms: Current status and future research needs. *Energy Environ. Sci.* **2011**, *4*, 4813−4834.

(5) Slate, A. J.; Whitehead, K. A.; Brownson, D. A. C.; Banks, C. E. Microbial fuel cells: an overview of current technology. *Renewable Sustainable Energy Rev.* **2019**, *101*, 60−81.

(6) Koch, C.; Harnisch, F. Is there a specific ecological niche for electroactive microorganisms? *ChemElectroChem* **2016**, *3*, 1282−1295.

(7) Doyle, L. E.; Marsili, E. Weak electricigens: a new avenue for bioelectrochemical research. *Bioresour. Technol.* **2018**, *258*, 354−364.

(8) TerAvest, M. A.; Ajo-Franklin, C. M. Transforming exoelectrogens for biotechnology using synthetic biology. *Biotechnol. Bioeng.* **2016**, *113*, 687−697.

(9) Alfonta, L. Genetically Engineered Microbial Fuel Cells. *Electroanalysis* **2010**, *22*, 822−831.

(10) Zhou, S.; Wen, J.; Chen, J.; Lu, Q. Rapid measurement of microbial extracellular respiration ability using a high-throughput colorimetric assay. *Environ. Sci. Technol. Lett.* **2015**, *2*, 26−30.

(11) Wang, Q.; Jones, A.-A. D., III; Gralnick, J. A.; Lin, L.; Buie, C. R. Microfluidic dielectrophoresis illuminates the relationship between microbial cell envelope polarizability and electrochemical activity. *Sci. Adv.* **2019**, *5*, eaat5664.

(12) Biffinger, J.; Ribbens, M.; Ringeisen, B.; Pietron, J.; Finkel, S.; Nealson, K. Characterization of electrochemically active bacteria utilizing a high-throughput voltage-based screening assay. *Biotechnol. Bioeng.* **2009**, *102*, 436−444.

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

(14) Hou, H.; Li, L.; Cho, Y.; de Figueiredo, P.; Han, A. Microfabcricated microbial fuel cell arrays reveal electrochemically active microbes. *PLoS One* **2009**, *4*, e6570.

(15) Hou, H.; Li, L.; Ceylan, C. Ü.; Haynes, A.; Cope, J.; Wilkinson, H. H.; Erbay, C.; de Figueiredo, P.; Han, A. A Microfluidic Microbial Fuel Cell Array that Supports Long-Term Multiplexed Analyses of Electricigens. *Lab Chip* **2012**, *12*, 4151−4159.

(16) Qian, F.; Baum, M.; Gu, Q.; Morse, D. E. A 1.5 μL microbial fuel cell for on-chip bioelectricity generation. *Lab Chip* **2009**, *9*, 3076−3081.

(17) Mukherjee, S.; Su, S.; Panmanee, W.; Irvin, R. T.; Hassett, D. J.; Choi, S. A microliter-scale microbial fuel cell array for bacterial electrogenic screening. *Sens. Actuators, A* **2013**, *201*, 532−537.

(18) Fraiwan, A.; Choi, S. Bacteria-Powered Battery on Paper. *Phys. Chem. Chem. Phys.* **2014**, *16*, 26288−26293.

(19) Choi, G.; Hassett, D. J.; Choi, S. A paper-based microbial fuel cell array for rapid and high-throughput screening of electricity-producing bacteria. *Analyst* **2015**, *140*, 4277−4283.

(20) Gao, Y.; Hassett, D. J.; Choi, S. Rapid characterization of bacterial electrogenicity using a single-sheet paper-based electrofluidic array. *Front. Bioeng. Biotechnol.* **2017**, *5*, 44.

(21) Tahernia, M.; Mohammadifar, M.; Hassett, D. J.; Choi, S. A fully disposable 64-well papertronic sensing array for screening electroactive microorganisms. *Nano Energy* **2019**, *65*, 104026.

(22) Tahernia, M.; Mohammadifar, M.; Gao, Y.; Panmanee, W.; Hassett, D. J.; Choi, S. A 96-well high-throughput, rapid-screening platform of extracellular electron transfer in microbial fuel cells. *Biosens. Bioelectron.* **2020**, *162*, 112259.

(23) Tahernia, M.; Mohammadifar, M.; Hassett, D. J.; Choi, S. A portable papertronic sensing system for rapid, high-throughput, and visual screening of bacterial electrogenicity. *Biosens. Bioelectron.* **2020**, *165*, 112348.

(24) Fraiwan, A.; Dai, C.; Sidhu, N. K.; Rastogi, A.; Choi, S. A Micro-sized Microbial Fuel Cell with Electrochemical Sensing Functionality. *Proceeding of the IEEE NEMS Conference 2014*; Apr. 13−16, 2014, Hawaii, USA, pp. 635−638.

(25) Cheng, K. Y.; Ho, G.; Cord-Ruwisch, R. Affinity of Microbial fuel cell biofilm for the anodic potential. *Environ. Sci. Technol.* **2008**, *42*, 3828−3834.

(26) Wagner, R. C.; Call, D. F.; Logan, B. E. Optimal Set Anode Potentials Vary in Bioelectrochemical Systems. *Environ. Sci. Technol.* **2010**, *44*, 6036−6041.

(27) Busalmen, J. P.; Esteve-Nuñez, A.; Feliu, J. M. Whole Cell Electrochemistry of Electricity-Producing Microorganisms Evidence an Adaptation for Optimal Exocellular Electron Transport. *Environ. Sci. Technol.* **2008**, *42*, 2445−2450.

(28) Pankratova, G.; Hederstedt, L.; Gorton, L. Extracellular electron transfer features of Gram-positive bacteria. *Anal. Chim. Acta* **2019**, *1076*, 32−47.

(29) Wu, W.; Yang, F.; Liu, X.; Bai, L. Influence of substrate on electricity generation of *Shewanella Ioihica* PV-4 in microbial fuel cells. *Microb. Cell Fact.* **2014**, *13*, 69.

(30) Aelterman, P.; Freguia, S.; Keller, J.; Verstraete, W.; Rabaey, K. The anode potential regulates bacterial activity in microbial fuel cells. *Appl. Microbiol. Biotechnol.* **2008**, *78*, 409−418.
(31) Scholz, F. Voltammetric techniques of analysis: the essentials. *ChemTexts* **2015**, *1*, 17.
(32) Ahmadi, M. M.; Jullien, G. A. Current-Mirror-Based potentiostats for Three-electrode amperometric electrochemical sensors. *IEEE Trans. Circuits Syst.* **2009**, *56*, 1339−1348.
(33) Souza, A. K. P.; de Moraes Cruz, C. A.; Marques, G. C.; de Castro, L. S. O.; Bezerra, T. B. A Compact Current Conveyor CMOS Potentiostat Circuit for Electrochemical Sensors. *Proceeding of the 2019 4th International Symposium on Instrumentation Systems, Circuits and Transducers (INSCIT)*; *2019*, 19078120.

https://dx.doi.org/10.1021/acsomega.0c03299
ACS Omega 2020, 5, 24717−24723