A Fully-Papertronic Biosensing Array for High-Throughput Characterization of Microbial Electrogenicity

Mehdi Tahernia, *Student Member, IEEE,* Maedeh Mohammadifar, Daniel J. Hassett, and Seokheun Choi, *Member, IEEE*

***Abstract*— For the first time, we report a low-cost, disposable fully-papertronic screening platform for rapid screening and identification of electroactive microorganisms. This novel papertronic device is capable of simultaneous characterizing the electrogenicity of 10's of the newly discovered, genetically engineered, bacteria. This work explored an exciting range of possibilities with the goal of fusing microbial fuel cell technology with 'papertronics,' the emerging field of paper-based electronics. Spatially distinct 64 sensing units of the array were constructed by patterning hydrophilic anodic reservoirs in paper with hydrophobic wax boundaries and utilizing 3-D multi-laminate paper structures. Full integration of a high-performance microbial sensor on paper can be achieved by improving the microbial electron exchange with the electrodes in an engineered conductive paper reservoir and reducing cathodic overpotential by using a solid electron acceptor on paper. Furthermore, the intrinsic capillary force of the paper and the increased capacity from the engineered reservoir allowed for rapid adsorption of the bacterial sample and promote immediate microbial cell attachment to the electrode, leading to instant power generation with even a small amount of the liquid.**

I. Introduction

Paper-based electronics or papertronics have attracted significant attention as a simple, low-cost, sustainable, flexible and disposable device platform [1, 2]. Several electronic devices have been realized on paper, including thin-film transistors (TFTs), solar cells, touch pads, light-emitting diodes, and electronic circuit [3]. Liquid-phase conductive metals, carbon-based nanomaterials, and conductive polymers have been successfully embedded into paper for stable electric functions even after repeated mechanical deformations [4-6]. Also, several smoothing techniques for the rough paper surfaces have been proposed to increase the substrate printability [7, 8]. While paper has been considered as a promising substrate for flexible electronics, it has also been mainly used for microfluidic assays and diagnostics [9-15]. Paper microfluidics has revolutionized public healthcare, both in developed and developing countries, as well as become a new class of point-of-care (POC) diagnostic devices that are portable, rapid, low-cost, and easy to assemble [11, 12]. To date, patterning processes of paper microfluidics have been well established in a programmed manner, such as photolithography, wax printing, and laser micromachining [13, 14]. Microfluidic channels in paper can be fabricated two-dimensionally or even three-dimensionally to transport fluids in both horizontal and vertical dimensions [15]. Thus, the paper-based microfluidic platforms are widely used to culture cells containing their growth media for diagnostic and pharmaceutical studies [16-18]. Multiple paper layers can form a 3-D model of a tissue or a biofilm while mass transport of nutrients and gasses into this 3-D system can be quantified along with the exploration of cellular motility and viability. Furthermore, a spatially distinct biosensing array for high-throughput analysis can be readily constructed on paper by patterning hydrophilic reservoirs with hydrophobic wax boundaries [19, 20].

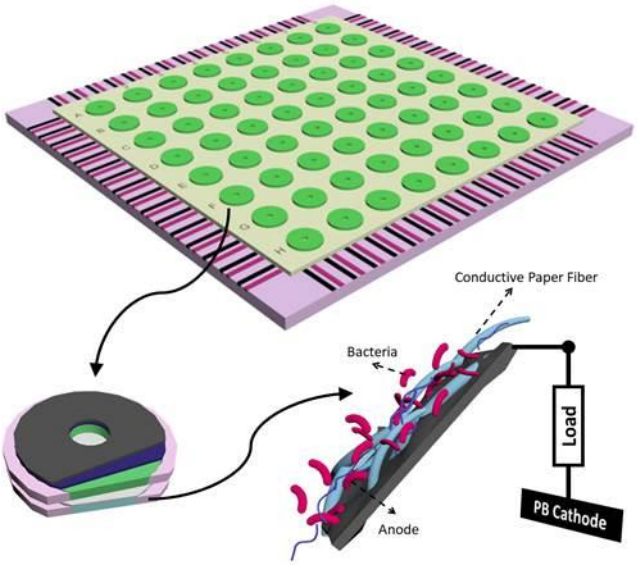

Figure 1. Schematic illustration of the fully papertronic biosensing array having 64 wells for high-throughput screening of bacterial electrogenicity

However, the full integration of both fluidic and electronic functions in paper has been hindered by difficulties in defining a novel electro-fluidic platform. Even the latest paper-based sensing array required plastic PCB boards [19] and suffered from low-performance for parallel measurements [20]. In this work, a complicated circuit with electronic and microfluidic components was mounted on a paper-based 3-D printed circuit boards (PCBs) to provide an exceptional high-throughput characterization of electrogenic bacteria with rapid power

*Research supported by NSF (ECCS #1503462, IOS #1543944, & ECCS #1703394) and the SUNY Binghamton Research Foundation (SE-TAE).

Prof. Choi is with the Department of Electrical & Computer Engineering at State University of New York (SUNY)-Binghamton, NY, 13902 USA. He is serving as an Associate Director for Center for Research in Advanced Sensing Technologies & Environmental Sustainability (corresponding author to provide phone: 607-777-5913; e-mail: sechoi@binghamton.edu).

M. Tahernia and M. Mohammadifar are with the Department of Electrical & Computer Engineering at State University of New York (SUNY)-Binghamton, NY, 13902 USA.

Prof. Hassett is with the Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, Cincinnati, OH, 45267 USA.

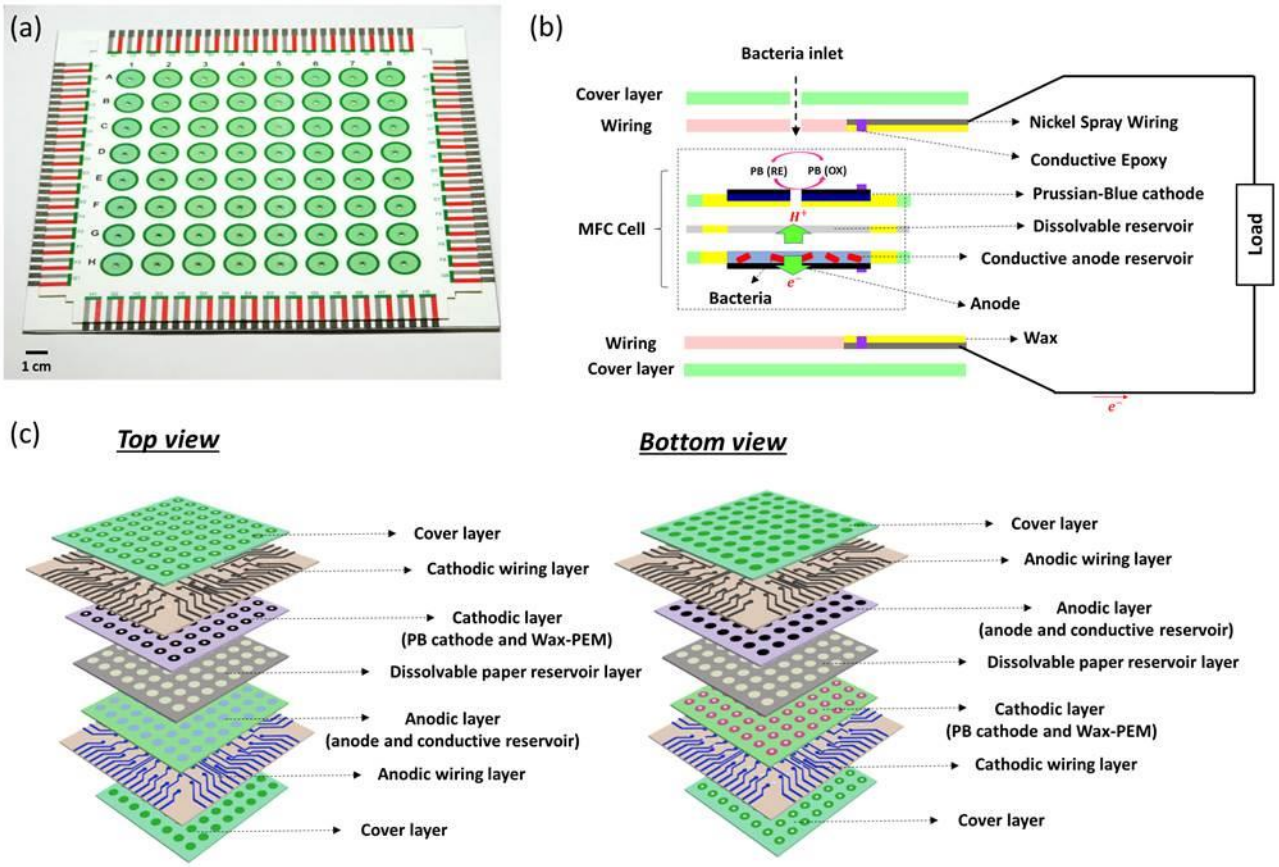

Figure 2. (a) Photo image of the 64-well biosensing array and schematic diagrams of a cross section (b), top (c) and bottom (d) view of the array showing the individual layers.

assessment. Microbial electron transfer capability, or "electrogenicity," creates a plethora of concepts and potential applications that offer environmentally sustainable advances in the fields of biofuels, wastewater treatment, bioremediation, desalination, and biosensing [21-26]. Despite its vast potential and remarkable research efforts, bacterial electrogenicity is arguably the most underdeveloped technology used to confront those challenges. Severe limitations are placed in the intrinsic energy and electron transfer processes of naturally occurring microorganisms. Considerable technology enhancement can be made with the new innovations in synthetic biology that regulates microbial electron transfer pathways and improves their capability for electricity generation [27]. What is needed is a high-throughput, rapid and highly sensitive test array for the electrogenicity of 10's of the newly discovered, genetically engineered, bacterial species. This is by no means a simple challenge, as the accurate and parallel quantitative measurements of bacterial electrogenicity require a long measurement time (~ 10's of days), continuous introduction of organic fuels (~10's of milliliters), complex device architectures, and labor-intensive operation.

This work created the ability to achieve rapid (30 min.), sensitive (10-fold improvement), and high-throughput (64 wells) characterization of bacterial electrogenicity from a single drop of culture (10's of microliters). This work used a fully integrated papertronic sensing array that inherently produced favorable conditions for easy, rapid, and sensitive controlling of a microbial liquid sample. The high-throughput array was batch-fabricated through all-printing processes of 3-D capillary-driven sensors and printed circuit boards on papers.

II. Materials and Methods

A. Device Fabrication

The sensing array consisted of seven functional paper layers: (i) cathodic cover layer, (ii) cathodic wiring layer, (iii) cathodic layer, (iv) dissolvable paper reservoir layer, (v) anodic layer, (vi) anodic wiring layer, and (vii) anodic cover layer. Two cover layers were used to seal the device. The cathodic/anodic wiring layers were connected to the cathodic/anodic layers by using silver epoxy. The anodic layer (v) was first fabricated on a filter paper by patterning hydrophilic regions with printed wax boundaries and then engineered to be conductive by pipetting a 40 µL mixture of 1wt% PEDOT:PSS (Sigma-Aldrich) and 5wt% dimethyl sulfoxide (Sigma-Aldrich) into each anodic well, followed by addition of 20µL of 2wt% 3-glycidoxypropy-trimethoxysilane (Sigma-Aldrich) [28-31]. Then, the anodic layer was attached to the wax-patterned dissolvable reservoir layer (iv) to improve the wicking force of the liquid. This polymer treatment with the dissolvable paper (iv) attachment patterned the non-conducting papers

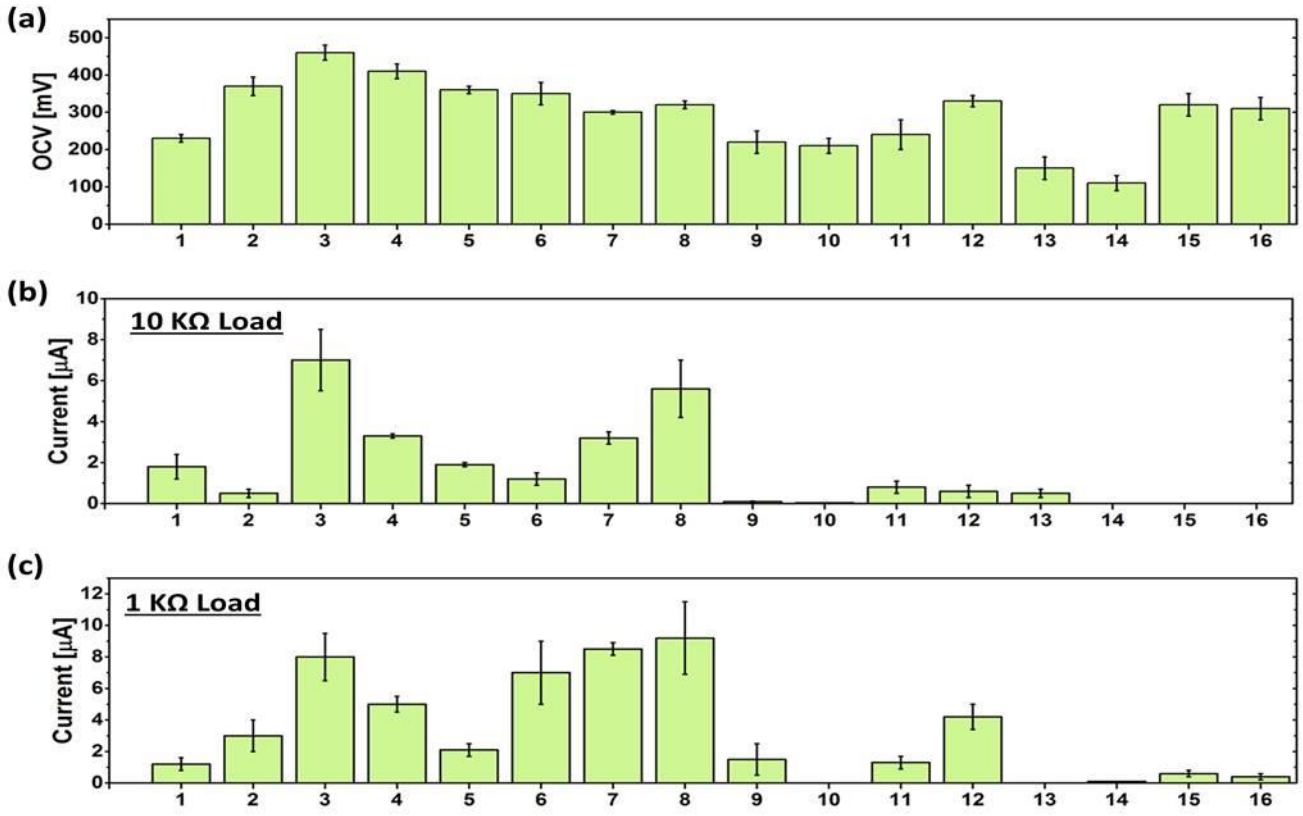

Figure 3. (a) Open circuit voltages (OCVs) and currents generated from each biosensing well through 10kΩ (b) and 1kΩ (c). Each sample was loaded onto four biosensing wells in the array generating error bars. (*wapR* (#1), *pslpel* (#2), *S. oneidensis MR-1* (#3), *PA0962::Gm* (#4), *pilY1* (#5), *PAO1* (#6), *moaA1::Gm* (#7), *pmpR* (#8), *lasIrhll* (#9), *norCB* (#10), *pmbA::Gm* (#11), *mvfR* (#12), *relA* (#13), *pilTnirS* (#14), *acnC::Gm* (#15) and media (#16))

with conducting materials while enabling each anodic well to have open pores and hydrophilic features for bacteria-containing liquid sample introduction.

B. Prussian-Blue/Graphene-based solid-state cathode

8mg of Prussian Blue (PB) nanoparticles and 3 mg of graphene in a mixture of 30 μL of PEDOT:PSS and 100 μL of isopropyl alcohol were prepared. Then, 5 μL of 5 wt% Nafion was added and sonicated for 30 min. The mixture was screen printed on the cathodic paper layer predefined by printed wax boundaries. Graphite ink (ERCON, E3449) was then printed on the cathodic layer as a current collector. The PB-based cathode allowed high energy recovery efficiencies and stable/reliable performances because of its intrinsically-formed open-framework chemical structure with wide channels. Furthermore, the PB-based solid-state cathode was more applicable to paper-based technologies because of its simple fabrication process and low-cost material.

C. Selected hypothesis-driven genes in P. aeruginosa

Our previous work indicated how strategic genetic modifications affect the electrochemical activity of bacteria [19, 20]. In this task, the working hypothesis and innovation is using the selected hypothesis-driven genes in *P. aeruginosa* as model exoelectrogens to form the customized synthetic microbial community described above. The *P. aeruginosa* possess genes for aerobic and anaerobic respiration, arginine and pyruvate fermentation, quorum sensing and a myriad of other cellular processes. Adaptation to micro-aerobic or -anaerobic environments is, therefore, a routine yet essential trait *P. aeruginosa* uses to occupy certain niches in the environment, especially when encased in surface-attached communities known as biofilms. This bacterium is capable of releasing electrons through polar surface appendages known as pili. The pili act as conductive nanowires, shuttling electrons within the bacteria as a result of metabolizing feedstock that include raw sewage, fertilizer run-off and animal waste. The bacteria with some genetic manipulation digest other organic products, including cellulose-degradation products like cellobiose from plants. The rationale for using *P. aeruginosa* is, therefore, that it is clearly one of the world's most metabolically versatile organisms, able to use well over 300 different carbon sources for growth. In this work, fourteen *P. aeruginosa* mutant strains and *S. oneidensis MR-1* were used: *wapR* (#1), *pslpel* (#2), *S. oneidensis MR-1* (#3), *PA0962::Gm* (#4), *pilY1* (#5), *PAO1* (#6), *moaA1::Gm* (#7), *pmpR* (#8), *lasIrhll* (#9), *norCB* (#10), *pmbA::Gm* (#11), *mvfR* (#12), *relA* (#13), *pilTnirS* (#14), *acnC::Gm* (#15). Each sample was loaded onto four biosensing wells in the array generating error bars. All microorganisms were cultivated in L-broth (LB) medium for 24 hours. Before testing, the cell titers were controlled by monitoring the optical density at 600 nm after centrifugation and suspension in fresh LB-broth medium. LB medium (#16) was used as control. The potentials between the anodes and the cathodes were measured with a data acquisition system (National instrument, USB-6212), and recorded every 30s via a customized LabView interface. The Open circuit voltage (OCV) was measured for the first five minutes. Then, 10kΩ and 1kΩ resistors were subsequently connected between the anodes and

cathodes to measure the current flow through the resistor by Ohm's law (I = V/R).

III. Results and Discussion

The fully-papertronic biosensing array exploited the dissolvable paper's capability to rapidly wick fluid and attract bacteria to the anode, resulting in rapid current generation upon the loading of the 10s' of micro-liters of bacteria-containing liquid sample. Within 30 min, we successfully determined the bacterial electrogenicity of fifteen bacterial species. We first compared the OCVs generated from each bacterial strain in the array. The OCVs varied significantly between the sensors, obviously indicating that each bacterial strain showed the different thermodynamic reactions (Figure 3a).

Figure 3b and 3c show the current generation through 10kΩ and 1kΩ resistor, respectively. The current produced from the media (#16) clearly showed differences from the other bacterial samples, demonstrating that the electrical current was generated by microbial metabolism. With both 10kΩ and 1kΩ resistors, *S. oneidensis MR-1*(#3) and *P. aeruginosa* pmpR (#8) generated the highest current outputs, followed by *PAO1* (#6), and *moaA1::Gm* (#7). Other bacterial mutants including *lasIrhll* (#9), *norCB* (#10), *relA* (#13), *pilTnirS* (#14), *acnC::Gm* (#15), generated very low or no current.

IV. Conclusion

We created a fully-papertronic microbial sensor array by integrating paper-based anodes, cathodes, PCBs, and reservoirs and measured bioelectricity generation of electrogenic bacteria. Our innovative biosensing platform achieved a large parallel electrogenic analysis of fifteen of bacterial species with simple, sensitive, and rapid quantification. Furthermore, this new sensing array provided in-depth understanding of extracellular electron transfer pathways that are relevant to their genetic engineering. This work will enable the development of new types of paper-based biosensing assays in simple, convenient, and easy-to-use packages.

References

[1] S. Thiemann, S.J. Sachnov, F. Pettersson, R. Boolstrom, R. Osterbacka, P. Wasserscheid, J. Zaumseil, "Cellulose-based lonogels for paper electronics," Advanced Functional Materials, 24, pp. 625-634, 2014.

[2] J. Ahn, J. Seo, T. Lee, D. Kwon, I. Park, T. Kim, J. Lee, "Extremely robust and patternable electrodes for copy-paper-based electronics," ACS Appl. Mater. Interfaces, 8, pp. 19031-19037, 2016.

[3] D. Tobjork and R. Osterbacka, "Paper electronics," Advanced Materials, 23, pp. 1935-1961, 2011.

[4] A. Asadpoordarvish, A. Sandstrom, C. Larsen, R. Bollstrom, M. Toivakka, R. Osterbacka, L. Edman, "Light-emitting paper," Advanced Functional Materials, 25, pp. 3238-3245, 2015.

[5] Y.L. Han, H. Liu, C. Ouyang, T.J. Lu, F. Xu, "Liquid on paper: rapid prototyping of soft functional components for paper electronics," Scientific Reports, 5, 11488, 2015.

[6] W.J. Hyun, O.O. Park, B.D. Chin, "Foldable graphene electronic circuits based on paper substrates," Advanced Materials, 25, pp. 4729-4734, 2013.

[7] M. Santhiago, J. Bettini, S.R. Araujo, C.C.B. Bufon, "Three-dimensional organic conductive networks embedded in paper for flexible and foldable devices," ACS Appl. Mater. Interfaces, 8, pp. 10661-10664, 2016.

[8] H. Zhu, Z. Fang, C. Preston, Y. Li, L. Hu, "Transparent paper: fabrications, properties, and device applications," Energy & Environmental Science, 7, 269-287, 2014.

[9] A.K. Yetisen, M.S. Akram, C.R. Lowe, "Paper-based microfluidic point-of-care diagnostic devices," Lab Chip, 13, pp. 2210-2251, 2013.

[10] D.M. Cate, J.A. Adkins, J. Mettakoonpitak, C.S. Henry, "Recent developments in paper-based microfluidic devices," Analytical Chemistry, 87, pp. 19-41, 2015.

[11] A.W. Martinez, S.T. Phillips, G.M. Whitesides, "Diagnostics for the developing world: microfluidic paper-based analytical devices," Analytical Chemistry, 82, pp. 3-10, 2010.

[12] S. Choi, "Powering point-of-care diagnostic devices," Biotechnology Advances, 34, pp. 321-330, 2016.

[13] X. Li, D.R. Ballerini, W. Shen, "A perspective on paper-based microfluidics: current status and future trends," Biomicrofluidics, 6, 011301, 2012.

[14] X. Jiang, Z.H. Fan, "Fabrication and operation of paper-based analytical devices," Annual Review of Analytical Chemistry, 9, pp. 203-222, 2016.

[15] A.W. Martinez, S.T. Phillips, G.M. Whitesides, "Three-dimensional microfluidic devices fabricated in layered paper and tape," PNAS, 105, pp. 19606-19611, 2008.

[16] B. Mosadegh, B.E. Dabiri, M.R. Lockett, R. Derda, P. Campbell, K.K. Parker, G.M. Whitesides, "Three-dimensional paper-based model for cardiac ischemia," Advanced Health Materials, 3, pp. 1036-1043, 2014.

[17] D. Lantigua, Y.N. Kelly, B. Unal, G. Camci-Unal, "Engineered paper-based cell culture platforms," Advanced Health Materials, 6, 1700619, 2017.

[18] G. Choi, and S. Choi, "Monitoring electron and proton diffusion flux through three-dimensional, paper-based, variable biofilm and liquid media layers," Analyst, 140, pp. 5901-5907, 2015.

[19] G. Choi, D. Hassett, and S. Choi, "A paper-based microbial fuel cell array for rapid and high-throughput screening of electricity-producing bacteria," Analyst, 140, pp. 4277-4283, 2015.

[20] Y. Gao, D. Hassett and S. Choi, "Rapid characterization of bacterial electrogenicity using a single-sheet paper-based electrofluidic array," Frontiers in Bioengineering and Biotechnology, 5, 44, 2017.

[21] D.R. Lovley, "Electromicrobiology," Annu. Rev. Microbiol. Vol. 66, pp.391-409, 2012.

[22] B.E. Rittmann, "Opportunities for Renewable Bioenergy Using Microorganisms," Biotechnology and Bioengineering, Vol. 100, pp. 203-212, 2008.

[23] H. Wang, H. Luo, P.H. Fallgren, S. Jin, Z.J. Ren, "Bioelectrochemical system platform for sustainable environmental remediation and energy generation," Biotechnology Advances, Vol. 33, pp.317-334, 2015.

[24] B.E. Logan, "Exoelectrogenic bacteria that power microbial fuel cells," Nat. Rev. Microbiol., Vol. 7, pp.375-381, 2009.

[25] J. Babauta, R. Renslow, Z. Lewandowski, H. Beyenal, "Electrochemically active biofilms: facts and fiction," A review, Biofouling: The journal of Bioadhesion and Biofilm Research, Vol. 28, pp.789-812, 2013.

[26] B.E. Logan, "Scaling up microbial fuel cells and other bioelectrochemical systems," Appl. Microbiol. Biotechnol., Vol. 85, pp.1665-1671, 2010.

[27] M.A. TerAvest, C.M. Ajo-Franklin, "Transforming exoelectrogens for biotechnology using synthetic biology," Biotechnology and Bioengineering, Vol. 113, pp. 687-697, 2016.

[28] M.M. Hamedi, A. Ainla, F. Guder, D.C. Christodouleas, M. Fernandez-Abedul, G.M. Whitesides, "Integrating Electronics and Microfluidics on Paper", Adv. Mater., Vol. 28, pp. 5054-5063, 2016.

[29] M.M. Hamedi, V.E. Campbell, P. Rothemund, F. Guder, D.C. Christodouleas, J. Bloch, G.M. Whitesides, "Electrically Activated Paper Actuators", Advanced Functional Materials, Vol. 26, pp. 2446-2453, 2016.

[30] Y. Gao and S. Choi, "Stepping Towards Self-powered Papertronics: Integrating Biobatteries into a Single Sheet of Paper," Advanced Materials Technologies, 2, 1600194, 2017.

[31] M. Mohammadifar and S. Choi, "A Papertronics, On-demand and Disposable Biobattery: Saliva-activated Electricity Generation from Lyophilized Exoelectrogens pre-inoculated on Paper," Advanced Materials Technologies, 2, 1700127, 2017.