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ARTICLE TYPE

Instant power generation from an air-breathing paper and pencil based bacterial bio-fuel cell

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We present a low cost, disposable microbial fuel cell fabricated on a paper based platform, having a start-up time in the tune of 10 s. The platform deploys ordinary pencil strokes for graphite electrode deposition. The device uses a membrane-less design in a one-time injection (OTI) mode or a continuous capillary driven flow mode (CPF), where oxygen from the atmosphere is used up at the cathode for water formation, leading to the generation of bioelectricity. The performance of the fuel cell is evaluated using two bacterial strains, namely, *Pseudomonas aeruginosa* IIT BT SS1 and *Shewanella putrefaciens*. These flexible devices are shown to sustain bacteria for a period of at least one hour, resulting in the generation of almost 0.4 V using *P. aeruginosa* and a maximum current of 18 µA using *S. putrefaciens* without the use of any additional catalysts.

Microbial fuel cells (MFCs) harness clean and renewable bioelectricity from almost any biodegradable matter such as wastewater or matter from soil sediments, by making use of microbes as biocatalysts.[1] Although development of micro-scale portable MFCs has already been reported by researchers, the requirement of syringe pumps or other flow distribution systems in many of the concerned techniques makes it impractical to be used in remote locations to drive devices. Paper platform, on the other hand, eliminates the need for any external pumping devices by allowing fluid transport through capillary action.[2] Further, the paper based platforms are bio-compatible and retain liquids for a long period of time in their active forms.[3] Paper-based fuel-cells can be employed in implantable bioelectronics devices, wearable electronics and can also be integrated with sensors having low power requirements. [4, 5]

Biofuel cells can be widely categorized into two types namely, enzymatic biofuel cells (EBC) and microbial fuel cells (MFC). Various paper based EBC have been developed involving ionic-liquid functionalized nanotubes, employing cathodic and anodic enzymatic biocatalysts, as well as porous carbon electrode based EBC.[6-8] A common drawback of the paper based EBC happens to be limited half-lives of the enzymes, temperature dependency and the higher costs involved in purifying enzymes. These limitations motivate the use of microbes for catalysis in paper based platforms, so as to generate power for longer periods, by utilizing inherent enzymes which are more resistant to temperature fluctuations.[10]

Fraiwan et al. developed a paper based MFC for the first time using *Shewanella oneidensis* and ferricyanide as catholyte.[11] Later, they also developed a paper based battery using various low cost membranes.[12] Their device is a double chambered MFC in which catholyte chamber containing ferricyanide solution (which is the most widely used catholyte in MFC since it produces high power density[13]) gets reduced. However, following their design considerations, it needs to be noted that when an electron acceptor is being used, constant re-filling of the catholyte is needed in order to ensure a constant power supply. Moreover, the use of this chemical may induce a perceptible level of toxicity.[14] Besides, too much dependence on requirement of such chemicals may potentially limit the application of paper based devices in terms of their portability.

In this communication, we, for the first time, report the development of an air-breathing paper biofuel cell which generates power using a paper based platform. Instead of employing ferricyanide solution, we deploy a paper-based air-cathode in place of a two chambered device, thereby eliminating the need for any additional laboratory chemicals. Use of oxygen as catholyte is advantageous over others as it generates water as sole end product and has a high oxidation potential.[15] Integrated with the paper platform, we employ pencil graphite-based electrodes that have already shown tremendous utility in a wide gamut of applications.[16] In fact, paper based pencil-stroked graphite electrodes have been successfully deployed for electro-osmosis and other functionalities.[17] Analogous considerations, in the present scenario, enable us to produce bio-compatible electrodes with minimal resources, besides significantly reducing the cost. The amount of graphite may be varied through the number of pencil strokes. Our studies have further revealed that the bacterial adhesion is greatly enhanced due to the graphite on paper electrodes.

Typically, a MFC consists of an anode, cathode, and a proton exchange membrane. The exoelectrogens present in the anodic chamber consume the organic matter and donate electrons to the anode as a part of their respiration, which results in the generation of ATP and thus aids the survival of the bacteria. The protons generated in this process are carried to the cathode through the separator and electrons flow through the external circuit. At the cathode, reduction of the catholyte (oxygen in this case) takes place, resulting in the production of electricity.

Anode: $\text{Acetate} + 4H_2O \xrightarrow{P.\ aeruginosa} 2HCO_3^- + 8e^- + 9H^+$

$\text{Lactate} + 2H_2O \xrightarrow{S.\ putrefaciens} \text{Acetate} + HCO_3^- + 4e^- + 5H^+$

Cathode: $O_2 + 4\ H^+ + 4\ e^- \longrightarrow 2\ H_2O$

It is well known that some specialized microorganisms called exoelectrogens have the ability to donate electrons externally via three mechanisms: (a) Production of own soluble electron shuttling molecules which carry electrons from the surface to the electrode, (b) Cytochrome C formation on cell membranes to mediate electron transport upon direct contact with electrodes and (c) Conduction of biofilms through the pili.[18] In the present study, *Pseudomonas aeruginosa* IIT BT SS1 undergoes the dominant mode of electron transfer through the electron shuttler pyocyanin. The addition of external mediator indicates a higher current generation. In *Shewanella putrefaciens*, an electron transfer through direct contact with the electrode is observed. The mechanisms for the electron transport are discussed in supplementary information S2.

Our device contains layers of Whatman no. 1 filter papers assembled in the form of a stack, which would form various compartments of the device as shown in Fig. 1.

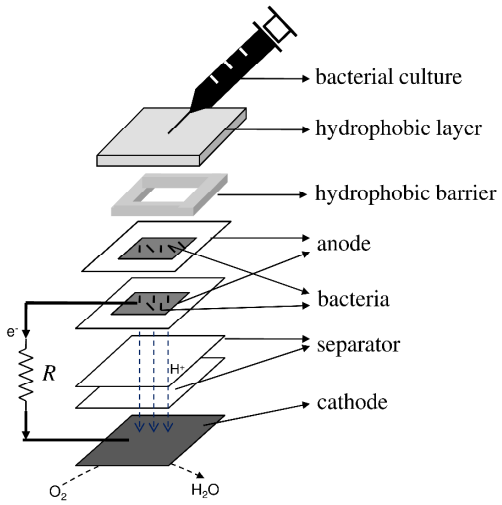

Fig. 1 Schematic of the paper based bacterial fuel cell

A hydrophobic layer of wax-paper is used in order to prevent the air penetration into the anodic chamber. This is followed by the anodic chamber containing graphite deposited from a 6B pencil (Faber-Castell), stroked 2 times over an area of 2 cm×2 cm. One more layer of pencil stroked graphite underneath the first layer also serves as anode. It is connected to a stainless steel wire using conductive graphite paste (Siltech Corporation Inc., Bangalore, India). . The pencil stroking deposits graphite on the filter paper and the amount of the graphite deposited is proportional to the number of strokes (XRD analysis of the electrodes and the SEM study are included in the supplementary information. Refer to Figures S3 and S4). A two layered anode is selectively adopted in the device in order to retain more bacteria in the anode. Two more layers consisting of untreated Whatman no. 1 filter paper, underlying this, serve as separators between the anode and the cathode. The last layer containing graphite (of about 4 cm×4 cm), also deposited from the pencil (10 strokes), acts as cathode, and is exposed to air.

Whatman no. 1 Filter paper is chosen as separator since it is inexpensive, porous, hydrophilic, and it can hold the fluid for longer periods of time, allowing a good exchange of the electrolyte between electrodes. Two layers of separators are used in order to prevent the bacteria from reaching the cathode. Further explanation and SEM analysis of cathode is included in S5. Although some back diffusion of oxygen to anode can still occur in spite of the multiple layered structure, performance can be enhanced if this can be totally mitigated. 1 mL insulin syringe is used to inject 400 µL anolyte containing bacteria per batch. The injected fluid passes though the anode and separator and finally to the cathode, which takes around 10 s. All voltage readings are recorded using Data Acquisition system (NI Instruments, Texas) in 1 min interval. For measuring the current flow across the circuit, a 10 kΩ resistance is used as load. Two facultative anaerobes, namely *Shewanella putrefaciens* (ATCC BAA1097™) and *Pseudomonas aeruginosa* IIT BT SS1 (lab isolate), are chosen as biocatalysts in the present study. Both the cultures are grown aerobically in minimal media for 36 hours at 37°C prior to inoculation. *P. aeruginosa* is grown in media with acetate as carbon source, which has the following composition: 0.8 g Sodium acetate, 2.4 g K_2HPO_4, 1.4 g KH_2PO_4, 1 g NH_4Cl, 0.15 g $CaCl_2$, 25 mg $MgSO_4.7H_2O$, 2.9 g NaCl, 2 g yeast extract. For growth of *S. putrefaciens*, sodium acetate in the media is replaced by 3 mL sodium lactate solution (60 %v/v). The pH of the media is adjusted to 7 before autoclaving. All experiments are carried out at room temperature. Pyocyanin (Sigma-Aldrich, India) is used to study the effect of mediator in power generation.

The device shown in Fig. 1 is operated in two modes – One Time Injection (OTI) and Continuous flow mode (CPF). (The actual images of the device operating in both the modes are shown in the supplementary document S1. In the OTI mode, 400 µL bacterial culture is injected once and the device characteristics are monitored. On the other hand, in the CPF mode, the paper part of the anode is extended onto a petri-dish reservoir containing media. Due to capillarity and a continuous evaporation, a passive pumping is set up. This allows the device to run autonomously.

In the OTI mode, a maximum OCV of 530 mV is observed in the case of *P. aeruginosa* without the use of any membrane to separate the cathode and anode. This kind of unprecedented power generation at such a rapid rate is unreported so far. The decaying nature of the potential and current is attributed to the evaporation through the sides of the device.

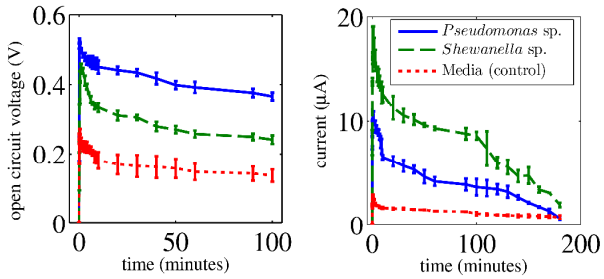

Fig. 4 (a) Temporal variation of the open circuit voltage and (b) current for the paper MFC using both *P. aeruginosa*., *S. putrefaciens*. and control in an OTI mode.

Although the open circuit voltage (OCV) values are lower for *S. putrefaciens*, the values of current production are almost double for this strain. This might be due to very less internal resistance owing to the increased adhesion of the bacteria as highlighted earlier. The devices with both the strains start voltage

generation within 10 s of inoculation. While the start-up times of conventional MFC last for few days, those of microfluidic MFCs last from few hours to minutes. The start-up time of the system discussed here is found to be very less, that is, in the order of seconds. The mediator might be responsible for carrying the electrons from the closely placed bacteria to the anode, or there is a quick adhesion of the bacteria to the surface of the anode. Since SEM images of the bacteria *P. aeruginosa* show fewer bacteria adhered to the electrode surface, the presence of the exogenous phenazine compounds in the supernatant might be responsible for shuttling the electrons to graphite. In the case of *S. putrefaciens*, however, there is a prominent adhesion to the graphite flakes, indicating that a direct contact with the electrode is facilitating the electron transfer. (Refer to supplementary information S4). A higher current generation using *S. putrefaciens* strain also supports this fact. There is also a predominant bacterial population attached onto the cellulosic fibers of the paper and along the pores of the paper matrix.

The current values for both the organisms drop to zero in a period of about one and a half hour owing to evaporation. The diffusion of the media through the paper stack and the simultaneous flow of electrons through the external circuit are responsible for this current generation. Hence, current generation is most likely to be diffusion controlled. This suggests that the hydrophilic paper matrix can effectively hold the media for a period of at least 1 h thereby eliminating the need for constant feeding, indicating longer periods of retaining capability. The influence of area of cathode and anode on the device characteristics are also depicted in the supplementary information S7). Also, the sharp fall in the voltage and current values after a few minutes indicates that there is no biofilm growth. Decrease in the current production after 1 h might be due to the reduction of metabolically active bacteria in the system. The evaporation of the water molecules through the cathode surface as well as sides might result in accumulation of salts in the system which poses an osmotic stress on the bacteria, resulting in the decrease in their activity. Thus, the paper matrix acts as a versatile platform being able to retain large volumes of bacteria in their active forms. The graphite electrodes are such that the graphite flakes are deeply integrated with the paper matrix. The ability of paper to hold media for sufficient time along with the good electrode-paper matrix integration allows enhanced contact between the bacteria (in its active form) and the electrode.(Refer to S4)

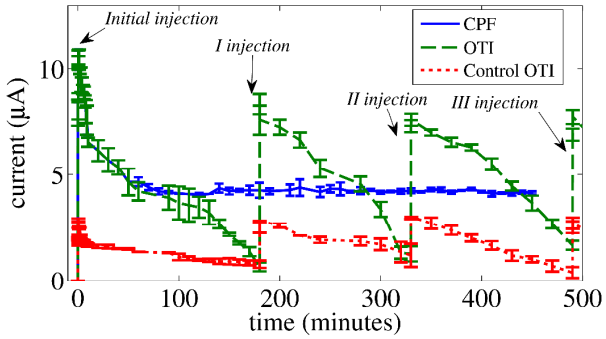

Fig. 5 Demonstration of the two operating modes of the device under consideration. OTI stands for one time injection (with intermittent refill injections highlighted by the peaks occurring in Fig. 5) and CPF stands for Capillary flow mode. Both these modes were tried using *P. aeruginosa*. Re-filling was done as soon as the current values dropped to access the re-usability of the device. 1 X PBS was used in the CPF mode.

In order to maintain a sustained power output, two modes of re-filling the device are adopted. The first one is to run the device by intermittent feeding where media are fed into the system using a syringe, and in the second approach 1X PBS (Phosphate buffered saline, pH 7.2) is placed in petri dish. This acts as reservoir from where the device can itself take in buffer, by means of capillary action. The paper based matrix allows for evaporation through the sides to the ambient, thereby driving a net flow of media from the interior to the exterior. A maximum current production of about 8 μA is obtained after the first injection done at 180 min, while the value drops to 6 μA after second injection. This shows that the device may be effectively refilled and used.

For the fabrication of the capillary flow device, a small strip of Whatman no. 1 paper, having dimensions of 2 mm ×5 cm, is attached to the anode. The other end of this strip is dipped into a reservoir containing PBS through which constant uptake of the buffer takes place through capillary action. This simple design enables the uptake of any biodegradable organic wastes directly into the anodic chamber of the device without the use of any external pump, and can result in the production of bioelectricity depending on the nature of the organic wastes. In this mode of re-fill, interestingly, a stable current generation profile is observed as long as the buffer is supplied. The V-I characteristics as well as P-I characteristics of the device depicted in Fig. 6 show that the strain *S. putrefaciens* is able to sustain a larger power at higher currents as compared to *P. aeruginosa*, as attributed to the respective mechanisms of electron transfer discussed previously.

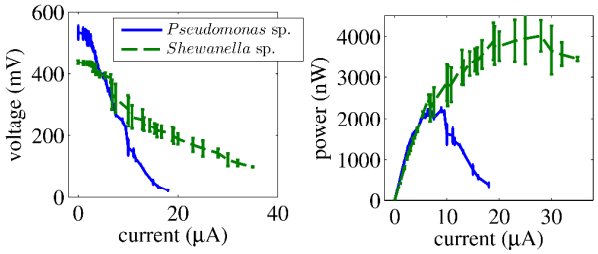

Fig. 6 V-I and P-I characteristics of the device

It can be inferred that a maximum power of about 3.5 μW can be generated from the device using *S. putrefaciens* strain using just pencil and paper in an OTI mode. This makes the device suitable for integration with various applications in locations with limited resources. This simple design eliminates the need for any sophisticated instruments for fabrication. Further, the inexpensive electrode deposition method and the sustained power supply from a tiny volume of bacterial culture makes the device highly attractive. Interestingly, the paper matrix itself may effectively hold considerable amounts of the bacteria and sustain the device in its active form, making the same an ideal platform for next generation power devices.

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Notes and references

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1 D. R. Lovley, "Bug Juice: Harvesting Electricity with Microorganisms.," *Nature Reviews. Microbiology*, 2006, **4**(7), 497–508.
2 (a). D. M. Cate, J. A. Adkins, J. Mettakoonpitak, and C. S. Henry, "Recent Developments in Paper-Based Microfluidic Devices.,"Anal. Chem., 2015, 87 (1) , 19–41; (b). A. K. Ellerbee, S. T. Phillips, A. C. Siegel, K. A. Mirica, A. W. Martinez, P Striehl, N Jain, M Prentiss and George M. Whitesides, "Quantifying Colorimetric Assays in Paper-Based Microfluidic Devices by Measuring the Transmission of Light through Paper," Anal. Chem. 2009, 81, 8447–8452; (c). B. Li, L Fu, W Zhang, W Feng, L Chen, "Portable paper-based device for quantitative colorimetric assays relying on light reflectance principle," Electrophoresis, 2014, 35, 1152–1159; (d). B Li, W Zhang, L Chen and B Lin, "A fast and low-cost spray method for prototyping and depositing surface-enhanced Raman scattering arrays on microfluidic paper based device," Electrophoresis, 2013, 34 (15), 2162-2168; (e). A. K. Yetisen, M. S. Akram and C. R. Lowe, "Paper-based microfluidic point-of-care diagnostic devices," Lab Chip, 2013, 13, 2210-2251.
3 X. Li, D. R. Ballerini, and W. Shen, "A Perspective on Paper-Based Microfluidics: Current Status and Future Trends.," *Biomicrofluidics*, 2012, **6**(1), 11301–113.
4 Y. Wang, L. Ge, C. Ma, Q. Kong, M. Yan, S. Ge and J. Yu, "Self-Powered and Sensitive DNA Detection in a Three-Dimensional Origami-Based Biofuel Cell Based on a Porous Pt-Paper Cathode.," *Chemistry*, 2014, **20** (39), 12453–62.
5 S. Berchmans, A. J. Bandodkar, W. Jia, J. Ramirez, Y. S. Meng, and J. Wang, "An epidermal alkaline rechargeable Ag-Zn printable tattoo battery for wearable electronics", *J. Mater. Chem. A*, 2014, **2**(38), 15788-95.
6 L. Zhang, M. Zhou, D. Wen, L. Bai, B. Lou and S. Dong, "Small-Size Biofuel Cell on Paper.," *Biosens. Bioelectron.*, 2012, **35**(1), 155–59.
7 X. E. Wu, Y. Z. Guo, M. Y. Chen, X. D. Chen, "Fabrication of Flexible and Disposable Enzymatic Biofuel Cells", *Electrochim. Acta*, 2013, **98**, 20–24.
8 I. Shitanda, S. Kato, Y. Hoshi, M. Itagakia and S. Tsujimura, "Flexible and High-Performance Paper-Based Biofuel Cells Using Printed Porous Carbon Electrodes.," *Chem. Commun.,* 2013,**49**, 11110-12.
9 S. C. Bartona and P. Atanassovb, "Enzymatic Biofuel Cells For Implantable And Micro-Scale Devices," *Prepr. Pap.-Am. Chem. Soc.*, *Div. Fuel Chem*. 2004, **49**(2), 476
10 T. H. Nguyen, A. Fraiwan, S. Choi, "Paper-based batteries: A review", *Biosens. Bioelectron.*, 2014, **54**, 640–49.
11 A. Fraiwan, S. Mukherjee, S. Sundermier, H.-S. Lee and S. Choi, "A Paper-Based Microbial Fuel Cell: Instant Battery for Disposable Diagnostic Devices.," *Biosens. Bioelectron.*, 2013, **49**, 410–14.
12 A. Fraiwan and S. Choi, "Bacteria-Powered Battery on Paper.," *Phys. Chem. Chem. Phys.*, 2014, **16**(47), 26288–93.
13 J. E. Mink, R. M. Qaisi, B. E. Logan and M. M. Hussain, "Energy Harvesting from Organic Liquids in Micro-Sized Microbial Fuel Cells," *NPG Asia Materials*, 2014, **6**(3), e89.
14 A. E. Franks and K. P. Nevin, "Microbial Fuel Cells, A Current Review," *Energies* 3, 2010, **5**(28), 899–919.
15 R. K. Arun, S. Halder, N. Chanda and S. Chakraborty, "A Paper Based Self-Pumping and Self-Breathing Fuel Cell Using Pencil Stroked Graphite Electrodes.," *Lab Chip*, 2014, **14**(10), 1661–64.
16 N. Kurra and G. U. Kulkarni, "Pencil-on-Paper: Electronic Devices.," *Lab Chip*, 2013, **13** (15), 2866–73.
17 P. Mandal, R. Dey and S. Chakraborty, "Electrokinetics with "paper-and-pencil" devices"", *Lab Chip*, 2012,**12**, 4026-4028
18 D. R. Lovley, "Electromicrobiology.," *Annual Review of Microbiology*, 2012, **66**, 391–409.