INVITED ARTICLE

TECHNOLOGY

A paper-based microbial fuel cell operating under continuous flow condition

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Microbial fuel cells have gained popularity as a viable, environmentally friendly alternative for the production of energy. However, the challenges in miniaturizing the system for application in smaller devices as well as the short duration of operation have limited the application of these devices. Here, the capillary motion was employed to design a self-pumped paper-based microbial fuel cell operating under continuous flow condition. A proof-of-concept experiment ran approximately 5 days with no outside power or human interference required for the duration of operation. *Shewanella oneidensis* MR-1 was used to create a maximum current of 52.25 μA in a 52.5 μL paper-based microfluidic device. SEM images of the anode following the experiment showed biofilm formation on the carbon cloth electrode. The results showed a power density of approximately 25 W/m^3 and proved unique capabilities of the paper-based microbial fuel cells to produce energy for an extended period of time.

***Keywords*: Paper-Based Microfluidic Devices; Microbial Fuel Cell; Continuous Flow Condition; Capillary.**

INNOVATION

In this work, the design of a proof-of-concept paper-based microbial fuel cell is reported. The device runs for 5 days and shows the production of current as a result of biofilm formation on anode. The capillary action is used to guide the liquids through the microbial fuel cell system and to eliminate the need for external power. By making a more environmentally friendly and compact paper-based design, the available applications of microbial fuel cells is ever expanding.

INTRODUCTION

The high power densities found in microfluidic fuel cells have made them a prime candidate for power generation in practical applications. Microbial microfluidic fuel cells have lower power densities than their electrochemical counterparts, but they have desirable attributes such as low environmental impact, high adaptability, and neutral pH[1,2]. The chemicals found in microfluidic fuel cells are often hazardous to humans as well as the environment[3]. For example, methanol is extremely toxic to humans when inhaled, ingested, or it comes in contact with skin. While the system has proved to be an effective way to produce power, the hazards associated with the chemicals used lessen the appeal for everyday use. Alternatively, microbial fuel cells are characterized as environmentally friendly and non-toxic. They are also sustainable energy production devices and can be used to recover energy from wastewater and other sources[4–7]. Microbial fuel cells have been created using a non-conductive plastic[8] or polydimethylsiloxane (PDMS)[9]. Although these methods have aided greatly in the miniaturization of the systems, they require some form of external pump to function[9,10]. The study done by Vigolo *et al.* showed the power output from this type of microbial fuel cell at varying flow rates. The optimum flow rate was found to be 15–20 μL/min for maximum of 0.57 Volts. Although the voltage output for this system was impressive, a fairly significant amount of liquid was used during the experiment.

The pumping mechanisms most commonly used in microscale fuel cells are syringe pumps because they can apply a slow and steady flow into the system. However, they are often quite large which limits their applications in smaller devices. Additionally, when taking into account the outside power needed to run the equipment, most current systems have a net negative power output. The challenge now is to find an alternative that eliminates the need for any type of external pump. Taghavi *et al.* have reported a self-sustainable system powered by a wearable microbial fuel cell. The system employs energy generated through human motions such as walking to circulate urine through the fuel cell[11]. Because cellulose fibrous materials such as paper, are inexpensive, lightweight, and widely available, one such alternative would be incorporating the paper in microfluidic fuel cells[3,12–14]. New developments have been made by incorporating the capillary action of paper[15–18]. Paper-based microfluidic devices operate similarly to open channel devices, and also benefit from allowing gas diffusion to occur between the pores of the paper[19]. While advances to this technology have mostly been used in the medical field as a means to provide a cheaper method of diagnostics, they help greatly with the miniaturization of the systems. In the study done by Esquivel *et al.*, a y-shaped design was used with anolyte and catholyte flowing side by side[3]. This type of system relied entirely on laminar flow to discourage the fuel and the oxidant from mixing. Using 75 μL of anolyte and catholyte at higher concentrations of methanol in the anolyte, the tests lasted about 2–6.5

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Received 4 March 2016; accepted 26 April 2016; published online 6 June 2016; doi:10.1142/S2339547816400124

minutes. The power capability of this type of system has been proven by the study done by Thom *et al.*, where enough power was generated to light an LED[13]. The stacked cell approach was used in this experiment with various cells stacked on top of one another. The path of flow for the liquid was dictated by hydrophobic wax printed on the paper. Their four-cell system was able to light a red (λ = 630 nm) LED for anywhere between 1.4 and 8.2 minutes depending on the amount of electrolyte used in the system. Clearly the time of these tests were relatively short and would not be effective for use in smaller hand held devices that require longer lasting power. Previous work by Fraiwan *et al.* demonstrates short time (about 1 hour) operation of paper-based microbial fuel cells generating power[20]. There are some studies on the effect of flow rates on the formation of biofilm on surfaces and most of them report biofilm formations occurring at operating time of longer than 1 hour[21,22]. This is important because the biofilm plays a vital role in current production of a microbial fuel cell: increased biofilm size and thickness ultimately leads to increased current production[23,24]. Individual bacterial cells metabolize electron-rich substances in a complex process involving many enzyme-catalyzed reactions[25]. The electrons are then free to travel to the anode through one of many modes of electron transport. Electron transport is very complicated, and evidence suggests that it is unique to each type of bacteria.[26] For *Shewanella oneidesis* MR-1, the most predominantly known ways of shuttling electrons from the individual bacteria cells to the anode are through direct contact[27], excreted soluble redox molecules[26,28–30] and biological nanowires[31]. Of these, it is widely believed that excreted soluble redox molecules serving as extracellular electron shuttles makes up for as much as 70% of electron transfer mechanisms from individual bacterial cells to the electrode, travelling distances >50 µm away from the cell surface[28]. Moreover, a control experiment developed by Jiang *et al.* identifies that direct contact between individual *S. oneidesis* cells and the electrode has little impact on the current generation, supporting a mediated electron transfer mechanism[27]. Biofilm helps with the adsorption of the redox molecules to the electrode, which makes it important to have in high power density microbial fuel cells[28]. There are not many studies on power production from paper-based microbial fuel cells running for few days. Without enough time for biofilm to form, the reported current and power data would predominantly be associated with extracellular electron transfer, which does not fully represent electrical producing capabilities of microbial fuel cells.

Here, we report the design of a proof-of-concept paper-based microbial fuel cell that operates under continuous flow condition for 5 days. The system uses the capillary action to pump the fluids through the system and shows the production of current with the onset of biofilm formation on anode. By integrating the use of paper as a pumping mechanism to guide the liquids through a microbial fuel cell system, the need for external power is eliminated. The longer duration of use and ability to operate individually may increase the number of situations where microbial fuel cells can be applied.

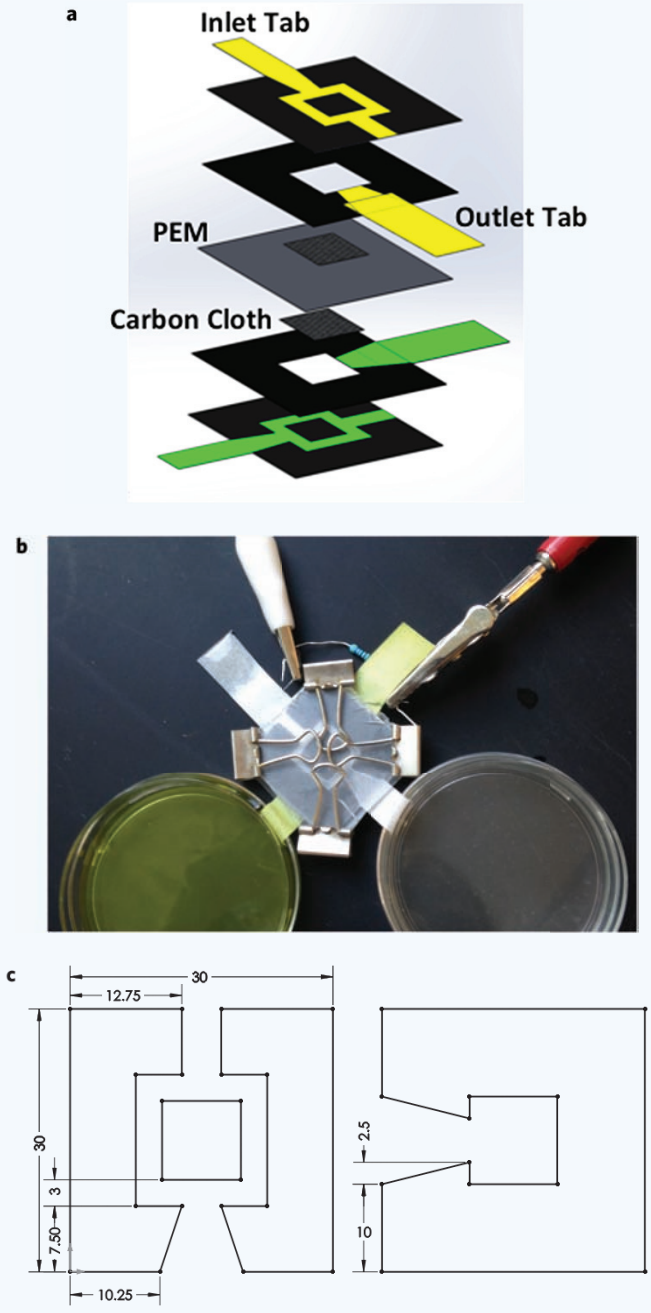

Figure 1 (**a**) An expanded set-up of the system including colored flows of the *Shewanella oneidensis* (yellow) and the potassium ferricyanide (green). (**b**) A photograph of the paper-based microbial fuel cell. (**c**) The two-dimensional geometry of the printed wax design (units in mm).

MATERIALS AND METHODS

In a paper-based microfluidic fuel cell the anolyte and catholyte flow side by side, relying on laminar flow (or low Reynolds numbers) to prevent cross over. In this process a small amount of mixing occurs between the two flows. Proton exchange membrane (PEM) (Nafion® N1110, Ion Power, New Castle, DE) is placed between the two chambers. PEM is used to separate the two liquids, as well as to allow the positively charged ions released in the biocatalytic breakdown of the anolyte to flow from the anode to the cathode. *Shewanella oneidensis* MR-1 in growth medium was used as the anolyte and potassium ferricyanide (III) (Sigma Aldrich, St. Louis, MO) was used as the catholyte. Potassium ferricyanide is non-hazardous, non-toxic, and produces its own mediators[32]. It has been proven effective in many microbial fuel cells[33].

S. oneidensis MR-1 was prepared as previously reported[34] and was grown over a 2 day period on a petri dish coated with a 2.85% tryptic soy broth (Sigma Aldrich, St. Louis, MO) and 2.4% Bacto Agar (BD, Sparks, MD) solution. Samples were then harvested and added to liquid 3% tryptic soy broth batch and allowed to grow in an incubator at 29–30°C over 24 hours. The paper-based device consists of several paper sheets stacked vertically on top of one another with a wax printed chamber design on each layer to guide the liquid flow. The overall dimensions of the MFC are a 30 mm × 30 mm wax-printed chromatography paper

square (Chromatography Paper CAT No: 3001-917 Grade: 1 CHR. Typical thickness: 0.18 mm, linear flow rate: 130 mm/30 min) that contains a path laid out for the liquid to flow through (**Fig. 1c**). The final chamber area is approximately 290 mm^2 with a volume of 52.5 µL.

The liquid path on the paper was produced by printing hydrophobic wax, which was then briefly heated at 160°C to melt through to the opposite side. Another wax printed layer placed underneath the initial layer allows the bacteria to pool around the carbon cloth (Fuel Cell Earth, Stoneham, MA) and encourages the growth of biofilm. Between these two layers a rectangular cut tab of plain cartography paper was placed for the liquid bacteria to leave the system. The carbon cloth electrodes are placed in direct contact on either side of the PEM to minimize the distance needed for ions to travel between electrodes. The system is held together with mini binder clips and thick pieces of plastic to shield the paper from any outside physical interference. A detailed set up is shown in **Fig. 1**. The carbon cloths on the anode and cathode sides are both connected to a small piece of 0.01" titanium wire (Alfa Aesar, Ward Hill, MA) that extends out of the system with the outlet tab. These wires attach to either side of a 470 Ω resistor (RadioShack, Fort Worth, TX) which completes the circuit. The anode was designated as the top section of the fuel cell to prevent leakage of the potassium ferricyanide into the anode compartment. Should there be any leakage between the layers, it would cause more damage for the potassium ferricyanide to leak into the bacteria than vice versa. The two sides were set up to flow perpendicular to one another so that the outlet tabs do not touch.

To begin the experiment, 4 mL of potassium ferricyanide and 4 mL of *S. oneidensis* in liquid medium were placed in separate, small petri dish cover reservoirs. The inlet tabs were then dipped into the corresponding reservoir and held in place with the petri dish base (**Fig. 1b**). To measure the voltage created by the system, a multimeter (Texas instrument DAQ) was connected in parallel around the resistor. An in-house Labview program was used to collect and record the voltage output of the cell across the resistor every minute for the duration of the experiment. The resultant power and current values were calculated using the Joule-Lenz Law and Ohm's Law. The reservoirs ran out of liquid approximately 100 hours after the experiment was started and continued to produce current for another 16 hours. The experiment was replicated for different time periods of 1 hour, 18 hours, 29.5 hours, and 100 hours to study the formation of biofilm over time. A scanning electron microscope (SEM) (Nikon, JSM-6700F) at an acceleration voltage of 15 kV was used to determine the creation of biofilm on the carbon cloth.

Prior to testing the microbial fuel cell for current production, an experiment was conducted to confirm the structure would prevent any leakage between the potassium ferricyanide and the liquid anolyte solution containing tryptic soy broth and *S. oneidensis*. For this test, 4 mL of dyed DI water replaced the anolyte and catholyte so that any mixing could easily be observed. In a real test, any cross contamination identified would render the electrical results inconclusive. The system was left to run for approximately 100 hours and no contamination was observed. It is important for the test to run for this extended duration to allow for the formation of biofilm, which influences the primary mode of electron transport in *S. oneidensis* and increases the current production[21,22,29]. The lack of contamination proved that the system's design was capable of sustaining flow for an extended period of time without leaking and was therefore usable for this experiment.

To begin the test, the inlet tabs were secured into their respective 4 mL anolyte and catholyte reservoirs and the fuel cell was left to operate without any human interference. At the same time, the data acquisition program was started. The liquid flows through the paper due to the capillary phenomena and has a linear flow rate of 4.33 mm/min, and a maximum volumetric flow rate of 2.34 µL/min calculated at the smallest cross-sectional area. The flow rate decreases over time as the paper channel is filled. Otherwise, the reservoir would have emptied before the 27 hour mark if this rate was maintained throughout the experiment. This lower flow rate, compared to similar experiments[3,9], facilitate the flow-based power production reduction. It also allows a more uniform biofilm production over an extended period of time.

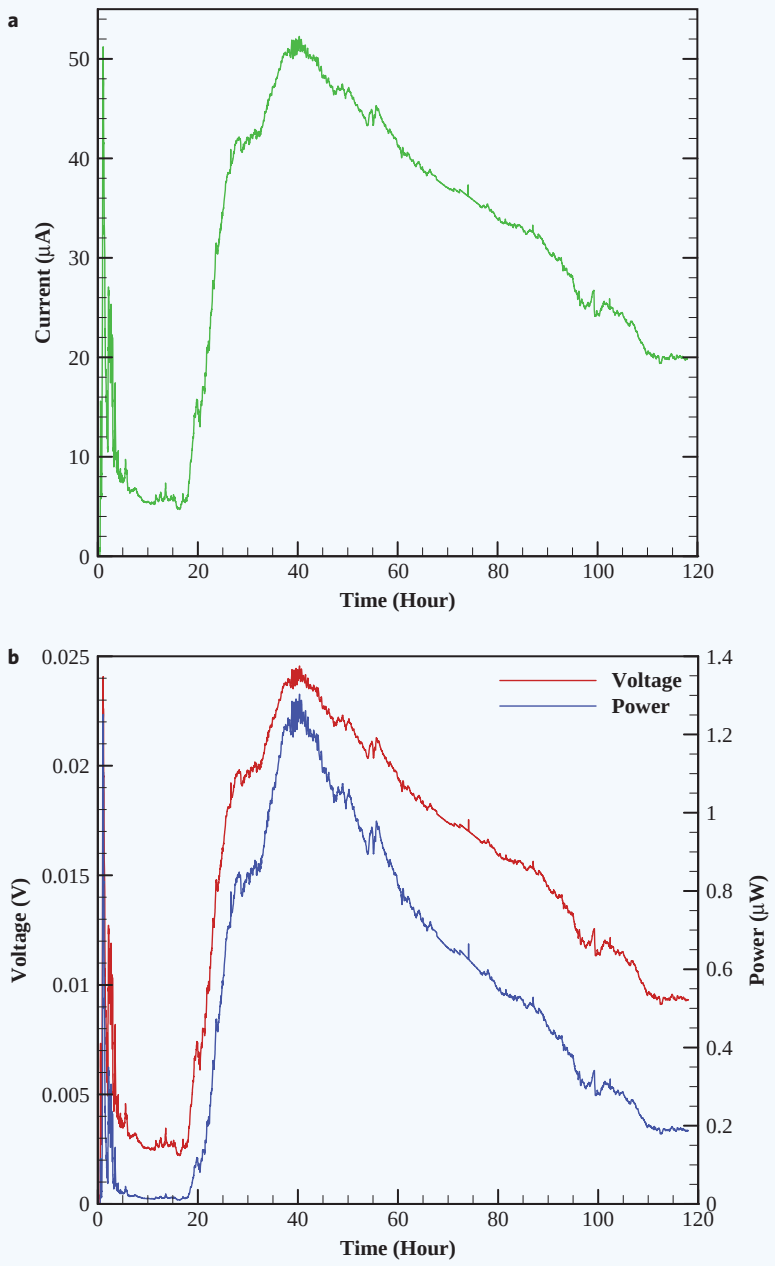

Figure 2 (**a**) The output current and (**b**) output voltage and power values collected over the duration of the test, the approximate time of biofilm formation is around hour 18.

RESULTS AND DISCUSSIONS

In this process, the dextrose in the medium is the carbon energy source. The positively charged ions pass through the PEM from the anode to the cathode, completing the circuit. The electrical energy created by the system is measured by reading the voltage across the external resistor throughout the test. The current was found through the manipulation of ohms law, which is plotted in **Fig. 2a** versus time.

An initial spike in current data occurs as a result of the fluid flow through the anode and cathode chambers at the beginning of the test. The peak of power output at the beginning of the test is due to the release of a buildup of loose electrons and protons from the catalyzed tryptic soy broth medium that was made in the time it took the anolyte to reach the carbon cloth anode. Once it reached the anode, the accumulation of electrons and protons was able to enter the electrical circuit all at once, creating the spike in power. Following this, the current immediately drops to a background value of approximately 6 μA. This trend confirms that when there is no biofilm formation on the anode to produce current and that nearly all power generation is flow powered at this time. As the anode is not pre-inoculated, the lag in power production is believed to be due to the *S. oneidensis* needing to attach to the carbon cloth. Bacteria on the carbon cloth gradually formed biofilm around the 18 hour mark. This agrees with other research, where *S. oneidensis* has been found to produce a biofilm in aerobic situations at the 12–24 hour mark at room temperature[35,36].

Bacteria groups together to form a bacterial continuum — biofilm — that has the ability to continue cellular respiration. Stationary bacteria on the carbon cloth produces power consistently with nutrition flowing over the biofilm. SEM images were taken at the 1 hour, 18 hour, 29.5 hour, 39.5 hour, and 100 hour marks of different fuel cells operating under exact similar experimental conditions to show the formation of biofilm over time at critical points in the experiment, and typical morphologies from these scans are presented in **Fig. 3**.

At 1 hour, there is very little biofilm, which confirms our hypothesis that power at this time point is attributed to other electron transport mechanisms (**Fig. 3a**). As shown in **Fig. 2**, there was an increase in current and power at the 18 hour mark which is when the biofilm was starting to form (**Fig. 3b**). At 29.5 hours, a power peak is obtained and SEM images show an increase in the biofilm density (**Fig. 3c**). The maximum potential energy output for this section after the biofilm formation is 0.0245 V and a power output of 1.3 μW at the 39.5 hour mark. The paper-based MFC reported by Fraiwan *et al.* generates a current of 74 μA[20]. While the reported current is slightly higher than what we obtained, the period of time in which our MFC can create electricity is longer than the reported device. **Figure 3d** shows that there is a substantial amount of biofilm growth on the anode compared to the 1 hour SEM image. After the output tab and carbon cloth are filled, the delivery of nutrition progressively decreased to the biofilm formation on the anode. The power output decreased proportional to the decrease in flow and delivery of nutrition to the *S. oneidensis* biofilm in the system as the anolyte dried out in the paper channel. This lack of nutrition and ability to get more nutrition for the bacteria's biofilm causes the output voltage to decrease gradually until approximately the 100 hour mark, when the reservoirs ran out of the liquid mediums. After five minutes without flow, the biofilm could begin to degrade (**Fig. 3e**)[21]. A morphology of individual bacteria cells is shown in **Fig. 4**. The biofilm acts as a constant source of electrons through cellular respiration[37], and without this, the current will begin to decrease. This explains the decrease in power at the 100 hour mark.

The experiments were conducted in an uncontrolled environment where there were slight fluctuations of room temperature and light.

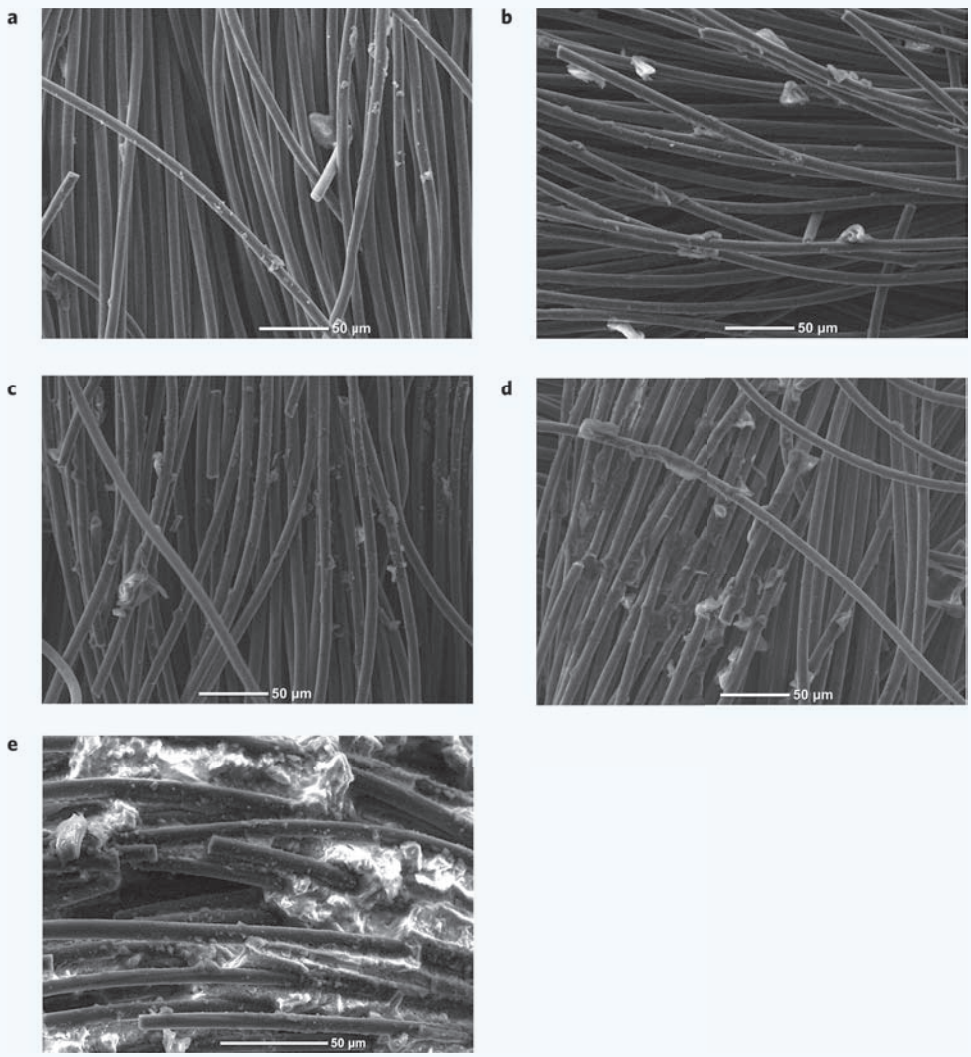

Figure 3 SEM images of biofilm formation on the carbon cloth anode. (**a**) Biofilm after 1 hour of operation. The lack of biofilm indicates power production at this time is flow based. (**b**) Biofilm after 18 hours of operation. Biofilm begins to form producing current as indicated by **Fig. 2**. (**c**) Biofilm after 29.5 hours of operation, aligning with the power peak of **Fig. 2**. The density of biofilm continues to increase. (**d**) Biofilm after 39.5 hours of operation when the maximum power output was reached. (**e**) Biofilm at the end of the experiment.

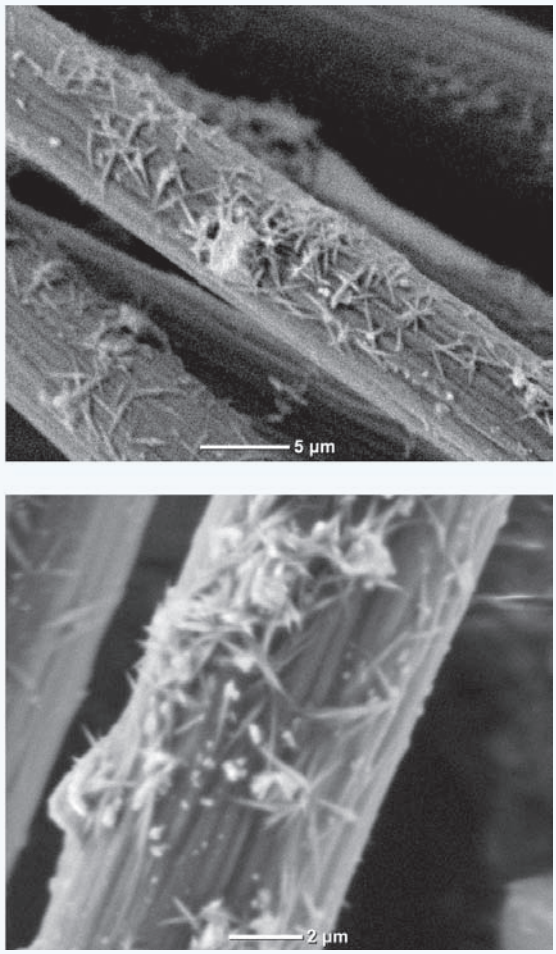

Figure 4 SEM images of biofilm on the carbon cloth anode. The non-uniformity of the bacteria might be because of the biofilm degradation due to the lack of nutrition. The microscopy was performed several days after the end of the experiment.

These fluctuations explain slight jumps in value in **Fig. 2**. Through the data collected, we could identify a pattern that is consistent with biofilm formation with a long-term experiment. There is an increase in current for the time after the biofilm formed on the carbon cloth. This provides evidence that for a viable microbial fuel cell inoculated with *S. oneidensis*, biofilm formation is a key factor in the ability for a microbial fuel cell to act like a battery. Power generated in this device contributes to a net positive power output because there is no outside power needed for the device to operate.

CONCLUSIONS

In this work, we produced a paper-based microbial fuel cell that runs for 5 days. This proves that paper-based systems that have been used to eliminate the need for pumps in microfluidic fuel cells can also be applied to microbial fuel cells. This is extremely important in the advancement of these devices and the expansion of their applications. The paper-based MFC produced 1.3 μW of power and 52.25 μA of current yielding a power density of approximately 25 W/m^3 for this experiment. These results show that the paper-based microbial fuel cells can create power in an environmentally friendly way without the use of any outside power. All power created in this device is usable because no electricity is needed to run the fluids through the device.

The biofilm formation on the carbon cloth during the test provides further evidence that the current measured was the result of the biochemical reaction taking place[23,34,38]. The capillary action of the paper provides a passive pump to draw the *Shewanella oneidensis* solution and the potassium ferricyanide from their respective reservoirs, as well as store the used material outside of the system in the outlet tabs.

Before any paper-based microbial fuel cell can be used commercially, further advancements need to be made. In order to attempt to control the voltage output and create constant current, adjustments must be made to make the flow rate constant with time through the paper. Optimizing the flow to prevent dry out is critical in the next steps of controlling these values. Possible solutions include larger area inlets and outlets, or incorporating multiple inlets and outlets throughout the fuel cell. Controlled environment tests will also aid in the regulation of the systems output and yield more stable results. In addition, further work is required to eliminate the need for using Nafion and ferricyanide for practical applications of this technology.

ACKNOWLEDGMENTS

This work was partially funded by the Iowa State University and the William March Scholar fund. We would like to thank Ashley Christopherson, Peter Meis, Luke Wagner, and Pouya Asrar for their assistance with this project.

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