A PDMS Fabricated Miniature Microbial Fuel Cell

Ismail.M.Ali, Dr. R.P.Phadke

Abstract: This study presents a new miniature - microbial fuel cell (MFC) platform which has been built by using polydimethylsiloxane (PDMS). The MFC design includes PDMS soft chambers featuring carbon cloth electrode, gold nanoparticles and a delivery system of electrolytes (mcL). Bioelectricity was generated using *Pseudomonas aeruginosa* cultivated on simple nutrient medium. *P.aeruginosa* in the anode chamber forms biofilm and generates electrons which flow from anode to cathode through external load and generates current while protons pass through the salt bridge to cathode chamber. These mini-MFC exhibited fast start-ups, reproducible voltage generation, and enhanced power densities up to approximately 2.1mV.

Index Terms: Carbon cloth, Gold nanoparticles, Miniature microbial fuel cell (mini-MFC), Polydimethylsiloxane, *Pseudomonas aeruginosa.*

1 INTRODUCTION

Microbial fuel cell represents a completely new approach to waste water treatment with the production of clean energy. A MFC converts chemical energy, available in a bio-convertible substrate, directly into electricity[6, 14].To achieve this, bacteria were used as a catalyst to convert substrate into electrons. The microorganisms use the produced energy to grow and maintain their metabolism. However, by using a MFC a part of this microbial energy can be harvested in the form of electrical energy (electricity). [1, 3, 4] Recently, there has been a growing interest in miniaturized MFC devices, not only because of their potential uses for portable power, but for their usefulness in studies of well-defined microbial communities to determine the maximal potential and fundamental limits of MFCs.[2,7,9,10,13,1] Miniaturized MFC chambers and electrodes have received increasing attention because they are able to provide unique platforms for fundamental studies of microbes, screening environmental strains, and potentially powering small portable electronic elements. Examples of miniature MFCs include: (1) a rationally designed 1.2 mL MFC that produces higher power and current densities than most macroscopic counterparts[12]; (2) a 24-well, 650 µL MFC array used to isolate an electrogenic strain that produces 2.3-fold higher power than the wild-type *Shewanella oneidensis* MR-1.[7] Many studies in the mL-to-L regime of MFC reactors have suggested that high-surface area carbon electrodes can enhance the rate of extracellular electron transfer by microbes, and therefore increase power generation.[8]

- Ismail Mansoor Ali has pursued Master's degree in Microbiology from Department of Microbiology, Ramnarian Ruia College (Mumbai University).
- E-mail: ismailmansoorali@gmail.com
- Dr. Ravindra Phadke is the Associate Professor and Head of Microbiology Department at Ramnarian Ruia College (Mumbai University).PH-09102224143098 Ext 261.
- E-mail: raviphadke@yahoo.com

In contrast, the use of carbon-based electrodes in sub-150 µL MFCs has not been reported, most likely because traditional carbon electrodes are bulky.[5] To overcome this limitation, we designed a miniature-MFC to incorporate thin carbon cloth electrodes into a sub-150 µL chamber embedded in a thin polydimethylsiloxane (PDMS) frame. In this work we observed that when 150 mcl of *P.aeruginosa* culture was slowly injected in the anode chamber, the voltage increased to 9.4mV within approximately 21.5 hours, followed by a decrease in the voltage, and again after 45 hours the voltage increased to ~5mV followed by a rapid decrease to the baseline.

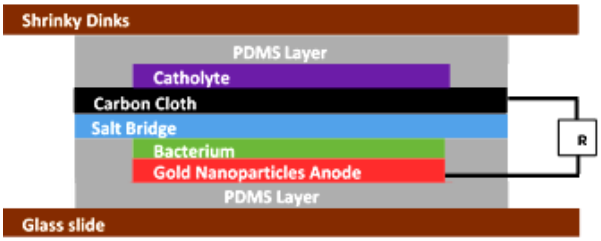
2 EXPERIMENTAL METHODS

2.1 Chemicals and materials used

Polydimethylsiloxane (purchased from Dow Corning) , 1M KOH (purchased from Loba Chemi) solution and 4% agarose (salt bridge), Shrinky Dinks, Carbon Cloth (Cathode) , 2mM Citric Acid (Catholyte solution) (purchased from Loba Chemi), Nutrient Broth (purchased from Hi Media) , Gold Nanoparticles (localling prepared by the Chemistry Department, Ramanrian Ruia College) suspended in 4% agarose (Anode), *Pseudomonas aeruginosa* lab culture, exp-EYE Data Logger (Zyxware Technologies Pvt.Ltd, Kerela).

2.2 Mini-MFC Fabrication, assembly and operation

The mini- MFC was fabricated as per the schematic-01. Using micro-fabrication technique, various PDMS layers were prepared for making the anode and the cathode chamber. The dimension of the PDMS layer in this setup was 0.133cmX2.1cm and height of the well was 0.266cm. To create the PDMS molds a 10:1 mixture of PDMS base and PDMS curing agent was poured on the glass slide and baked for 30 minutes at 90^{o}C. Gold nanoparticles embedded in 4% agarose layer was placed in the anode well. A layer of salt bridge was placed over the anode chamber. Carbon cloth layer (cathode) of the dimension on 2.4cmX2.4cm was placed over the salt bridge layer. A PDMS Mask was added on to the cathode to form wells for the catholyte solution followed by the addition of a PDMS top layer for the cathode chamber. On to the cathode chamber shrinky dink was used as a final layer for the insertion of 4 sets of 0.3mm diameter tubings through the setup, a) for addition and removal of anolyte and, b) for addition and removal of the catholyte in the MFC chambers. The entire assembly was then clipped together using a pair of paper clippers (Figure: - 01). The chambers were electrically connected with thin copper wires. These electrodes were connected to 10kΩ resistor and the system was attached to expEYES data logger for the recording of the readings (Figure 02).



Schematic 01: Fabrication of Mini-MFC

Assembly of the entire MFC device was carried out in a laminar air flow unit chamber so as to keep the system sterile. The MFC setup was exposed to UV for 15 minutes before carrying out the bacterial inoculation. 24 hours old fresh culture of *P.aeruginosa* lab culture was injected into the anode chamber through the tubing under aseptic condition. 2mM citric acid solution was injected in the cathode chamber through cathode tubing.

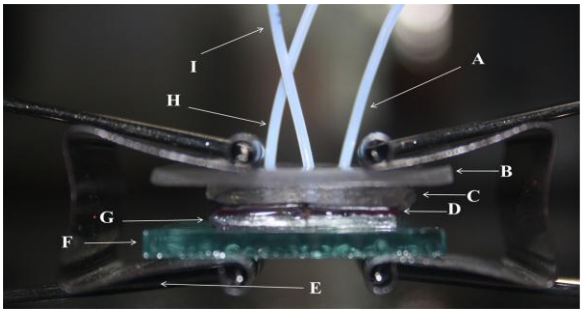


Fig 01: Lateral View of Mini-MFC Setup

A: Inlet for Anolyte, B: Shrinky Dinks, C: PDMS layer, D: Gold Nanoparticles, E: Clamps, F: Glass Slide,
G: PDMS Layer, H: Outlet for Catholyte, I: Inlet for Catholyte.

The setup was operated at ambient conditions. No extra precautions was taken to exclude residual oxygen in the feeding medium, because *P.aeruginosa* is a facultative strain that can scavenge dissolved oxygen quickly, maintaining a low oxygen level in the anode chamber.

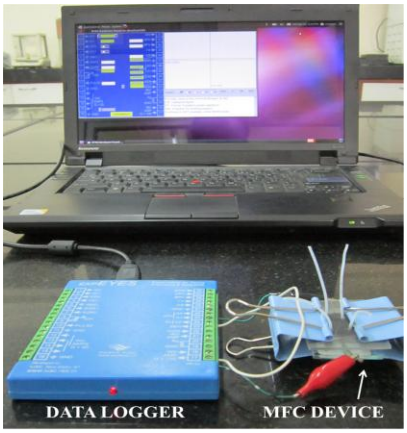


Fig 02: MFC device connected with Data Logger

2.3 Bacterial culture conditions, and biofilm staining procedure

P.aeruginosa was chosen as a model for inoculation in the MFC device due to its electrogenic property and, as it is found to thrive in hypoxic conditions and has thus colonized in artificial environments. The strain was cultured aerobically in nutrient broth medium at 37^{o}C for 18-24hrs with shaking at 120rpm. The culture was used without any further treatment as an inoculum in the MFC operations. After each MFC operations, the bacterial biofilm from the anode chamber is studied. The biofilm layer was rinsed with distilled water and then stained with 0.1% aqueous crystal violet dye, and this layer is observed through a Phase contrast Microscope (Moitic) attached to a 5 mega-pixel CCD camera (Figure 03).

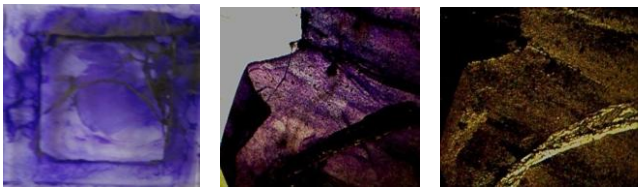


Fig 03: Biofilm formation in anode chamber

A: - Stained biofilm layer in anode chamber
B: - 450X, C: - 450X using Phase contrast microscope

3 Results and Discussions

3.1Voltage generation and data logger readings

A series of electrical measurements were carried out to evaluate the performance of the mini-MFC in electricity generations. The voltage change before and after the bacterial inoculation was recorded. The MFC device without the addition of the culture showed a flat stable background voltage of 0V (Data not shown). Upon inoculation with 150 µl of 24 hours old fresh lab culture of P.aeruginosa of O.D. 0.26, a steady increase of voltage up to 9.4mV was observed within 21.5 hours, followed by a decrease in voltage, and again after 45 hours the voltage increased to ~5mV followed by a rapid decrease to the baseline within 52.5 hours (Figure 04). The MFC started up almost immediately upon inoculation, in contrast to the lag period of days or months that was commonly observed in large scale devices. This fast response feature agreed with several previous reports of sub-10 µL reactors and a 1.2 mL miniature reactor. It is believed that the high sensitivity to bacterial inoculum is a generic advantage of miniaturized, high surface-area-to-volume (SAV) ratio devices. The shortened diffusion distance enables the electrode to be more responsive to the electrochemical change in the micro-chamber. The voltage evolution was monitored as a function of time and concentration of the culture. The MFC device was inoculated with two different O.D. of culture; and the voltage generated was compared. The culture concentration used was at OD 0.26 and at OD 0.42 and it was observed that the culture OD with 0.26 gave a high voltage of 9mV within 20 hours of run of the MFC setup. The voltage decreases with time and again after 45 hours of run there was a rise of ~5mV followed by a rapid decrease to baseline. Within 52.5 hours the setup shows a baseline reading indicating the death of the culture due to lack of nutrients (Figure 05). Whereas, in the culture OD with 0.42 it was observed that the MFC setup gave

a high voltage of ~5.5mV and a reduction in the voltage was observed with time. Within 21.5 hours the entire setup shows 0mV reading indicating the death of the culture due to lack of nutrients.

Electrical measurement and calculations

An external resistor (R) was connected between electrodes of the mini-MFC to close the circuit. Current through the resistor (I) was calculated via Ohm's law: I = V/R. Output power (P) was calculated via P = V*I. Q. Fang in his research work [11] observed that when 2mL of S.oneidensis culture was slowly injected to fill the anode tubing/chamber, and then the end of tubing was sealed for batch mode growth a, current increased to 700 nA within approximately 12 hours. In this project it was observed that when 150 mcl of P.aeruginosa culture was slowly injected to fill the anode chamber, the current increased to 9 mV which was 940 nA within approximately 21.5 hours. These mini-MFCs showed excellent reproducibility and consistent performance in voltage–time plot when operated in the batch-fed mode.

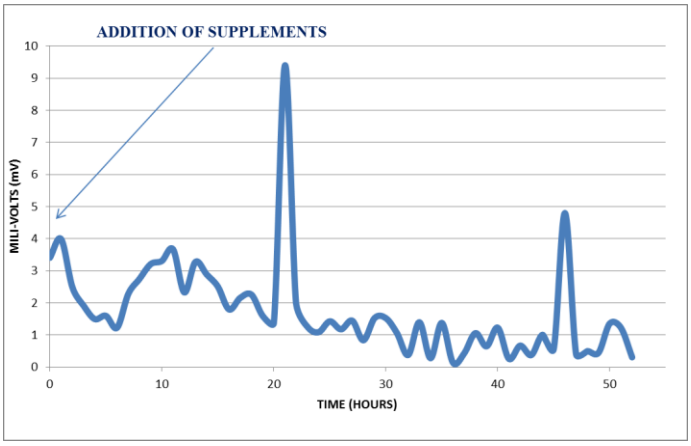


Fig 04:- Graphical Representation of Data Logger Readings for 52.5 Hours

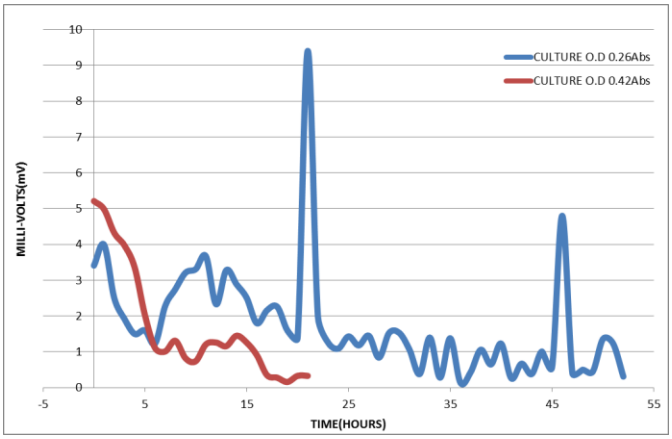


Fig 05:- Graphical Representation of Data Logger Readings of Pseudomonas aeruginosa at OD of 0.26 Abs and 0.42 Abs

3.2 Microscopic studies of the anode biofilm

Biofilm formation was observed in the anode chamber after the setup was run for 52.5 hours with the culture density of OD 0.26 using Phase contrast microscope attached with a 5 mega-pixel CCD camera. Sample from biofilm layer was isolated on the Nutrient agar plate and showed only P.aeruginosa colonies, confirming that the mini-MFC was not contaminated by other organisms during operation and was thus able to offer a clean environment for bacterial growth. Microscopic studies also confirmed that negligible amount of the bacterial cells were found on the salt bridge and the inner walls of the PDMS chamber, as these insulting materials did not provide an appropriate surface in favor of bacterial growth.

4 Conclusion

A novel dual chambered micro-MFC device was designed and fabricated to investigate the electrogenic bacteria-anode interaction for development of an on-chip energy supply. The mini-MFC device we developed could serve as a versatile platform for studying the interactive effects of cell growth, electrode activity/reactivity, and ion transport at the micro-nano-scale.

Acknowledgment

We would like to thank the microbiology department of Ramnarain Ruai College for providing the fascilities to carry out this assessment, and Mr. Amit Dhakulkar, Homi Baba Centre for Science Education, for his expertise on the data logger device, and Mr. Tejas Patade (Chemistry department, Ramnarain Ruia College) for providing with the Gold nanoparticles.

References

[1] B.E. Logan, Microbial Fuel Cells, John Wiley & Sons, New York, 2008.

[2] B. R. Ringeisen, E. Henderson, P. K. Wu, J. Pietron, R. Ray, B. Little, J. C. Biffinger and J. M. Jones-Meehan, Environ. Sci. Technol., 2006, 40, 2629–2634.

[3] D. R. Bond, D. E. Holmes, L. M. Tender and D. R. Lovley, Science, 2002, 295, 483–495.

[4] D. R. Lovley, Nat. Rev. Microbiol., 2006, 4, 497–508.

[5] Fang Qian, Zhen He , Michael P. Thelen , Yat Li, 2011. A microfluidic microbial fuel cell fabricated by soft lithography. Bioresource Technology 102(2011)5836-5840

[6] H. Pham, J. Jae Kyung, In Seop Chang, And B. Kim. Improvement of Cathode Reaction of a Mediatorless Microbial Fuel Cell (2004).Journal Of Microbiology and Biotechnology. 14(2), 324-329.

[7] Hong, S.W., Chang, I.S., Choi, Y.S., Kim, B.H., Chung, T.H., 2009. Responses from freshwater sediment during electricity generation using microbial fuel cells. Bioproc. Biosyst. Eng. 32, 389–395

[8] Logan, B.E., Hamelers, B., Rozendal, R., Schroder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., 2006. Microbial fuel cells: methodology and technology. Environ. Sci. Technol. 40, 5181–5192.

[9] M. Chiao, K. B. Lam and L. Lin, in Proceedings of 16th IEEE Micro Electro Mechanical Systems Conference, Kyoto, Japan, 2003, pp. 383–386.

[10] M. Chiao, K. B. Lam and L. Lin, J. Micromech. Microeng., 2006, 16, 2547–2553.

[11] Q. Fang, B. Mary, Q. Gu And M. Daniel (2009) A 1.5 Ml Microbial Fuel Cell For On-Chip Bioelectricity Generation. Lab on Chip. 10.1039/ B 910586g.

[12] Ringeisen, B.R., Henderson, E., Wu, P.K., Pietron, J., Ray, R., Little, B., Biffinger, J.C., Jones-Meehan, J.M., 2006. High power density from a miniature microbial fuel cell using Shewanella oneidensis DSP10. Environ. Sci. Technol. 40, 2629– 2634

[13] S. R. Crittenden, C. J. Sund and J. J. Sumner, Langmuir, 2006, 22, 9473–9476.

[14] T. Makoto, T. Akimasa, A. Tatsuya, K. Hirokazu, N. Matsuhiko. Structural Studies of Enzyme-Based Microfluidic Biofuel Cells (2007). Science Direct Journal of Power Sources.178 (2008) 53-58.

[15] Y. Z. Fan,H. Q.Hu andH. Liu, J. Power Sources, 2007, 171, 348–354.