Bioresearch Communications

Volume 03, issue 02, July 2017



Journal Homepage: www.bioresearchcommunications.com

Original Article

A Mediator-Less Double Chambered Microbial Fuel Cell Using Sewerage Sludge With Output of Relatively Higher Voltage and Higher Efficiency

Saifuddin Sarker^{1,a}, Md. Belal Chowdhury^{1,a}, S. M. Shah Riyadh², Tanvir Hossain¹, H. M. Syeed Uddin³ & H. M. Syfuddin^{1*}

¹Department of Biochemistry and Molecular Biology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh ²Institute of Energy, University of Dhaka, Dhaka-1000, Bangladesh.³Department of Microbiology, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh

ABSTRACT: Microbial fuel cell (MFC) has become a great alternative source for electricity generation along with waste water management. Due to low voltage and low power output, it is yet to be applied in commercial field. During last few decades, there have been numerous efforts to develop MFCs. Here, we report an MFC that has been calibrated by using available ingredients in local markets and using local sewerage sludge. The voltage differences are found to be comparatively higher and the efficiency of the cell also shows the potential of the sewerage sludge in this manner. We have connected five cells in series connection to test the current load and it gave out potential output by supplying current for illumination of an LED for 144 hours, as far as recorded, without any interval.

KEYWORDS: Microbial Fuel Cell, Sewerage Sludge, Green Energy, Bioconversion, Mediator-less MFC.

Article History Received: 30 April, 2017

Accepted: 17 June, 2017



Scan the QR code to see the online version or,visitwww.bioresearchcommunication.com Corresponding author

H. M. Syfuddin Email: syfuddin-bmb@sust.edu

Citation: Sarker S., Chowdhury B., S. M.Riyadh, Hossain T., H. M. Uddin, H. M. Syfuddin.2017.A Mediator-Less Double Chambered Microbial Fuel Cell Using Sewerage Sludge With Output of Relatively Higher Voltage and Higher Efficiency. Biores Comm. 3(2), 422-429.

INTRODUCTION

In the growing world of green energy, microbial fuel cell has become one of the major focal points to generate power where simultaneous treatment of waste water can be carried out. With the help of microorganisms as the biocatalysts, microbial fuel cell produces electricity by conversion of chemical energy to electrical energy¹. The basic mechanism of a microbial fuel cell is very simple where anaerobic bacteria oxidize the organic matter in the anode chamber by the process of biocatalysis and the electrons are transferred to the cathode chamber to let the reduction reaction take place. The phenomenon of bioconversion has shaped the direction of numerous researches around the world and thus microbial fuel cell research has created many branches of potential utilization.

There have been several reports for the past few years, where the authors endeavored to increase the output power of MFCs by executing several developments like selection of specific electricity generating bacteria, using mediator producing microorganisms or optimization of electrode surface.^{2,3} In spite of the efforts, MFCs have not been proved to be useful in support for the commercial electronic devices due to the low voltage and low power output. A crucial step is carrying out the researches of the power electronics based harvester circuits for the improvement of harvest and power boost.^{4,5} To make it more viable for use, single chambered

microbial fuel cells have also been tried.^{6,7,8,9} As the development of the single chambered cells are in primary step, we have focused our study on double chambered microbial fuel cell using local ingredients because of having some advantages. A comparative analysis on electricity generation in double and single chamber MFC is reported by Luo et al., (2009).¹⁰ They experimentally showed double chamber MFC had a better that performance when it came to voltage generation, current generation and power density outcome on account of area and volume. In the removal of COD, double chamber MFC was found to be more efficient compared to single chamber MFC reported by Hampannavar and Pradeep (2011).¹¹ In the double chamber, different conditions can be maintained in each compartment on interest. But the single chamber MFC has the low coulombic efficiency generally because of diffusion of oxygen into anode i.e. consumption of oxygen by the bacteria without an ion permeable membrane (single chamber) than the double chamber design.¹² In addition, in some single chamber MFC, a membrane for ion exchange having high cost, is used.¹³ The dependence of power production basically lies in the specific substrate concentration, the used bacterial species and most importantly the design of MFCs.^{14,15} The most

importantly the design of MFCs.^{14,15} The most interesting aspect of MFCs, reported by Rabaey et al., is that mixture of bacteria has been found to be better than a single species.¹⁴

Here in this paper, we report a microbial fuel cell which is double chambered and in the anode chamber, crude sewerage sludge has been used as the source of microbes and their energy source. The compartments of the anode and cathode were constructed using Polyvinyl Chloride (PVC) sheets available in local markets and the U-shaped salt bridges between the compartments were prepared using specific concentration of Sodium Chloride (NaCl) and Agar. Different sizes of compartments were used to contain different volumes of sludge in the anode and correspondingly same volume of distilled water in the cathode. No mediator was used in the anode and in the cathode compartments. Observation of 168 hours revealed the highest output voltage of about 726 mV without any significant change with respect to different volumes, which is in accordance with the previous researches.¹⁵

The current load was tested by using Light Emitting Diode. In this research, the potentiality of the sewerage sludge as the microbial source and fuel has been thoroughly studied and, as far as our knowledge goes, there has been no report of such study in Bangladeshi sewerage sludge till date.

MATERIALS AND METHODS Salt Bridge Preparation

Salt bridges were prepared by mixing 0.01 N NaCl with agar. At first 20ml 0.01 N NaCl was prepared and 0.4g of agar was mixed with that solution. The volumetric flask containing solution was placed in a hot bath tub. After reaching 85^oC temperature, the agar started to dissolve. The solution was kept in bath tub for 15 minutes for proper mixing of agar. The hot solution was poured into U-shaped PVC pipes and then rested to cool.

Samples

Sewerage sludge was collected from sewerage system of Sylhet city of Bangladesh.

MFC Preparation and Operation

MFCs of different shape were prepared for performing experiment using 8.0mm PVC sheets to build the compartments. Effective volumes of different MFC compartments were 12000ml, 750ml 1500ml and respectively. Every compartment was washed with 70% ethanol. The lid of each anode compartment was drilled for insertion of salt bridge and connecting wire of electrodes. Coiled copper wires were used as electrodes. The electrodes (anode and cathode) were fixed with lids by glue. In anode compartment sewerage sludge was inoculated and distilled water was used in cathode ensuring the absence of other mineral ions. Generated electrons travel through close circuit from anode to cathode trigger the reaction of oxygen coming from distilled water with the proton traveling through the salt bridge, and thus reduces oxygen in presence of water to generate hydroxyl ion.^{16,17} Here, distilled water acts as source of oxygen in cathode chamber of MFC as oxygen is the most common oxidizer. pH was measured on the both compartments prior to the experiment to ascertain neutrality. Whole experiment was conducted at room temperature. The anode and cathode compartments were connected by a U-shaped salt bridge for proton exchange. The lid of anode compartment was sealed for providing anaerobic condition resulting in a complete setup for operation, as shown in Figure.1.





Figure.1. Graphical Design of double chamber MFC.

Sample preparation for Gram staining

A small amount of sludge was taken into test tubes containing Luria Broth (LB) media. After 24 hours of incubation at 37^oC temperature in an incubator, bacterial culture was spread in a petri dish containing Luria Agar (LA) media. LA media with sample's bacteria was incubated at $37^{0}C$ temperature. Subsequently, 24 hours of incubation, bacterial colonies were found and single colony of bacteria was isolated and transferred to a glass slide for performing Gram staining (Gram's Kit, **REF**: SK001, Staining Micromaster Laboratories Pvt. Ltd) observation. Following Gram staining, the slide was shifted to a microscope, using x100 lens for observation.

RESULT AND DISCUSSION

The open circuit voltages (OCV) and voltages with different loads (100K Ohm, 10K Ohm, 1 K Ohm and 560 Ohm) were measured using a multimeter (Model: Veyron VL-9205 AC/DC Voltage / Current / Capacity / Resistance Digital Multimeter) at a fixed time interval of 24 hours. All currents were calculated using the Ohm's Law.

Generated Voltage Differences

As shown in Figure.2, the graphical representation of OCV of three MFCs was measured for a time span of 168 hours. The anode and cathode of three double chamber MFCs had an effective volume of 750ml, 1500ml and 12000ml respectively. The three MFCs were operated with identical parameters (same fuel cell construction, electrode surface area, salt bridge, sewerage sample, sample's pH and operational temperature) except volumes. Voltage difference for each MFC were reached at 726mV for 750ml, 712mV for 1500ml,



Figure.2 Voltage generation graph: Open circuit voltage (OCV) for 12000ml MFC, 1500ml MFC and 750ml MFC with time. (—) represent 12000ml MFC, (—) 1500ml MFC and (—) 750ml MFC.

727mV for 12000ml after 24 hours of operation for identical. The highest generated OCV were 733mV for 750ml at day-2, 729mV for 1500ml at day-5 and 727mV for 12000ml at day-1. These potentials were measured prior the external resistance were connected with a parallel connection. During experimental period, these OCV were seemed to be mostly constant with MFCs' internal resistances which were measured in a range of mega ohm for each MFC. Large volume MFC indeed generated more electrons but due to the accumulation of electrons near the electrode, electrons of distant location could not make it to the electrode generating similar output as the lower ones. But as the summation of electrons was higher in large volume MFC than smaller one, it could thereby produce stable current compared to the smaller volume. То eradicate this problem. manv researchers employed various mediators in anode chamber to centralize the electrons towards electrode since mediators are expensive and some are toxic.18,19,20,21,22 Mediators such as thionine, methyl viologen, methyl blue, humic acid, neutral red and so on play an important role on facilitation of generated electron from MFC to electrodes.²³

There was a constant volume i.e. 750ml for each of MFCs but varying electrode length to measure OCVs. The electrode lengths were 100 ± 2 cm, 200 ± 2 cm and 300 ± 2 cm. As shown in figure.3, the comparison of generated OCVs for different electrode length with time was made. The highest picked OCV were 736mV at day-7 for 300 ± 2 cm electrode among three MFCs. 733mV at day-2 and



578mV at day-3 were the highest OCVs for 200 ± 2 cm and 100 ± 2 cm electrode length MFCs respectively. It is apparent that, in low electrode length, the generated OCV was lower than other two MFCs and a significant decrease of OCV with time was observed. In 200 ± 2 cm and 300 ± 2 cm electrode MFCs, there were relatively constant OCVs. This settles a relationship between generated



Figure.3. Grouped bar diagram of generated voltage for constant volume in varying electrode length with respect to time.

OCV with electrode surface length. During experiment, there were provided anaerobic condition, unchanged pH (6.98) and room temperature for better growth of microorganism like electrogenic bacteria. Crude sludge contains undefined ions that help in osmotic balance, chemicals and nutrients required for bacterial growth. Bacteria can live and reproduce according to their respective growth pattern using the sludge as their growth media and those conditions (physical) required for growth and thus they survived and generated electricity without addition of any minimal media.

Current Generation

As shown in Figure.4, the generated current with gradually decreased resistance from 100K ohm to 560 Ohm had a range from 0.00632mA to 0.248mA for 750ml MFC, 0.00694mA to 0.257mA for 1500ml MFC and 0.00608mA to 0.0768mA for 12000ml MFC over the experimental period. To measure the current for each MFC, the external resistance of 100K Ohm, 10K Ohm, 11K Ohm and 560 Ohm were used as loads.



(C)

Figure.4. Current generation graph: current for 100K Ohm, 10K Ohm, 1K Ohm and 560 Ohm for 750ml (A), 1500ml (B) and 12000ml(C) MFC with time.

The recorded highest current for each MFC was obtained on different days. The highest currents were 0.248mA for 750ml MFC at day-3, 0.257mA for 1500ml MFC at day-7 and 0.0768mA for 12000ml MFC at day-7 respectively. There was a fixed electrode length or surface area (200 cm in



length, 65 ± 2 sq. cm in area) of copper wire used for 750ml, 1500ml and 12000ml MFC.

An increase of voltage with different loads was observed when blowing was carried out in the cathode, containing distilled water. It may be due to increased flow of oxygen that gives electron to the cathode.

Light Emitting Diode (LED) Test

After 7 days of experiment, three MFCs identical in volume of 750ml varying on electrode length and other two MFCs identical in electrode length but vary in volume i. e. 12000ml and 1500ml respectively were connected in series connection which is further connected to a light emitting diode (LED) in a parallel connection. Open circuit voltage (OCV) of series connected MFCs was 3.06V, recorded prior to connecting the LED. When LED was connected in parallel to the series connection of five MFCs, it started to emit light and initial close circuit voltage and current with LED were 1.67V and 0.052mA respectively. As shown in Figure.5, the voltages of five MFCs connected in series and the current with LED were recorded over 144 hours. After 144 hours, the voltage and current were found to be 1.60V and 0.013mA respectively. Decrement of voltage and current occurred due to the resistance of LED that depends on the intensity of emitting light. And even after 3 weeks of initial connection, LED was found to be emitting light and voltage and the current was recoded as 1.615V and 0.015mA, which indicates the high efficiency and stability of the MFCs.



Figure.5. Voltage of five MFCs connected in series and, current after discharging with LED at different time.

Efficiency of MFC

Polarization curve, a powerful tool for analysis of MFC, reflects the overall performance of anode chamber, cathode chamber, or for the whole

MFC.^{13,24,25} Polarization curve represents the cell voltage and power density in terms of current (or current density).¹³ Generally, microbial fuel cell possesses infinite resistance while obtaining OCV. As substrate consumption by microbes increases gradually, generation of electrons and protons also increases, which results the acceleration of bioelectricity production.²⁴

Polarization curve were generated by using varying external resistance of $100K\Omega$, $10K\Omega$, $1K\Omega$ and 560Ω after the generation of steady voltage.²⁴ As shown in figure.6A, after 24 hours of initial setup, maximum power density for 12000ml MFC was found to be 1.93132mW/m^2 at external resistance of $10 \text{ K}\Omega$. For 1500ml and 750ml MFC, maximum power densities were found to be 4.5507mW/m^2 and 5.22147mW/m^2 , both at $10 \text{K}\Omega$ external load.





Figure.6. Polarization curve: (A) Voltage and power density with generated current at day-1 for 12000ml MFC, 1500ml MFC and 750ml MFC. (B) Voltage and power density with generated current at day-1 for 12000ml MFC, 1500ml MFC and 750ml MFC. (C) Voltage and power density with generated current at day-1 for 12000ml MFC, 1500ml MFC and 750ml MFC. Electrode surface area for each MFC was 65.99 cm². Black and red lines represent the voltage and power density respectively.

At day-4, as shown in Figure.6B, after 96 hours of initial setup, maximum power density was found to be 2.17668mW/m² at 10K Ω external load for 12000ml MFC. Maximum power density of 1500ml MFC was found to be 6.18329mW/m² at 1K Ω external load. 750ml MFC topped at day-4 for highest density which power was 7.40119mW/m² for 1K Ω load. At final day, shown in Figure.6C, after 168 hours of setup, maximum power density of 12000ml MFC was found to be 2.00779mW/m^2 for $10 \text{K}\Omega$ load, 7.94671mW/m^2 for 1500ml MFC at 1KΩ load. For 750ml MFC, maximum power density was found to be 5.29907mW/m² at 1K Ω load.

These results suggest that MFC volume of 750ml and 1500 ml (and ranges between them) are suitable for maximum power density and load of $1K\Omega$ resistance.

Gram Staining Observation

For the preliminary identification of bacteria, Gram Staining was performed and observed under a microscope using X100 lens. Distinct color and different shape of bacteria were found such as bacteria with dark purple color and red or pink color (Figure.7) which indicates the presence of gram positive bacteria and gram negative bacteria respectively in sample. Both gram negative and positive bacteria are electrogenic and thus generate electricity. The gram's straining experiment gives the primary evidence of gram negative and positive bacteria in the sludge. It is better if molecular and physical identifications of microorganisms could be possible to perform but those techniques were out of range for this experiment.



Figure.7. Observations of Gram staining using x100 lens.

Microbial fuel cells were operated using the crude sewerage sludge and locally available products were used to construct the whole set up that are cost-effective rather than single chamber which is a costly process including operation, engineering for design and electrode materials^{13,26,27} The highest voltage was around 733 mv. After 20 days with LED, the voltage dropped to 1.615V and the current dropped to .015 mA. There are more to investigate in this field like the nature of the microorganisms and their genetic variability in Bangladesh. The design of the cells can be developed to get more power.

ACKNOWLEDGMENTS

We would like to thank Mr Maruf Hossain, Arafat Islam Ashik and Mahedi Hasan for their assistance during the experiment.

REFERENCES

- 1. Allen, R. M., & Bennetto, H. P. (1993). Microbial fuel-cells. *Applied biochemistry and biotechnology*, *39*(1), 27-40.
- 2. Bond, D. R., & Lovley, D. R. (2003). Electricity production by Geobactersulfurreducens attached to electrodes. *Applied and environmental microbiology*, 69(3), 1548-1555.
- 3. Park, D. H., & Zeikus, J. G. (2003). Improved fuel cell and electrode designs for producing



electricityfrommicrobialdegradation. Biotechnologyandbioengineering, 81(3), 348-355.

- Wu, P. K., Biffinger, J. C., Fitzgerald, L. A., & Ringeisen, B. R. (2012). A low power DC/DC booster circuit designed for microbial fuel cells. *Process Biochemistry*, 47(11), 1620-1626.
- Kim, Y., Hatzell, M. C., Hutchinson, A. J., & Logan, B. E. (2011). Capturing power at higher voltages from arrays of microbial fuel cells without voltage reversal. *Energy & Environmental Science*, 4(11), 4662-4667.
- 6. Mohan, S. V., Raghavulu, S. V., & Sarma, P. (2008). Biochemical evaluation N. of bioelectricity production process from anaerobic wastewater treatment in a single microbial chambered fuel cell (MFC) employing glass wool membrane. Biosensors and Bioelectronics, 23(9), 1326-1332.
- Goud, R. K., Babu, P. S., & Mohan, S. V. (2011). Canteen based composite food waste as potential anodic fuel for bioelectricity generation in single chambered microbial fuel cell (MFC): bio-electrochemical evaluation under increasing substrate loading condition. *international journal of hydrogen energy*, 36(10), 6210-6218.
- Khilari, S., Pandit, S., Ghangrekar, M. M., Das, D., & Pradhan, D. (2013). Graphene supported α-MnO 2 nanotubes as a cathode catalyst for improved power generation and wastewater treatment in single-chambered microbial fuel cells. *Rsc Advances*, 3(21), 7902-7911.
- Liu, H., Cheng, S., & Logan, B. E. (2005). Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environmental* science & technology, 39(2), 658-662.
- Luo, H. P., Liu, G. L., Zhang, R. D., & Jin, S. (2009). Comparison of power generation in microbial fuel cells of two different structures. Huan jing ke xue= Huanjing kexue/[bian ji, Zhongguo ke xue yuan huan jing ke xue wei yuan hui" Huan jing ke xue" bian ji wei yuan hui.], 30(2), 621-624.
- 11. Hampannavar, U. S., & Pradeep, N. V. (2011). Treatment of distillery wastewater using single chamber and double chambered MFC. International Journal of Environmental Sciences, 2(1), 114.

- 12. Cheng, S., Liu, H., & Logan, B. E. (2006). Increased performance of single-chamber microbial fuel cells using an improved cathode structure. *Electrochemistry Communications*, 8(3), 489-494.
- Logan, B. E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., ... & Rabaey, K. (2006). Microbial fuel cells: methodology and technology. *Environmental science & technology*, 40(17), 5181-5192.
- 14. Rabaey, K., Boon, N., Höfte, M., & Verstraete, W. (2005). Microbial phenazine production enhances electron transfer in biofuel cells. *Environmental science & technology*, 39(9), 3401-3408.
- 15. Liu, B. F., Ren, N. Q., Tang, J., Ding, J., Liu, W. Z., Xu, J. F., ... & Xie, G. J. (2010). Bio-hydrogen production by mixed culture of photo-and dark-fermentation bacteria. *international journal of hydrogen energy*, 35(7), 2858-2862.
- 16. Oliot, M., Galier, S., de Balmann, H. R., & Bergel, A. (2016). Ion transport in microbial fuel cells: Key roles, theory and critical review. *Applied Energy*, 183, 1682-1704.
- 17. Logan, B. E. (2008). *Microbial fuel cells*. John Wiley & Sons.
- Taskan, E., Özkaya, B., & Hasar, H. (2014). Effect of different mediator concentrations on power generation in MFC using Ti-TiO2 electrode. *International Journal of Energy Science*, 4(1).
- 19. He, Z., Minteer, S. D., & Angenent, L. T. (2005). Electricity generation from artificial wastewater using an upflow microbial fuel cell. *Environmental science & technology*, 39(14), 5262-5267.
- 20. Ghangrekar, M. M., & Shinde, V. B. (2007). Performance of membrane-less microbial fuel cell treating wastewater and effect of electrode distance and area on electricity production. *Bioresource Technology*, 98(15), 2879-2885.
- Logan, B. E., Murano, C., Scott, K., Gray, N. D., & Head, I. M. (2005). Electricity generation from cysteine in a microbial fuel cell. *Water Research*, 39(5), 942-952.
- 22. Watanabe, K. (2008). Recent developments in microbial fuel cell technologies for sustainable bioenergy. *Journal of bioscience and bioengineering*, 106(6), 528-536.



- 23. Delaney, G. M., Bennetto, H. P., Mason, J. R., Roller, S. D., Stirling, J. L., & Thurston, C. F. (1984). Electron-transfer coupling in microbial fuel cells. 2. performance of fuel cells containing selected microorganism mediator—substrate combinations. *Journal of Chemical Technology and Biotechnology*, 34(1), 13-27.
- 24. Nandy, A., Kumar, V., & Kundu, P. P. (2013). Utilization of proteinaceous materials for power generation in a mediatorless microbial fuel cell by a new electrogenic bacteria Lysinibacillussphaericus VA5. *Enzyme and Microbial Technology*, 53(5), 339–344.
- 25. Nandy, A., Kumar, V., & Kundu, P. P. (2016). Effect of electric impulse for improved energy generation in mediatorless dual chamber microbial fuel cell through electroevolution of Escherichia coli. *Biosensors and Bioelectronics*, 79, 796-801.
- 26. Logan, B., Cheng, S., Watson, V., & Estadt, G. (2007). Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. *Environmental science & technology*, 41(9), 3341-3346.
- Zhou, M., Chi, M., Luo, J., He, H., & Jin, T. (2011). An overview of electrode materials in microbial fuel cells. *Journal of Power Sources*, 196(10), 4427-4435.

