Journal of Biological Engineering Research and Review, 2019; 6(1): 14-20
ISSN: 2349-3232 Online
DOI: 10.5281/zenodo.15355616

Research Article

Use of Glycerol as Substrate for Electricity Generation Using *Citrobacter sp.* in a Double Chambered Microbial Fuel Cell

Rabiya Sultana[1$], Nirab. C. Adhikary[2], Mohan. C. Kalita[3], Narayan. C. Talukdar[1], Mojibur. R. Khan[1*]

[1]Molecular Biology and Microbial Biotechnology Laboratory, Life Sciences Division, Institute of Advanced Study in Science and Technology, Paschim Boragaon, Garchuk, Guwahati-781035, Assam, India

[2]Physical Sciences Division, Institute of Advanced Study in Science and Technology, Paschim Boragaon, Garchuk, Guwahati-781035, Assam, India

[3]Department of Biotechnology, Guwahati University, Guwahati -781014, Assam, India

***Corresponding author. E-mail: mojibur.khan@gmail.com (M R Khan)**

[$]Co-corresponding author. E-mail: sultana.rabiya@ymail.com (R Sultana)

ARTICLE INFO:

Article History:
Received: 11/02/2018
Revised: 25/03/2019
Accepted: 27/03/2019
Available Online: 06/04/2019

Keywords:
Microbial fuel cell; Glycerol; Citrobacter; Renewable energy; Electricity; Confocal microscopy

Citation: Sultana R, Adhikary NC, Kalita MC, Talukdar NC, Khan MR. Use of Glycerol as Substrate for Electricity Generation Using Citrobacter sp. in a Double Chambered Microbial Fuel Cell. Journal of Biological Engineering Research and Review. 2019, 6(1), 14-20

Abstract: Microbial fuel cells (MFCs) have the potential to convert organic substrates into electricity thus facilitating the strategies of renewable energy production. In recent years the exploration for newer energy resources for MFC has widened and in this context, the use of glycerol in bioenergy production was investigated to check its efficacy in electricity generation. Thus, the power generation of a double-chambered MFC was observed with glycerol as the substrate and *Citrobacter sp.* as the bacterium of interest. Here, the MFC system yielded a power density of 79.42 mW/m^2 with carbon cloth as the electrodes and Nafion as the proton exchange membrane. Further, the MFC system was optimized for the ambient temperature, in which the maximum voltage and current were obtained at 35^0C. In the study, the *Citrobacter sp.* showed its best performance at the optimum temperature of 35^0C. Likewise, the optimal pH for the MFC system in which the electrical output was high was observed in the pH value of 7.4. Moreover, the anodic bacterial biofilm analysis under confocal microscope provided evidence of the presence of live bacteria which were responsible for the efficient current generation of the MFC system.

INTRODUCTION

Microbial fuel cells (MFCs) have emerged as a promising tool in the field of renewable energy and had seized the attention of the researchers as a potential technology for energy generation [1-2]. A microbial fuel cell (MFC) is a device that generates electricity from the organic substrate with the aid of microorganisms acting as biocatalyst termed as exoelectrogenic bacteria [3]. These bacteria possess the potential to oxidize the substrate liberating electrons and protons, thus generating electricity [4-5]. The electricity production primarily relies on the type of renewable biomass and carbon sources used in the study of MFC [6].

The use of substrates plays a pivotal role in MFC wherein they act as carbon sources for the generation of electricity [7, 8]. These substrates act as energy avenues [6] for efficient power generation [9-14]. MFCs are devices that target energy production with the aid of substrates that are easily available and versatile. Amidst the range of substrates, glycerol acts as an attractive fuel source which is found abundantly in nature. Further, glycerol occurs as the structural constituent of many lipids and is also one of the compatible solutes produced during osmoregulation in yeasts [15]. Moreover, glycerol is found abundantly as a biodiesel byproduct which could aid in the production of high-value goods by process of biotransformation. Altogether, its occurrence in nature has enabled many microorganisms to use glycerol as the primary source of carbon and energy. This potential of glycerol to substitute carbon sources in industrial fermentation processes have attracted the attention to be used as a fuel source. Evidence of glycerol/O_2 biofuel cells [16] confirms the usage of glycerol as an effective energy bank. Moreover, reports on fermentation of glycerol by a wide

range of microorganisms for product synthesis [17] together with the conversion of glycerol to ethanol [18] approves the application of glycerol for energy production. As glycerol is easily available and high-value compounds are obtained in larger amounts by its reduction as compared to sugars [19], hence glycerol is opted as an efficient carbon source for current production. Thus, the use of glycerol in microbial fuel cells for electricity generation has paved the way for newer ventures and a promising base for future energy applications.

Furthermore, together with the substrate, the choice of microorganisms in a microbial fuel cell play a crucial role in the generation of electricity. Emphasis should be laid on the selection of microorganisms since these microbes could efficiently influence the power production by oxidation of the substrates yielding electrons [20]. Earlier studies provided the evidence of *Geobacteraceae* [21], *Shewanella putrefaciens* [22], *Gammaproteobacteria* [23], *Betaproteobacteria* [24], *Clostridia* [25], *Pseudomonas* [26], *Rhizobiales* [27] for electron production from substrates. Altogether, studies on pure cultures of bacteria viz. *Rhodopseudomonas palustris* strain DX-1 [28], *Geobacter sulfurreducens* [29] depicted that these cultures yielded power densities which comes within the same range as that of mixed culture communities [5]. Likewise, in a study by Xu and Liu 2011, an amount of 88.1 mW/m^2 was attained in a single chambered MFC in which *Citrobacter* sp. (SX-1) was used as an electrogenic bacteria [30]. Further, Chen *et al.* 2017, in their study had used *Citrobacter freundii* (a species of *Citrobacter*) with a consortium of bacteria which was used to decolourize Victoria Blue R and generated electricity from wastewater sludge [31].

Recently, various modifications had been implemented in the construction of MFC to enhance the power output and also to cut short the operating expenses of the system [32-33]. Studies on double chambered MFC have yielded different current and power densities under different conditions. Moreover, working on the reactor configuration and operation parameters of the microbial fuel cell can also lead to the enhancement of power output [34].

The present study basically aims for the power generation of *Citrobacter* sp. taking glycerol as a substrate in a double-chambered microbial fuel cell for enhancement of electrical energy. Since previously no study was undertaken with *Citrobacter* sp. along with glycerol, hence, the double chambered MFC was optimized for operational parameters like temperature and pH and the power density thus obtained is 79.42 mW/m^2.

MATERIALS AND METHODS

Inoculum preparation

The *Citrobacter* (strain RCE3 JN673775) [35] for the MFC experiment was obtained from the Microbial Repository Centre of Institute of Bioresources and Sustainable Development (IBSD), Imphal, India.

The growth medium in the MFC system constituted of the minimal medium (M9 medium) which comprised of salts such as disodium hydrogen phosphate (Na_2HPO_4) 33 g/L; potassium dihydrogen phosphate (KH_2PO_4) 15 g/L, sodium chloride (NaCl) 2.50 g/L, ammonium chloride (NH_4Cl) 5g/L, together with addition of glycerol (0.4%), $MgSO_4$ (1M) and $CaCl_2$ (1M) [36]. For the inoculum preparation, about 200 mL of M9 medium was taken in an Erlenmeyer flask into which a loopful of pure culture of *Citrobacter* from a fresh Nutrient agar (NA) plate was inoculated and incubated for 24 hours in a shaker incubator (Scigenics Biotech, Orbitek) at 150 rpm at 28°C [37]. After the specified time, about 1mL of bacterial culture was collected from the Erlenmeyer flask kept in a shaker incubator and used as anolyte along with the M9 medium. The process of anolyte (M9 medium and inoculum) preparation was performed under laminar airflow hood (Hitech, HH 1200) in sterile conditions.

MFC design and operational conditions

The double-chambered microbial fuel cell was constructed from low-density polyethylene (LDPE) boxes of 191×156×37mm (L×W×H mm) which was obtained from Tarsons. The anodic and cathodic chambers comprised of 200mL volume each constituting carbon cloths (EC-CC1) as the respective electrodes with an area of 6.25 x 10^{-4} m^2. The Nafion (NRE-212) of size 2.5 cm × 2.5 cm acts as the proton exchange membrane (PEM) which remained in the middle of the two compartments for the transport of ions (protons) from the anodic to the cathodic compartment [Fig.1].

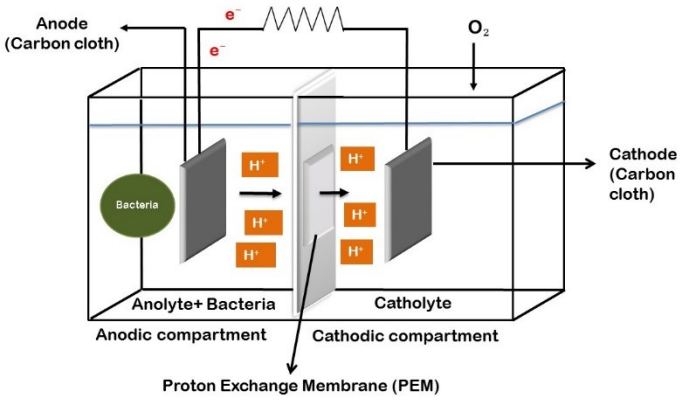

Fig. 1: Schematic diagram of double chambered microbial fuel cell

Prior to the experiment, the MFC chamber was sterilized by UV irradiation for about 20 minutes under a laminar airflow hood (Hitech, HH 1200). Subsequently, after the sterilization process, about 200 mL of minimal media (M9 media) with glycerol (0.4%) as the carbon source and the bacterial inoculum (1mL) was used as anolyte in the anodic compartment. Simultaneously, about 200mL distilled water along with NaCl (0.18 g/L) was used as catholyte in the cathodic compartment of the MFC system and the electrodes were connected by copper wire to the external circuit. The MFC experiments were executed in replicates of three in a temperature controlled incubator (CALTAN, NSW 152). The current and voltage thus generated were recorded by a voltmeter and ammeter respectively.

Experimental parameters

The double-chambered MFC system was optimized for operational conditions of temperature and pH taking glycerol as the carbon source and *Citrobacter* sp. (Strain RCE3 JN673775) as the bacterium of interest. For optimization of temperature, the MFC system was incubated at 25°C, 30°C and 35°C. For each individual temperature value, three MFC

replicates were run at each case. Altogether, for the optimization of the pH, experiments were performed at pH 6, pH 7, pH 7.4 and pH 8 for the desired pH taking three MFC replicates in each condition.

Confocal Microscopy

For the study of the bacterial biofilms in the anodic compartment of the double chambered microbial fuel cell, the carbon cloth in the anode chamber was removed by dismantling the fuel cell. Proper care was taken not to touch the surface of the anodic carbon cloth while dissembling. The removal of the anodic carbon cloth was followed by the staining of the bacterial biofilm by the LIVE/DEAD BacLight Bacterial Viability Kit (L7012) (Molecular Probes, Eugene). The staining was performed according to the guidelines provided with the kit. On completion of the staining protocol, the carbon cloth was placed gently on a glass slide, onto which few drops of BacLight mounting oil were added. The bacterial biofilm was studied under a confocal laser scanning microscope with a Leica DMi8 with a 10X objective lens wherein the two-dimensional images were prepared.

RESULTS AND DISCUSSION

The *Citrobacter* sp. belongs to Gammaproteobacteria, Enterobacteriales and Enterobacteriaceae and is abundantly found in soil, water and sewage. This bacteria acts as a significant bioremediation tool [37] since it has the unique capability to withstand both in the presence and absence of electron acceptors. Former reports on the performance of *Citrobacter* sp. for electricity generation exhibited a power density of 204.5 mW/m^2 with the strain *C. freundii* Z7 [38] and a current density of 205 mA/m^2 [30] with the strain *Citrobacter* SX-1 taking sodium citrate as a substrate in a single chamber air-cathode. In our study, we have opted for *Citrobacter* (strain RCE3 JN673775) which is a rhizospheric bacteria obtained from the roots of Mandarin orange [35]. Studies revealed that rhizospheric bacteria are believed to be endowed with the capability to generate electricity [39] and report from Xu and Liu for electricity generation by *Citrobacter* sp. have confirmed it [30].

Selection of substrates plays a very important role in microbial fuel cells for efficient power generation. These substrates get oxidized generating protons and electrons which in turn affects the power generation of the MFC system. Previous studies revealed the utilization of a varied number of substrates like glucose, acetate, butyrate, propionate etc. [6] in the study for microbial fuel cells with electron producing bacteria like *Geobacter sulfurreducens* [29], *Shewanella putrefaciens* [22] etc. The aforesaid experiments corroborated the growth of the bacteria to be related to the power efficiency of the fuel cell. More the growth of the bacteria, more is the electrical output.

Hence, in order to enhance the electrical output of a double-chambered microbial fuel cell, the *Citrobacter* sp. (strain RCE3 JN673775) along with glycerol as the substrate was used to study the operational conditions of the system. One of the crucial factors for electricity generation is the operating temperature of the MFC system. The optimal temperature effects the bacterial growth rate and power generation of the fuel cell and is one of the prerequisites for efficient energy generation. Thus, in our study, the operation of the MFC system for temperature optimization was performed at temperature values of 25°C, 30°C and 35°C with three MFC replicates in each case. The selection of the temperature range of the MFC system was based on the working temperature of the *Citrobacter* sp. which perform best at the temperature range of 22°C-35°C [40]. The voltage and current generated in the different temperature values showed a spike when the temperature was raised to 35°C as compared to that at 25°C and at 30°C. The maximum voltage attained by the system was found to be 599.5 mV and current of 82.8 μA at 35°C (Fig. 2).

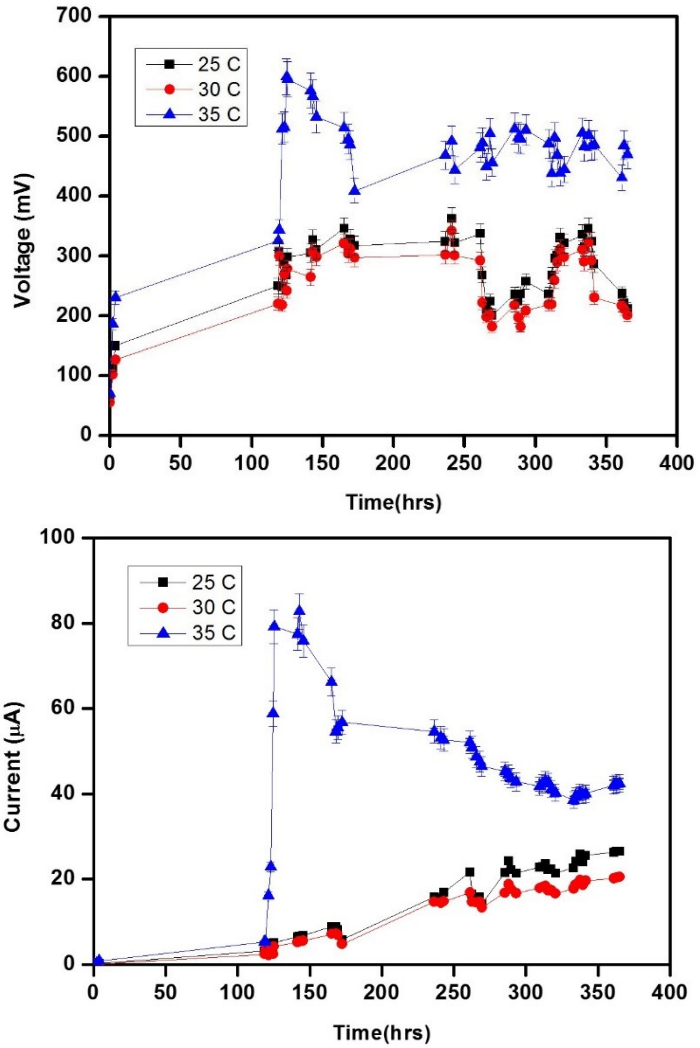

Fig. 2: Voltage and Current graphs during temperature optimization of the MFC system.

The spike of electricity could be attributed to the enhanced growth of the bacteria at 35°C due to optimal conditions as compared to that of other temperature values. The enriched bacterial growth resulted in the increased metabolic activity which in turn led to the oxidation of substrates yielding more energy. Further, glycerol promoted the energy efficiency of the bacteria thus acting as a potent carbon and versatile energy source for the MFC system [40-41]. Studies on glycerol utilization in the MFC system by *Bacillus subtilis* exhibited an upsurge of power generation proving to be a potential substrate for the generation of electricity in MFC systems [42].

Moreover, the *Citrobacter* sp. yielded a power density of 79.42 mW/m^2 (Fig.3) at 35°C which is maximum as compared to the power densities obtained at 25°C and at 30°C.

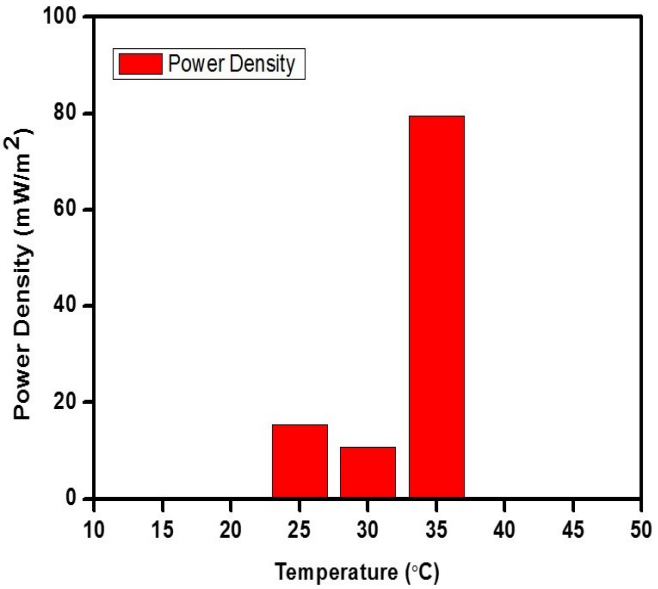

Fig. 3: Calculated power density of the MFC system at temperature values of 25°C, 30°C and 35°C

The spike in the power density clearly revealed the maximum activity of the *Citrobacter* sp. due to favourable growth conditions of the bacteria generating a maximum electrical output. The decrease of power density in the temperature values of 25°C and at 30°C showed that the bacterium did not meet the optimal requirements of growth which led to the decline of MFC performance.

Besides temperature, pH also influences the growth of the bacteria thus leading to variation in the energy output. Slight variations in pH could affect the bacterial growth in the anodic chamber due to which the power generation of the system could be altered. Hence, the current generation was monitored for the ambient pH of the anodic medium of the MFC system with glycerol as the substrate. The anodic medium in our study of the MFC system comprised of the minimal medium (M9 media). In order to check the efficacy of the *Citrobacter* sp. in the medium, different pH values of pH 6, 7, 7.4 and 8 were selected to determine the optimal pH. The choice of pH values was based on the pH of M9 medium (pH 7.4) and accordingly related to it, the lower and upper pH values were selected. The results revealed that amidst the pH values of pH 6, 7, 7.4 and 8, the optimum pH was obtained at pH 7.4 (Fig. 4). The experiment was operated with three MFC replicates in each case in a temperature controlled incubator at the optimized temperature of 35°C. In Fig. 4, the voltage and current generation increased at pH 7.4 while it decreased in the latter cases. The enhancement of electrical energy could be credited to the metabolic activities of the bacterium leading to the production of electrons and protons in the favourable pH [43]. Studies pertaining to pH made it quite evident that the production of electricity is directly influenced by the pH of the electrolyte of the MFC system [43]. Previous MFC experiments exhibited a decrease near acidic pH (5.2) which was gradually increased as the pH reached to the neutral range of pH 7 [44]. Further, earlier reports stated that the neutral pH provided the optimum environment for the bacteria at which the bacterial metabolism showed the maximum activity [45-47]. Henceforth, the optimised pH observed in our study clearly revealed the rise of power density due to the maximum activity of the bacterium in the neutral range of pH 7.4.

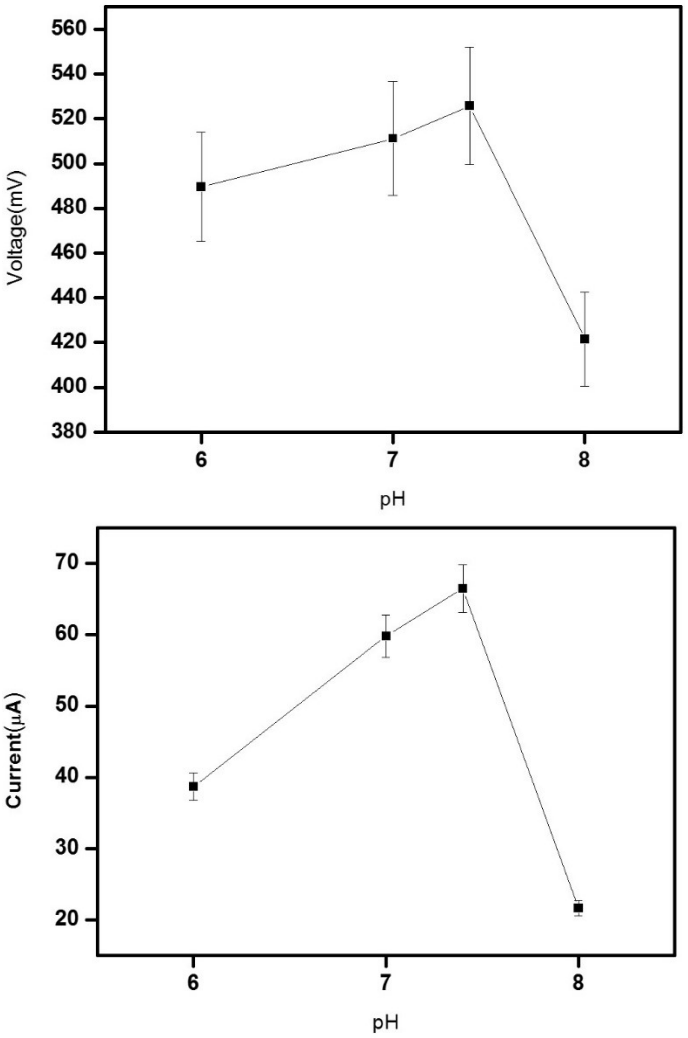

Fig. 4: The voltage and current output of the MFC at different pH of the media for *Citrobacter* sp. at the operational temperature of 35°C

Previous studies on the anodic bacterial biofilm illustrated that the power output from the MFC system was due to the attached bacterial cells onto the surface of the electrode [43, 48]. The cells of the bacteria get deposited on the electrode surface forming a layer resulting in a bacterial biofilm. Studies elucidated that the thickness of the biofilm is related to the power production i.e. thicker the biofilm higher is the energy output. Further, the inability of the bacteria to adhere to the anode surface acts as a limiting factor in the generation of electricity [49]. These findings provided an insight into the correlation of bacterial growth and current production in the MFC system in which the bacterial biofilm acts as a significant factor. Hence, in order to check the impact of bacterial growth in our study for electricity generation, anodic biofilm images were captured under Confocal Microscopy at the beginning and during the experiment.

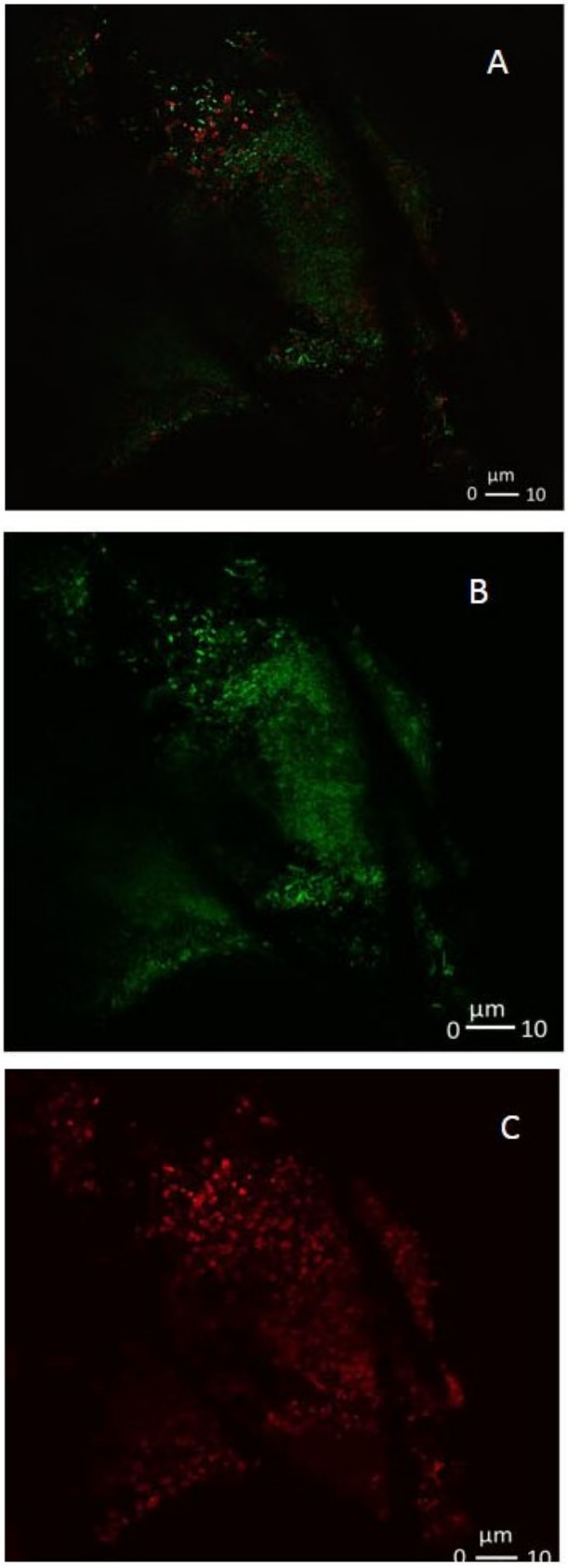

Fig. 5: Confocal microscopy image on anodic carbon cloth biofilm in a magnification of 10X. (A) Biofilm with live and dead *Citrobacter* in the carbon cloth; (B) Live bacterial biofilm with green fluorescence; (C) Dead bacterial biofilm with red fluorescence.

The results showed that in the anodic compartment, the *Citrobacter sp.* forms a layer of biofilm on the surface of the anodic carbon cloth wherein the carbon cloth fibres get completely coated by the bacterial biofilm. The use of the LIVE/DEAD BacLight kit (L7012) (Molecular Probes, Eugene) enabled the visualization of the viable cells by displaying the cells with a green fluorescence. And when the cells remained in a non-growing stage, the kit enabled its visualization with a red fluorescence (Fig. 5). Thus, these Confocal Microscopy images provided the evidence of living bacteria in the bacterial biofilm thus generating electricity which eventually increased as the biofilm layer got gradually deposited on the carbon cloth.

CONCLUSION

This study primarily focused on the optimization of operational parameters of temperature and pH in a double-chambered MFC system with glycerol as the carbon source. A power density of 79.42 mW/m^2 was observed with the bacterium *Citrobacter* sp. in the anodic chamber of the MFC system in the optimized temperature of 35°C and at an optimal pH of 7.4. Altogether, the images of the bacterial biofilm observed under confocal microscope indicated the role of bacterial biofilm in the production of electricity. These findings highlighted the role of glycerol as an efficient energy source for generating electricity with the bacterium *Citrobacter* sp. in a double-chambered MFC system.

Acknowledgement: This work was supported by the UGC, New Delhi for MANF fellowship (Ref No. F1-17.1/2015-16/MANF-2015-17- ASS-51613) and also the Institute of Advanced Study in Science and Technology (IASST).

Conflict of Interest: The authors declare no financial or commercial conflict of interest.

REFERENCES

1. Li M, Zhou M, Tian X, Tan C, Mcdaniel CT, Hassett DJ et al. Microbial fuel cells (MFC) power performance improvement through enhanced microbial electrogenicity. Biotechnol Adv 2018, 36(4):1316-1327.
2. Logan BE, Rabaey K. Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies. Science. 2012;337:686–90.
3. Logan BE. Exoelectrogenic bacteria that power microbial fuel cells. Nat Rev Microbiol 2009;7:375–81.
4. Debabov VG. Electricity from microorganisms. Mikrobiologiia 2008;77(2):149–57.
5. Lovley DR. The microbe electric : conversion of organic matter to electricity. Curr Opin Biotechnol 2008;1–8.
6. Pant D, Van Bogaert G, Diels L, Vanbroekhoven K. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol 2010;101(6):1533–43.
7. Chaudhuri SK, Lovley DR. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nat Biotechnol 2003;21:1229–32
8. Chae K, Choi M, Lee J, Kim K, Kim IS. Effect of different

substrates on the performance , bacterial diversity , and bacterial viability in microbial fuel cells. Bioresour Technol 2009;100(14):3518–25
9. Rabaey K, Lissens G, Siciliano SD, Verstraete W. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnol. Lett 2003; 25:1531-1535
10. 10 Logan BE, Murano C, Scott K, Gray ND, Head IM. Electricity generation from cysteine in a microbial fuel cell. Water Res 2005;39:942–52.
11. Liu H, Cheng S, Logan BE. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environ Sci Technol 2005;39(2):658–62.
12. Scott K, Murano C. Microbial fuel cells utilising carbohydrates. J Chem Technol Biotechnol 2007
13. Jang JK, Chang IS, Hwang HY, Choo YF, Lee JY, Cho KS, et al. Electricity generation coupled to oxidation of propionate in a microbial fuel cell. Biotechnol Lett. 2010, 32:79-85
14. Kiely PD, Regan JM, Logan BE. The electric picnic : synergistic requirements for exoelectrogenic microbial communities. Curr Opin Biotechnol 2011;22(3):378–85
15. Wang ZX, Zhuge J, Fang H, Prior BA. Glycerol production by microbial fermentation: a review. Biotechnol Adv 2001; 19:201–23
16. Arechederra, RL, Treu, BL, Minteer, SD.Development of Glycerol/O2 biofuel cell. J. Power Sources 2007; 173, 156–161
17. Yazdani, SS, Gonzalez, R. Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. Curr. Opin. Biotechnol 2007; 18, 213–219.
18. Yazdani, SS, Gonzalez, R. Engineering Escherichia coli for the efficient conversion of glycerol to ethanol and co-products. Metab. Eng 2008; 10, 340–351
19. Dharmadi Y, Murarka A, Gonzalez R. Anaerobic fermentation of glycerol by Escherichia coli: a new platform for metabolic engineering. Biotechnol Bioeng 2006; 94:821–9.
20. Lovley DR. Microbial fuel cells: novel microbial physiologies and engineering approaches. Curr. Opin. Biotechnol 2006, 327-332
21. Caccavo F, Lonergan DJ, Lovley DR, Davis M, Stolz JF, Mcinerneyl MJ. Geobacter sulfurreducens sp. nov., a hydrogen- and acetate-oxidizing metal-reducing microorganism.Appl. Environ. Microbiol 1994;60(10):3752–9.
22. Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH. A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. Enzyme Microb Technol 2002;30(2):145–52.
23. Kim, GT, Webster, G, Wimpenny, JWT, Kim, BH, Kim, HJ,Weightman, AJ. Bacterial community structure , compartmentalization and activity in a microbial fuel cell. J Appl Microbiol 2006;101, 698–710.
24. Kim JR, Jung S, Regan JM, Logan BE. Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. Bioresour Technol 2007; 98:2568-2577.
25. Rismani-yazdi H, Christy AD, Dehority BA, Morrison M, Yu Z, Tuovinen OH. Electricity Generation From Cellulose by Rumen Microorganisms in Microbial Fuel Cells. Biotechnol Bioeng 2007;97(6):1398–407
26. Freguia S, Hoi E, Boon N, Man K, Keller J, Rabaey K. Microbial fuel cells operating on mixed fatty acids. Bioresour Technol 2010;101(4):1233–8
27. Ishii S, Shimoyama T, Hotta Y, Watanabe K. Characterization of a filamentous biofilm community established in a cellulose-fed microbial fuel cell. BMC Microbiol 2008;12:1–12.
28. Xing D, Zuo Y, Cheng S, Regan JM, Logan BE. Electricity generation by Rhodopseudomonas palustris DX-1. Environ Sci Technol 2008; 42:4146-4151
29. Dumas C, Basseguy R, Bergel A. Electrochemical activity of *Geobacter sulfurreducens* biofilms on stainless steel anodes. Electrochimica Acta 2008; 5 (16): 5235-5241
30. Xu S, Liu H. New exoelectrogen Citrobacter sp. SX-1 isolated from a microbial fuel cell. J Appl Microbiol 2011;111(5):1108–15.
31. Chen C, Tsai T, Wu P, Tsao S, Huang Y. Toxic / Hazardous Substances and Environmental Engineering Selection of electrogenic bacteria for microbial fuel cell in removing Victoria blue R from wastewater. Taylor & Francis 2017;4529.
32. Kim JR, Min B, Logan BE. Evaluation of procedures to acclimate a microbial fuel cell for electricity production. Appl Microbiol Biotechnol. 2005;68(1):23–30.
33. Gil G, Chang I, Kim BH, Kim M, Jang J, Park HS, et al. Operational parameters affecting the performance of a mediator-less microbial fuel cell. Biosens Bioelectron 2003;18:327–34.
34. Liu, H, Cheng, S, Logan, BE. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environ. Sci. Technol. 2005; **39**, 658-662.
35. Thokchom E, Kalita MC. Isolation , screening , characterization , and selection of superior rhizobacterial strains as bioinoculants for seedling emergence and growth promotion of Mandarin orange (Citrus reticulata Blanco). Can J Microbial 2014; **60**, 85–92
36. Sambrook J, Russell RW. Molecular cloning: A laboratory manual, 3rd ed. Cold spring harbor laboratory press, cold spring harbor, N.Y. 2001
37. George MG. Bergey's manual of systematic bacteriology, Vol 2. *The Proteobacteria: Part B the Gammaproteobacteria* 2nd edn. Springer, New York 2005; 651.
38. Huang J, Zhu N, Cao Y, Peng Y, Wu P, Dong W. Exoelectrogenic bacterium phylogenetically related to *Citrobacter freundii* isolated from anodic biofilm of a microbial fuel cell. Appl. Biochem. Biotechnol 2015; 175, 1879–1891.
39. S. Ishii, Y. Hotta, and K. Watanabe, "Methanogenesis *versus* Electrogenesis: Morphological and Phylogenetic Comparisons of Microbial Communities," Biosci. Biotechnol. Biochem. 2008; vol. 72, no. 2, pp. 286–294.
40. Chung R, Moon DJ, Chang YN, Chung DS, Kong T, Kim J. The Cellulolytic Bacteria R . albus for Improving the Efficiency of Microbial Fuel Cell. Journal of Sustainable Bioenergy Systems 2018; 08(02):36-46
41. Du Z, Li H, Gu T. A state of the art review on microbial fuel cells : A promising technology for wastewater treatment and bioenergy. Biotechnol Adv 2007;25:464–82.
42. Roshan V, Chen C, Chen C, Chen H, Tseng M, Jean J, et al.

Glycerol degradation in single-chamber microbial fuel cells. Bioresour Technol 2011;102(3):2629–34.
43. Ishii, S, Shimoyama, T, Hotta, Y, Watanabe, K. Characterization of a filamentous biofilm community established in a cellulose-fed microbial fuel cell. BMC Microbiol 2008; 12, 1–12.
44. Ren ZY, Ward TE, Regan JM. Electricity production from cellulose in a microbial fuel cell using a defined binary culture. Environ. Sci. Technol 2007; 41 (13), 4781–4786.
45. Puig S, Serra M, Coma M, Cabré M, Balaguer MD, Colprim J. Effect of pH on nutrient dynamics and electricity production using microbial fuel cells. Bioresour. Technol 2010; 101(24) 9594–9599.
46. Yuan Y, Zhao B, Zhou S, Zhong S, Zhuang L. Electrocatalytic activity of anodic biofilm responses to pH changes in microbial fuel cells. Bioresour. Technol 2011;102 (13) 6887–6891
47. Behera M, Ghangrekar MM. Performance of microbial fuel cells in response to change in sludge loading rate at different anodic feed pH. Bioresour. Technol 2009;100,5114–5121.
48. Liu Y, Climent V, Berná A, Feliu JM.Effect of Temperature on the Catalytic Ability of Electrochemically Active Biofilm as Anode Catalyst in Microbial Fuel Cells. Electroanalysis 2011; 23(2)387–394.
49. Sharma Y, Parnas R, Li B. Bioenergy production from glycerol in hydrogen producing bioreactors (HPBs) and microbial fuel cells (MFCs). International Journal of Hydrogen Energy 2011; 36 (6):3853-3861.

About Author

Dr. Mojibur R. Khan is currently working as an Associate Professor at the Institute of Advanced Study in Science and Technology (IASST), Paschim Boragaon, Guwahati, Assam. He had graduated from Assam Agricultural University (Jorhat) in the year 1999 with specialization in Plant Breeding and Genetics and also completed M.Sc. in Agricultural Biotechnology (2002). He had completed his PhD on molecular plant-microbial interactions from University College Dublin (UCD) (National University of Ireland) under the guidance of Prof. Fiona Doohan in 2006.