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# Study of the Efficiency of a Double-chambered Microbial Fuel Cell using *Citrobacter* sp

**Rabiya Sultana[1*], Nirab. C. Adhikary[2], Mohan. C. Kalita[3], Narayan. C. Talukdar[4], Mojibur. R. Khan[5]**

[1,5]Life Sciences Division, Institute of Advanced Study in Science and Technology, Vigyan Path, Paschim Boragaon, Garchuk, Guwahati-781035, Assam, India.
[2]Physical Sciences Division, Institute of Advanced Study in Science and Technology, Paschim Boragaon, Garchuk, Guwahati, Assam, India
[3]Department of Biotechnology, Gauhati University, Guwahati -781014, Assam, India
[4]Molecular Biology and Microbial Biotechnology Laboratory, Life Sciences Division, Institute of Advanced Study in Science and Technology Boragaon, Garchuk, Guwahati, Assam, India

***Corresponding author:** sultana.rabiya@ymail.com Ph No. +919859488769*

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***Abstract-*** Microbial fuel cells (MFCs) are devices that use bacterial metabolism to convert chemical energy in organic matter to electrical energy from a wide range of organic substrates. In this work, the efficiency of a double-chambered microbial fuel cell was studied by taking *Citrobacter* sp.as the bacterium of interest, under different operational conditions with sucrose as the carbon source. Both the chambers of the MFC were separated by Nafion i.e. the proton exchange membrane (PEM) while the carbon cloths act as the respective electrodes. The maximum power density measured in this system was found as 125.67 mW/m². In this study, the growth condition of the bacteria was optimized for ambient temperature, which clearly revealed the temperature dependence of the MFC system for production of maximum current and voltage. Moreover, a pH optimization test of the MFC system was performed wherein the performance of the *Citrobacter* sp. was found to be better at pH 7.4 as compared to other pH values.

***Keywords*:** *Citrobacter*, double-chambered microbial fuel cell, proton exchange membrane, electrical power density, exoelectrogenic bacteria

## I. INTRODUCTION

Microbial fuel cells (MFCs), in recent years, had apprehended the attention as an alternative source of energy and hence varied aspects of MFC had been studied by many researchers [1-3]. The MFC technology involves certain micro-organisms termed as exoelectrogens which oxidize the organic matter to electricity generating ions (protons) and electrons, thus highlighting the biocatalytic efficiency of the microbes [4-7]. The concept of MFC could be dated to the year 1911 wherein M. C. Potter demonstrated the generation of electricity with the aid of bacteria like *Escherichia coli* and *Saccharomyces* [8] which was considered as the birth of microbial fuel cells [9]. Microbial fuel cells are present in different forms and structures. In case of a double-chambered microbial fuel cell, it generally consists of two chambers, i.e. the anodic and cathodic chamber separated by a membrane which allows only protons to pass through, known as the proton exchange membrane (PEM). The anodic compartment generally includes the substrate and the microorganism wherein the substrate gets oxidized yielding $CO_2$, protons and electrons. The protons can move through the proton exchange membrane to the cathodic compartment which thereby produces an electrical potential difference between the two chambers. When the electrodes are connected, electrons in the anodic chamber pass through an external electric circuit to the cathode [10-12]. Efforts have been made by many researchers to improve the performance [13-19] and also to reduce the construction and operating costs of MFCs [20-21] in the last few decades.

In recent years, varied modifications of the MFC were constructed for efficient power generation which include MFC models like microsized MFC with air-cathode MFC [22], multiwalled carbon nanotubes [23], mediator-less MFCs [24-25], microbial electrolysis cells, microbial desalination cells [9], miniature microbial fuel cell (mini-MFC) with electron mediators [26], a single chamber microbial fuel cell (SCMFC) containing eight graphite electrodes (anodes) and a single air cathode [22]. Further,

 

MFC designs like membrane less MFC with separated electrode chambers [27], separator electrode assembly (SEA) type MFC [28], mediator-less and membrane-less MFC (ML-MFC) [29], MFC with anaerobic fluidized bed membrane bioreactor (MFC-AFMBR) [30] and recirculation microbial desalination cell (rMDC) [31] were also worked out for the enhancement of the power output of MFC.

The purpose of the present study was to assess the performance of electricity generation of the bacterium *Citrobacter* sp. (Strain RCE3 JN673775) in a double-chambered MFC. In the present work, the construction of the dual chambered MFC was followed by the inoculum preparation for operation of the fuel cell. Further, to obtain the maximum electrical output of the MFC system, operational factors like temperature and pH were optimized with sucrose as the carbon source. Henceforth, Scanning electron microscopy (SEM) was performed to check the bacterial biofilm formation in relation to electricity generation.

## II. RELATED WORK

Various studies centering double chambered microbial fuel cell had been executed for enhancement of power efficiency. These include a report on double chambered MFC by Chaudhuri and Lovley 2003, wherein it was revealed that the maximum current obtained was 74 $mA/m^2$ at a voltage of 445 mV. Further, Kim *et al.* 2002, stated that their study on double chambered MFC yielded a current of approximately 40 μA, with a voltage of 0.6 V with a power density of $4.7 \times 10^{-3}$ $W/m^2$. Moreover, Rhoads *et al.* 2005 designed a double chambered fuel cell which comprised of reticulated vitreous carbon with *Klebsiella pneumoniae* (ATCC No. 700831) in the anodic compartment and *Leptothrix discophora* SP-6 in the cathodic compartment yielding power densities of 126.7± 31.5 $mW/m^2$ & 3.9± 0.7 $mW/m^2$ respectively. On the other hand, in a work of Xu and Liu 2011, using a single chambered MFC with carbon cloth/ platinum as electrodes and taking *Citrobacter* sp. (SX-1) as an electrogenic bacteria, an amount of 88.1 $mW/m^2$ was achieved. Moreover, Yu *et al.* 2017, worked on the double chambered fuel cell to check the electricity production and nitrite denitrification into the cathode. Recently, a species of *Citrobacter* i.e. *Citrobacter freundii* along with a consortium of bacteria was used to decolorize Victoria Blue R and resulted in the generation of electricity from wastewater sludge [32]. Till date, no study was reported stating the optimal parameters of *Citrobacter* sp. in a double-chambered microbial fuel cell. Lately, generation of electricity and Cr (VI) reduction in a double-chambered microbial fuel cell has also been reported by Li *et al.* 2018 [33] . Further work on double chambered MFC is performed wherein dynamic membranes are used as separators for efficient power generation [34]

## III. METHODOLOGY

### MFC Construction

A two-chambered fuel cell comprising of cathodic and anodic compartments of 200 ml each was constructed from low-density polyethylene (LDPE) boxes of 191×156×37mm (L×W×H mm) obtained from Tarsons. The electrodes in the microbial fuel cell constituted of carbon cloths (EC-CC1) as shown in Fig.1. The figure displayed that the chambers of the fuel cell were separated by a proton exchange membrane, i.e. Nafion (NRE-212) of size 2.5 cm × 2.5 cm that aid in the conduction of ions from the anodic compartment to the cathodic compartment. In the anodic compartment, about 1ml of the bacterial inoculum was inoculated with sucrose as the substrate while on the other hand, the cathodic compartment was filled with distilled water (200 ml) along with the addition of NaCl (0.18 $gl^{-1}$ ) to increase the conductivity of the compartment [35].

### Medium and Inoculum preparation

During the experiment, the minimal medium or M9 medium was prepared by adding salts viz. disodium hydrogen phosphate ($Na_2HPO4$) 33 $gl^{-1}$; potassium dihydrogen phosphate ($KH_2PO4$) 15 $gl^{-1}$, sodium chloride (NaCl) 2.50 $gl^{-1}$, ammonium chloride ($NH_4Cl$) 5 $gl^{-1}$, together with the addition of substrate i.e. sucrose (0.4%), $MgSO_4$ (1mol $l^{-1}$) and $CaCl_2$ (1mol $l^{-1}$). After preparation, the medium was autoclaved at 121°C for 15 min [36].

In this work, a bacterium named *Citrobacter* sp. (Strain RCE3 JN673775) which was obtained from the Microbial Repository Centre of Institute of Bioresources and Sustainable Development (IBSD), Imphal, India was used in the MFC experiments [37]. To prepare the inoculum, a fresh Nutrient agar (NA) plate was streaked with a stock culture of *Citrobacter*. Once the bacteria formed colonies, then a loopful of pure culture colonies was transferred to 200 ml of M9 medium in an Erlenmeyer flask and kept on shaking condition for incubation of 24 hours in a shaker (Scigenics Biotech, Orbitek) at 150 rpm at 28°C [38]. After 24 hours, about 1mL of bacterial culture was collected from the exponential phase of the bacterium in the Erlenmeyer flask and used as inoculum in the anodic chamber of the microbial fuel cell along with the M9 medium. The process for the preparation of the anolyte (M9 medium and inoculum) and catholyte was carried out under sterile conditions in a laminar airflow hood (Hitech, HH 1200).

### Operational conditions

After the MFC system was constituted with carbon cloths (EC-CC1) as the electrodes and Nafion (NRE-212) as the proton exchange membrane, the respective solutions of the anodic and cathodic chambers were prepared for the MFC experiment. Hence, about 200 ml of minimal media (M9 media) with sucrose (0.4%) as the carbon source along

 

with bacterial inoculum (1ml) was used as anolyte in the anodic compartment, while the catholyte comprised of 200 ml distilled water along with NaCl (0.18 $gl^{-1}$ ) in the cathodic compartment of the MFC system. The MFC chambers during the experiments were kept closed with their lids upon them. The experiments were operated taking three MFC replicates for each condition in a temperature controlled incubator (CALTAN, NSW 152) in a semi-aerobic condition in continuous mode which took place for about 200 hours.

**Experimental Conditions**

To optimize the required operating temperature of the MFC system, the microbial fuel cell was incubated at different temperatures viz. $25^0$C, $30^0$C and $35^0$C with three MFC replicate at each case. Furthermore, for pH optimization of the microbial fuel cell, experiments were conducted at different pH of different values viz. pH 6, pH 7 pH 7.4 and pH 8.

**Scanning Electron Microscopy (SEM)**

Bacterial biofilm on the anodic carbon cloth was examined using a scanning electron microscope (Zeiss, Sigma VP) at the end of the MFC experiment. Using sterile forceps and scissors, strips of the anodic carbon cloth were cut and air dried under laminar air flow (Hitech, HH 1200). The biofilm coated carbon cloth was then sputter coated with Au and the images were observed under SEM.

## IV. RESULTS AND DISCUSSION

In the anodic compartment of the microbial fuel cell, the microbes (*Citrobacter*) oxidize the organic substrate (electron donor) for growth, thus generating electrons and H+ ions (protons) [39]. The H+ ions (protons) produced were then passed through the proton exchange membrane (PEM) i.e. Nafion membrane towards the cathodic compartment and thereby a potential difference develops between the two compartments (Fig 1). The electrons generated in the anodic compartment were allowed to flow through the electrodes which were connected with an external circuit with the aid of copper wire. The generated current was then measured by a voltmeter and an ammeter at regular intervals.

The *Citrobacter* sp. is ubiquitous and commonly found in soil, water and sewage. Further, it is grouped in Gammaproteobacteria, Enterobacteriales and Enterobacteriaceae. *Citrobacter* is endowed with the capability to sustain both the presence and absence of electron acceptors and hence acts as an important bioremediation tool [40]. The *Citrobacter* (strain RCE3 JN673775) in our study is a rhizospheric bacteria which was isolated from the roots of Mandarin orange [37]. It is believed that the bacteria found associated with the rhizosphere, facilitate electricity generation [41] and since *Citrobacter* [37] belongs to the group of rhizospheric bacteria, hence its efficacy for electron production is checked in our microbial fuel cell studies.

Detailed research centering *Citrobacter* sp. in the microbial fuel cell has not been done till date. Only a few studies were performed involving *Citrobacter* sp. amidst which the isolation of a new strain of *Citrobacter* sp. (SX-1) by Xu and Liu on electricity production in a single chambered microbial fuel cell [41] could be mentioned. In our study, we have highlighted the power generation of the *Citrobacter* (strain RCE3 JN673775) in a double-chambered microbial fuel cell yielding a maximum power density of about 125.67 $mW/m^2$ which has not been reported yet.

In our study, the optimization of the operating temperature of the double chambered MFC system was worked out by taking three replicate MFCs of individual temperatures at 25°C, 30°C and 35°C with sucrose as a substrate in the anodic compartment. Since, *Citrobacter* sp. belong to the group of Gammaproteobacteria, Enterobacteriales and Enterobacteriaceae, its working temperature varies between 22°C-35°C [37] and hence the optimization of the MFC system has been performed in the temperature values of 25°C, 30°C and 35°C. The current and voltage were recorded accordingly and the power density obtained thereof is shown in Fig 3. The graph showed that the power density of the bacterium spiked at a temperature of 30°C yielding a value of about 125.67 $mW/m^2$. These results clearly depicted that the microbial growth of the *Citrobacter* sp. at 30°C exhibited the highest activity which resulted in the attainment of the highest electricity production of the MFC system.

It has been observed that the carbon source of a system plays a pivotal role in the generation of electricity. The carbon source acts as an energy bank for the microbial processes in the anodic compartment that affects the bacterial growth and ultimately influences the performance of the MFC system. Ieropoulos et.al. [42] in their study observed that sucrose was a better carbon source, yielding highest power and current output as compared to other carbon sources viz. glucose, fructose and maltose under same external circuit condition. The study stated that sucrose resulted in the rapid growth rate of bacteria while the other carbon sources (glucose, fructose and maltose) inhibited the growth rate [42]. Thus, the use of sucrose in our study has resulted in the increase in bacterial growth which is evident from the electrical output of the MFC system.

In Fig 3, it is shown that the power density increased when the temperature was raised from 25°C to 30°C while on the other hand, it decreased when the temperature was increased to 35°C. These changes in power density could be attributed to the growth and metabolic activity of the bacterium along with the increase or decrease of temperature. The decrease in MFC performance at 25°C and 35°C could be stated that the bacterium was sensitive to

 

lower (25°C) and also to a higher temperature (35°C) which affected the bacterial growth and ultimately the performance of the MFC system. Thus, the optimal temperature was obtained at 30°C yielding the maximum electrical output of the double chambered MFC system.

Altogether, the pH of the anodic medium also influences the power density and has significant effect in the energy production. The pH of the medium in the anodic chamber influences the metabolic efficiency of the bacterium and consequently affects the generation of protons and electrons. To investigate the pH effect of the minimal media (M9 media) in the anodic chamber for electricity generation of the double-chambered MFC, different pH values of the M9 media viz. pH 6, pH 7, pH 7.4 and pH 8 were opted for optimization. Since M9 media usually remains at the pH 7.4, hence pH values lower and upper than the pH value 7.4 were selected to check the efficacy of electricity generation by *Citrobacter* sp. at varied pH levels for optimization of the MFC system.

In Fig 4, it has been observed that the current and voltage produced at pH 7.4 was higher than that for pH 6, pH 7 and pH 8. This would result in the production of larger amount of electrons from the oxidation process in the anodic compartment of the double chambered MFC which would significantly contribute to the increase in electricity generation. The pH of the system directly influences the bacterial metabolism which affects the mechanism of electron and hydrogen ion generation [43]. Moreover, changes in the pH value tend the bacteria to respond accordingly and adjust different activities involving processes like degradation of amino acid, translocation of protons etc [43]. Altogether, based on the type of bacteria and its growth conditions, different changes are brought about in the physiological parameters like ion concentration, proton motive force etc [44]. According to previous findings, the optimum environment for the bacteria in the anodic chamber is found to be at neutral pH [45-47]. However, to clarify the aforesaid phenomena, further research is required in this field. Further, Ishii et al., 2008 in their observations reported that pH value is directly linked with the production of electricity. These results were influenced by the electrolyte of the MFC which was evident by the activity of microorganisms that showed more activity at optimal pH than at sub-optimal pH.

In our study, the voltage and current increased with increasing pH till the attainment of pH 7.4 and gradually declined at pH 8 (Fig 4). Similarly, Gil et. al performed experiments in a double-chambered MFC wherein it was observed that the optimal pH was achieved within the range of 7 to 8 yielding more energy than at pH 5 and pH 6 [48]. Besides, reports on low electricity production at acidic pH were also found in a two-chamber MFC wherein the power density consisted of only 10 % to that of the highest data observed [49]. Also, reports by Ren et al. [43] showed that electricity generation decreased at pH 5.2 for acidic fermentation products which were resumed gradually as the pH attained to 7.0. Hence, it can be concluded that bacteria within the neutral range of pH exhibit more activity as compared to the acidic and alkaline pH range.

Thus, for the optimization of the operating pH, MFCs with three replicates of individual pH values of the minimal media were run at the optimized temperature of 30°C in the double chambered microbial fuel cell. In Fig 4, it has been shown clearly that the voltage and current generated in case of pH 7.4 are maxima as compared to other pH values. The power enhancement in the pH 7.4 could be credited to the growth and metabolism of the bacterium at this specific pH, which clearly states that neutral pH provides the optimal conditions for the anodic bacterium which is in accordance with earlier findings [45-47]. Thus, based upon the electricity production by the bacterium in the anodic chamber at varying pH values, the optimal pH was observed to be at pH 7.4 wherein the bacterium exhibited the maximum current and voltage of the MFC system at 30°C.

The formation of bacterial biofilm on the anode has a direct effect on the production of electricity of the MFC system. You et.al [48] in their study stated that the decrease in bacterial growth on the anode leads to a decline in current production ultimately resulting in its termination. Thus, the growth of the bacteria exhibits some correlation to the production of electricity which shows the critical role of the bacterial biofilm in the MFC system. To investigate the correlation between the presence of bacterial biofilm and electricity generation, scanning electron micrographs were taken at the beginning and at the end of the MFC experiment. The results showed that the bacteria (*Citrobacter* sp.) formed a biofilm on the anodic carbon cloth after the experiment which resulted in electricity generation. While, on the other hand, the anodic virgin carbon cloth did not show any evidence of biofilm since the images were taken prior to the inoculum of the bacteria in the MFC experiment. Thus, due to the absence of bacterial biofilm in the virgin carbon cloth in the anodic chamber, there was no data observed in the case of current and voltage output. Hence, it was observed that the bacterial biofilm gradually developed resulting in a uniform layer forming a sheath wrapping the anodic carbon cloth which was examined with the aid of SEM as shown in Fig 5.

Thus, under optimized conditions, the MFC system was operated for 200 hours taking three replicates wherein the highest voltage of 590 mV and current of 213 µA values were obtained (Fig 6). Amidst the published reports, the aforementioned voltage and current generated in our experiment of double-chambered MFC with the *Citrobacter* sp. at 30°C and sucrose as the carbon source, is the maximum data generated till date.

 

## V. CONCLUSION AND FUTURE SCOPE

The present study reports the optimization of the MFC system for temperature and pH conditions with *Citrobacter* sp. as the bacterium of interest. A power density of 125.67 $mW/m^2$ was achieved in the optimum temperature of $30^0$C at the optimized pH value of 7.4. The enhancement of electricity generation could be attributed to the increase in bacterial growth of the bacterial biofilm on the anodic carbon cloth of the MFC system. These results indicate the importance of the double chambered MFC for efficient electricity generation using the *Citrobacter* sp. with sucrose as the substrate.

MFCs act as sustainable power supplies and possess potential for applications such as biosensors, wastewater treatment, medical devices, robotics etc. Hence, although the study of biofuel is still in its infant stage [49] but proper and extensive research in this field would lead us to attain our goal for an energy sustainable society.

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### Conflict of interest

The authors declare no financial or commercial conflict of interest.

### Author Contribution

Conceptualization – MRK, NCA, NCT; Methodology- MRK, NCA, NCT, MCK; Investigation- RS, Writing – RS, Original Draft - RS; Writing, Review & Editing - RS, MRK, NCA; Supervision- MRK, NCA, NCT, MCK

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**Figures**

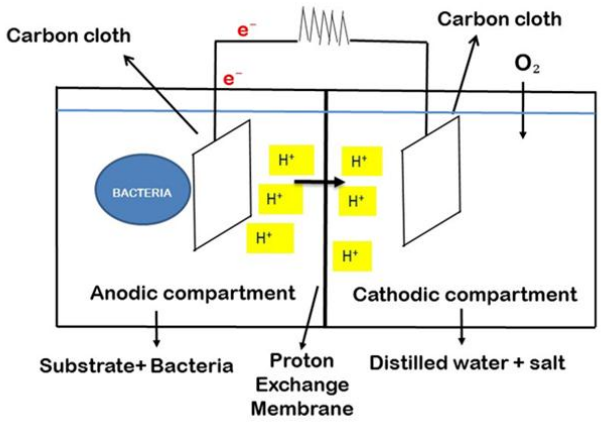


Figure 1. Schematic diagram of the double-chambered MFC system

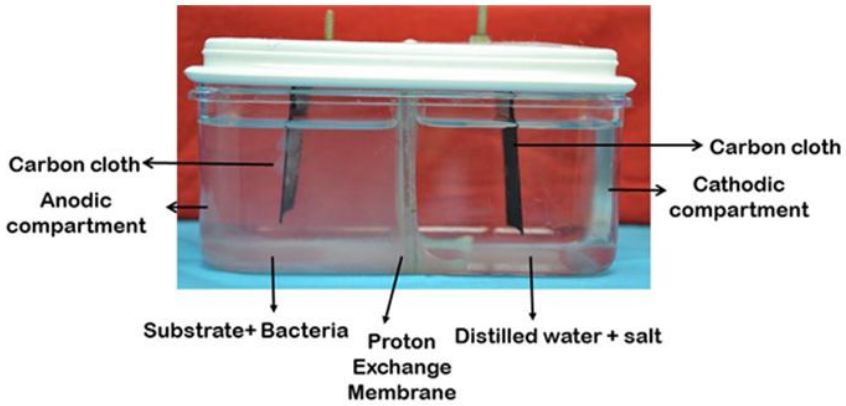


Figure 2. Photograph of the set-up of MFC

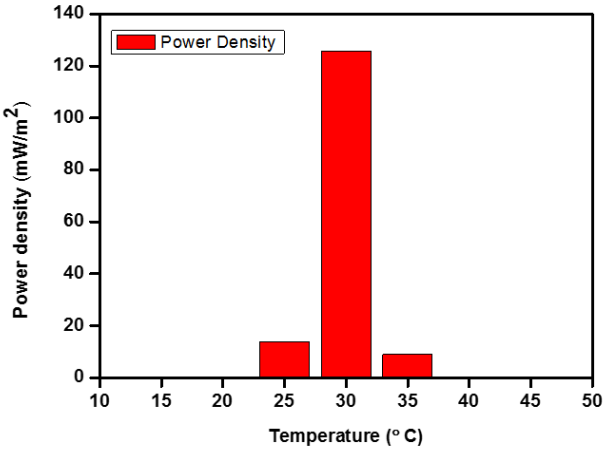


Figure. 3. Calculated power density of the MFC system at temperature values of 25°C, 30°C and 35°C

 

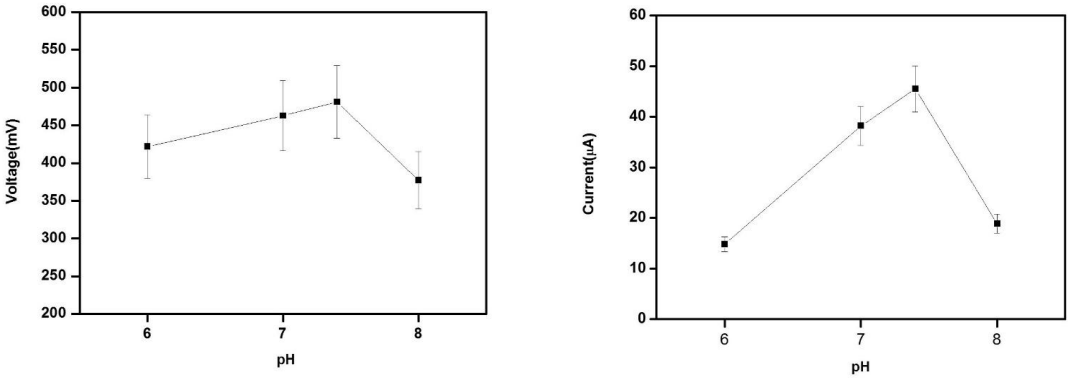


Figure 4. The voltage (A) and current (B) output of the MFC at different pH of the media for *Citrobacter* sp. at the operational temperature of 30°C

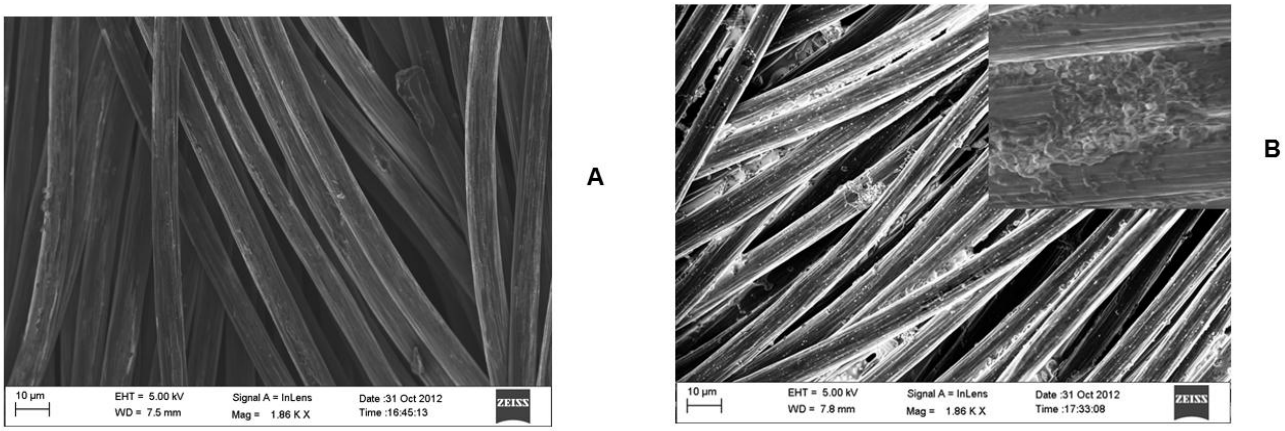


Figure 5. SEM images of the Anodic Carbon Cloth
A) Virgin Carbon Cloth-prior to experiment
B) Carbon Cloth-after experiment showing the magnified image in the inset

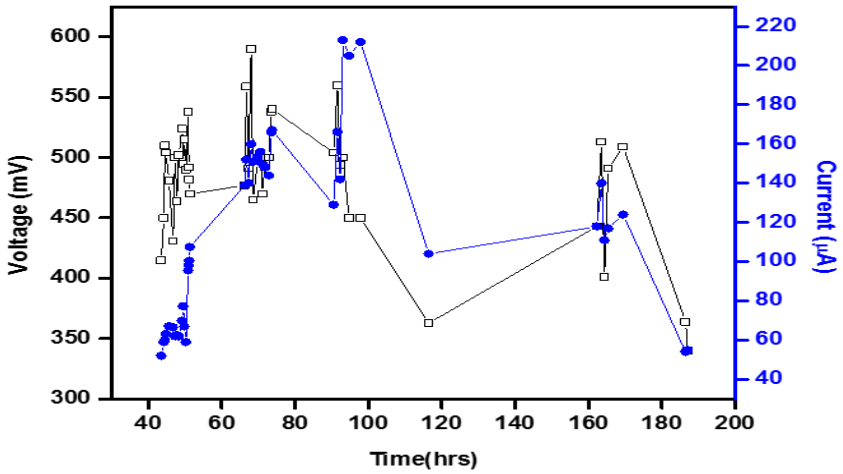


Figure 6. Current and voltage record of MFC at the optimized condition of 30°C at the operational pH of 7.4

 

## AUTHORS PROFILE

**Rabiya Sultana (RS)**
Ms. Rabiya Sultana is currently working as a PhD Research Scholar in the Institute of Advanced Study in Science and Technology (IASST) under Gauhati University. She has been awarded with the UGC Maulana Azad National Fellowship (MANF) and presently she is working as a UGC MANF PhD research fellow. Her research area deals with enhancement of electricity by microbial fuel cell (MFC).

**Nirab. C. Adhikary (NCA)**
Dr. Nirab. C. Adhikary is currently working at Physical Sciences Division in the Institute of Advanced Study in Science and Technology (IASST), Guwahati, Assam. He had completed his PhD in Experimental Plasma Physics from Gauhati University in the year 2010. He did his Postdoctoral Research work on Cryogenic Dusty Plasma Physics during 2010-11 at Yokohama National University, Japan. He has published several papers in journals of repute.

**Mohan. C. Kalita (MCK)**
Dr. M. C. Kalita is a Senior Professor and former Head (2007-2010) of the Department Department of Biotechnology, Gauhati University and also founder Head i/c (2009-2014), Department of Bioengineering and Technology, Gauhati University Institute of Science and Technology (GUIST). His diverse fields of research interests include Plant Biochemistry & Biotechnology, Ethno pharmacology and human diseases, Biofertilizers & Biopesticides, Microalgae bioenergy and biomitigation of $CO_2$, Nano biotechnology. Prof Kalita has published over 130 research papers of national and international repute.

**Narayan. C. Talukdar (NCT)**
Dr. Narayan. C. Talukdar is currently the Director of the Institute of Advanced Study in Science and Technology (IASST) and also the programme head of Biodiversity and ecosystem research, Division of Life Sciences. He was the former Director of the Institute of Bioresources and Sustainable Development (IBSD) and Scientist F in the Division of Microbial Resources, Imphal, Manipur, India. Prof Talukdar has published over 185 research items of national and international repute. His area of work deals with research on 1) interaction between macro and micro-organism, their implication in ecosystem functioning 2) Bio-inputs and Bioenergy and 3) Validation and integration of traditional knowledge based herbs of north east India.

**Mojibur. R. Khan (MRK)**
Dr. Mojibur R. Khan is currently working as an Associate Professor at the Institute of Advanced Study in Science and Technology (IASST), Guwahati, Assam. 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. He has published several research papers in journals of repute. His area of research are environmental genomics and alternative energy.

 