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Article

Sustainable Energy Generation of Anaerobic Electrogenic Bacteria from Soil Sludge by Microbial Fuel Cells

Mohamed Irfan Raja Sulthan[1], Selvakumar Sakthivel[2], Kanthimathi Muthusamy[3], Amutha Priya Thangarajan[4]*

[1]Department of Microbiology, Sri Ram Nallamani Yadava college of arts and science, Kodikurichi, Tenkasi 627804, Tamil Nadu, India, [2]Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, Tamil Nadu, India, [3]Sri Paramakalyani Centre for Excellence in Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, Tamil Nadu, India, [4]Department of Botany, G. Venkataswamy Naidu College, Kovilpatti, Tamil Nadu, India

ABSTRACT

Microbial fuel cells (MFCs) are an enticing environment friendly technology that employs electrochemically active microbes to convert organic materials into bioelectricity while causing no environmental damage, therefore combating global warming and energy scarcity. The present study's objective is to produce electricity using MFC and soil sludges. In both the presence and absence of oxygen supply, the electrogenic capacity of bacteria from different soil sludges, including river, agricultural, and garden sludges, was examined. A two-chambered MFC is constructed using an anode, a cathode, a proton exchange membrane, and a salt bridge as its constituent parts. Several observations indicate that this MFC may generate the maximum of up to 603 millivolts (0.603 volts) of electricity in the absence of an air pump. The two common electrogenic bacterial strains that are isolated from all the soil sludge samples are *Clostridium* spp. and *Geobacter* spp. However, since the activity of the bacteria gradually decreases and the voltage produced is not exceptionally high, this experiment has the drawback that it does not produce a voltage that lasts long. Although it has certain challenges, an MFC is an alternative essentially a solution for the renewable energy produced by bacterial activity that needs additional consideration, research, and development.

Key words: Microbial fuel cells, Electricity generation, Soil sludges, Electrogenic bacteria.

1. INTRODUCTION

The necessity for environmentally friendly energy sources is justified by the imminent energy scarcity and global warming due to excess utilization of non-renewable resources and greenhouse gas emissions. As the burning of fossil fuels release carbon dioxide (CO_2) and other pollutants into the atmosphere, the environment suffers from the use of non-renewable resources widely [1]. The use of biogenic bacteria in microbial fuel cells (MFCs) offers new insight into the production of energy utilizing a variety of substrates, which has drawn a lot of scientific attention to the topic. MFCs are a bio-electrochemical device that uses the activities of microbes to transform chemical energy found in organic substrates into electrical energy. To tackle global warming and the energy crisis, this alluring green technology employs electrochemically active microorganisms to convert organic materials into bioelectricity with no harm to the environment [2].

The use of organic wastes as substrate in MFC makes it an ecofriendly device that offers a dual benefit of bioelectricity generation and waste management [3-5]. MFCs uses electrogenic bacteria to convert the chemical energy of a particular substrate contained in wastes into electrical energy [6,7]. Redox reactions are the foundation for how MFCs technology functions. The bacteria oxidize the organic material and it releases protons, electrons, and CO_2. Bacteria removes electrons from the substrates which then move across a wire under a load (resistor) to the cathode where they combine with protons and oxygen to form water. When these electrons flow from the anode to the cathode, they generate the current and voltage to make electricity [8]. This is how bioelectricity is produced using the bacteria's natural metabolism with the help of MFCs. In anode chamber, substrates such as carbohydrates in the form soil sludge is used, the bacteria breakdown the organic matter in sewage and releases electrons, as a result, bioelectricity is produced in MFC.

Extensive research has been conducted to improve MFC performance through the fabrication of a new stack of MFCs. The electrogenic ability of bacteria from a different soil sludge such as river, agriculture lands, and garden sludge in presence and absence of air pump was also investigated in the present study.

2. MATERIALS AND METHODS

2.1. Materials

Analytical grade chemicals used in the present work and were purchased from Sigma Aldrich.

***Corresponding author:**
Amutha Priya Thangarajan,
E-mail: herbalpriya.t@gmail.com

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2.2. Collection of Sludge Samples

Thick, soft sludge samples were collected separately from three different sources such as river sludge, agricultural sludge, and garden mud sludge. The sludge samples collected had different physical traits such as color and texture. The samples were aseptically collected and transferred to the laboratory using sterile gloves and sterile polythene bags.

2.3. Isolation of Soil Bacteria

Soil samples were weighed (1 g) aseptically and were diluted with 100 ml sterile distilled water (10^{-2} dilution) and the sample was then serially diluted from 10^{-2} to 10^{-7} dilutions. From the serially diluted sample, 0.1 mL was taken from 10^{-6} to 10^{-7} dilution. The bacterial colonies were isolated on nutrient agar plates by spread plate technique. Then, all the plates were incubated for 24 h at 37°C in anaerobic incubator. Further, the isolates from the nutrient agar plates were streaked on the nutrient agar slants to get pure culture and for storage.

2.4. Isolation and Identification

To identify the exoelectrogenic bacteria, gram staining and biochemical test procedures were performed as described in Bergeys manual of determinative bacteriology [9].

2.5. MFC Chamber Construction

A two-chambered MFC is constructed as described by Li *et al*. [10] with minor modifications with its components including anode, cathode, and proton exchange membrane – a salt bridge. Two plastic containers were used as the chambers of MFC (Figure 1). One is an anaerobic anode chamber (Red container) and another one is an aerobic cathode chamber (Green container). Both the container lids had holes punched in them for the insertion of copper wire. For the salt bridge, holes were bored in sides of each container's wall. A folded iron mesh gauze was used as electrodes, the folds were bind together with copper wire. Both the electrodes were externally connected with the copper wire. One of the containers was filled with three kilograms of the soil sludge and the other container was filled with distilled water. The electrodes were kept submerged in them. The electrode immersed in container containing distilled water acts as cathode and the electrode in soil container acts as anode. The lids were closed tightly and sealed with the help of adhesive [11].

The salt bridge was prepared with a cotton wick/rope, salt, and water. 98 g of NaCl was dissolved in 100 mL of sterile distilled water and heated for 2 mints. The cotton rope is allowed to be soaked well in the salt water until the wick completely absorbs the salt water. This wick acts as a medium for proton transfer which will be flowed from water filled container. To prevent the salt bridge from detachment and leakage, it was tightly glued. This salt bridge acts as a proton exchange membrane through which only protons are permitted to diffuse over and it connects the two chambers of MFC. The electron counter flows through the external circuit through copper wire. The copper wire from both the containers was connected to the multi-meter probes using crocodile clips. The black probe was connected to the anode and red probe was connected to the cathode. The electrons flow from anode toward cathode and this current flow could be detected with a multitester and measured in millivolts (mV) with the help of multimeter. To enhance the efficiency of electron transmission, an air pump was turned on as it supplies more oxygen. The experiment was carried out at room temperature. The power output across the terminals was estimated by recording the readings from 20 to 200 mints for every 20 mints. The results were recorded as mean value.

3. RESULTS AND DISCUSSION

3.1. Identification of Electrogenic Bacteria

The two exoelectrogenic bacteria that were isolated from the soil sludges were identified as strain (1) *Clostridium* spp. and strain (2) *Geobacter* spp. through gram staining and a series of biochemical tests (Table 1). These two electrogenic bacterial strains are commonly present in all the three soil sludges (river, agricultural, and garden). Out of all other bacterial strains, these two exoelectrogenic bacteria were found to be potential worthy of our research.

According to Bond and Lovley [12], the bioelectricity production in MFC is solely by the bacterial cells attached to the electrode. Several reports revealed that low level detection of oxidized acetate (<10 µM), hydrogen metabolized (3 Pa), and electron transfer to electrodes rate (0.21–1.2 mM of electrons/mg of protein/min) [13]. Moreover, current production by this MFC is 65 mA/m^2 or poised-potential 163–1143 mA/m^2.

Water evaporation-induced electricity generators have recently attracted extensive research attention as an emerging renewable energy–harvesting technology that harvests electricity directly from water evaporation. However, the low power output, limited available

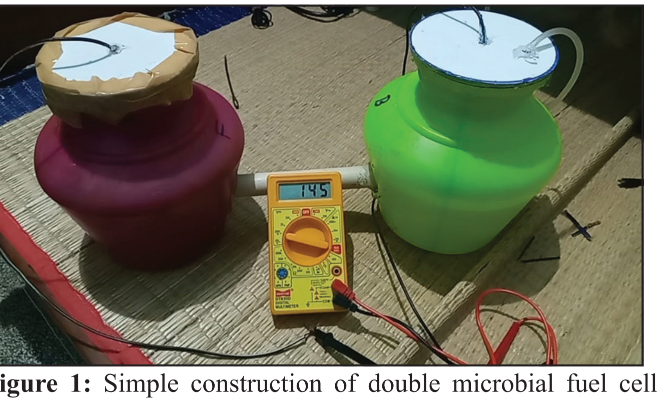

Figure 1: Simple construction of double microbial fuel cell chamber.

Table 1: Identification of exoelectrogenic bacteria.

Characteristics	Strain 1	Strain 2
Morphological		
Grams staining	Positive	Negative
Shape	Rod	Cocci
Growth type	Anaerobe	Anaerobe
Motility	Negative	Positive
Biochemical		
Indole	Negative	-
Voges Proskauer	Negative	-
Oxidase	Negative	Negative
Catalase	Negative	Negative
H_2S production	Negative	Positive
Urease	Negative	Positive
Nitrate	Positive	Positive
Starch hydrolysis	Positive	Negative
Gelatin hydrolysis	Positive	Negative
Carbohydrate fermentation	Positive	Negative
Genus identified as	*Clostridium* spp.	*Geobacter* spp.

material, complicated fabrication process, and extremely high cost have restricted wide applications of this technology. The MFC can generate continuous electric power with a maximum output power density of ~685.12 μW/cm^2, which is two orders of magnitude higher than that of previously reported analogous devices [14]. The superior performance of the device is attributed to the intrinsic properties of the *Geobacter sulfurreducens* biofilm, including its hydrophilicity, porous structure, and conductivity. This study not only presents the unprecedented evaporating potential effect of *G. sulfurreducens* biofilms but also paves the way for developing hydrovoltaic technology with biomaterials [14].

3.2. Electricity Generation

The voltage output from the river, agriculture, and garden sludges in the absence of air pump was recorded, the readings were noted until it stopped showing a better output. For the river, agricultural, and garden sludges, in the presence of air pump, the output generated was between 0.150 V and 0.346 V (Figure 2a), 0.110 V and 0.236 V (Figure 3a), and 0.100 V and 0.127 V (Figure 4a), respectively. In the absence of air pump, the output measured for river, agricultural, and garden sludges were between 0.346 V and 0.603 V (Figure 2b), 0.236 V and 0.502 V (Figure 3b), and 0.100 V and 0.177 V (Figure 4b), respectively.

Without the oxygen supply (i.e., in absence of air pump), the voltage range keeps on changing for every 2 min, from 346 mV, it increases to 388 mV, 420 mV, and so on, in river sludge MFC, from 100 mV to 120 mV, 140 mV, and so on, in agricultural sludge MFC, and from 236 mV to 250 mV, 262 mV, and so on, in garden sludge MFC. While comparing the results from these three sludges, the voltage output from river sludge was higher in absence of oxygen. Based on our results (Figures 2-4), it shows a lot of differences in the voltage output which could be detected by the multimeter from different soil samples. Based on the results obtained, it is evident that there are several variations in the amount of bioelectricity produced by MFC through recordings in the multimeter. This result depicts that this MFC is a low cost and energy efficient approach and it has been demonstrated that the isolated electrogenic bacteria could flourish in anaerobic environment. Each sludge type will have a distinct community of microbes and each species of microorganism will provide a varied performance to the MFC. This variable location in sample collection is a significant parameter which determines the number and type of microbiological communities in which the exo-electrogenic bacteria present in each sludge. The two common exo-electrogens identified

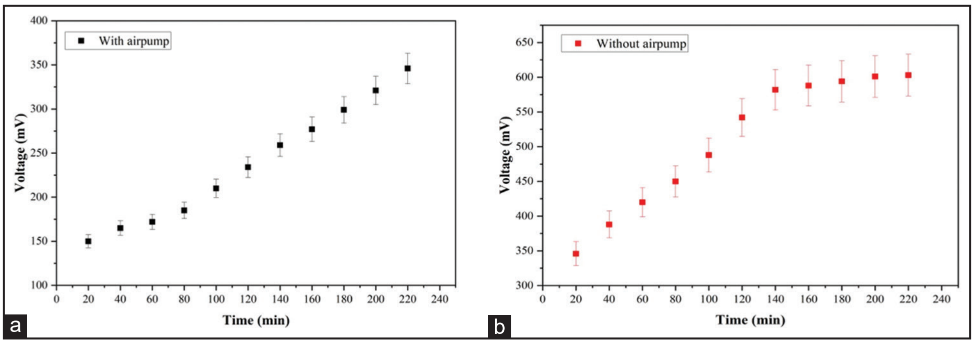

Figure 2: Voltage output from river sludge (a) output from with airpump and (b) output from without airpump.

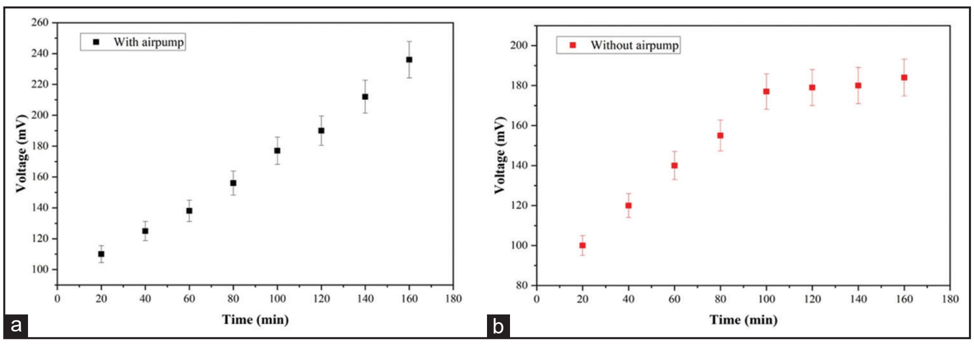

Figure 3: Voltage output from agricultural sludge (a) output from with airpump and (b) output from without airpump.

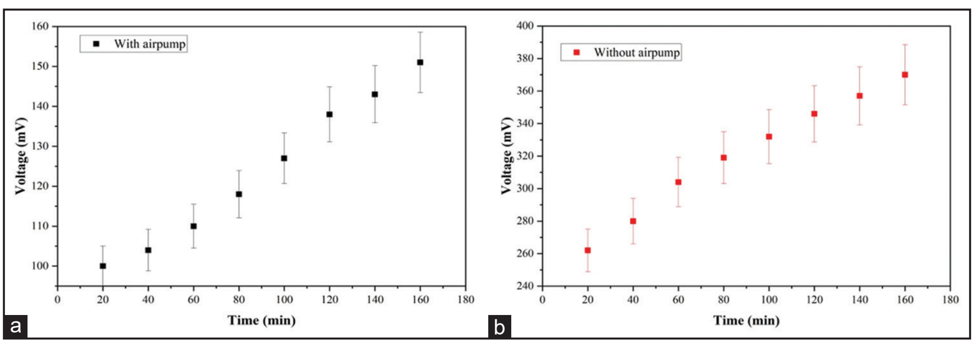

Figure 4: Voltage output from garden sludge (a) output from with airpump and (b) output from without airpump.

were *Clostridium* spp. and *Geobacter* spp., they acts as catalyst in all the three MFCs [15,16]. Bacteria belonging to the genus *Geobacter* were identified and were found more prominently in the anodes of MFCs than in the bulk soil. The findings confirmed that *Geobacter* spp. was responsible for bioelectricity generation [17]. Jenol *et al*., [16] stated that *Clostridium* spp. is an excellent exo-electrogen capable of generating starch-powered bioelectricity. Hence, specific bacterial species will be supplied with oxygen in a waste water treatment system to boost the efficiency of the operation [18-23]. As different bacterial species function in different ways, the pathway or kind of electrode attached to the substrate will also influence their performances [24].

4. CONCLUSION

The two exoelectrogenic bacteria *Clostridium* spp. and *Geobacter* spp. were found to be potential worthy of our research. The highest output of MFC in absence of oxygen is 603 mV and in presence of oxygen, 100 mV was recorded as the lowest output. This difference demonstrates that providing anaerobic environment for MFC will give more efficient results. Thus, *Clostridium* spp. and *Geobacter* spp. can be inferred to be potential exoelectrogenic bacteria in MFCs. This MFC experiment, which converts microbe activity inside mud into electricity through redox reaction, was chosen as a green alternative because it has the potential to generate renewable energy. Despite the fact that MFCs have yet to be created with economically acceptable pricing of installed power and with a maximum capacity, research on MFCs has been moving quickly and steadily.

5. AUTHOR CONTRIBUTIONS

All the authors are contributed equally.

6. CONFLICTS OF INTEREST

All the authors declared that there are no conflicts of interest.

7. ACKNOWLEDGMENT

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***Bibliographical Sketch**

Dr. T. AMUTHA PRIYA M.Sc (Env. Biotech) M.Sc. (Bot), Ph.D, did her Doctoral Research in "Micropropagation and Molecular Marker analysis of Calotropis gigantean" at Department of Plant Science, Manonmaniam Sundaranar University, Tirunelveli. She has extensive hands-on training experiences in Genetic diversity Analysis with genetic markers, plant tissue culture, plants dye preparation and using different materials like cotton, palm leaf, coir etc. She is excellent in experimental researches in these areas as evidenced from research and review papers published in peer reviewed journals also presented papers in national and international conferences. In addition, she has vast teaching and administrative experience in academic institutions. Currently, she is an Assistant Professor, Department of Botany, G. Venkataswamy Naidu College, Kovilpatti 628502, Tamil Nadu, India.