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# Growth Curve Analysis of *Rhizobium leguminosarum* Using Voltage Produced by Microbial Fuel Cell

**Shrirang R. Maddalwar[1*], Arti S. Shanware[2]**

[1]Student, Department of Biotechnology, Rajiv Gandhi Biotechnology Centre, Nagpur, India
[2]Director, Department of Biotechnology, Rajiv Gandhi Biotechnology Centre, Nagpur, India

***Address for Correspondence:** Mr. Shrirang R. Maddalwar, Student, Department of Biotechnology, Rajiv Gandhi Biotechnology Centre, Nagpur-440020, India

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**ABSTRACT**

Microbial fuel cells could be used for the study growth rates of aerobic microbial species on the basis of voltage produced by them in the microbial fuel cell assembly. A fresh culture of *Rhizobium leguminosarum* was added in the anode chamber of a microbial fuel cell assembly and subsequent voltage produced by it was recorded after every fifteen minutes. The 24 ml/hr of air was pumped into the anode chamber to maintain the dissolved oxygen level and resistance of 12 ohm was applied across the electrodes. This process was studied in triplicates and voltage data was recorded. The graph plotted of voltage against time suggested that the growth curve of the species in the microbial fuel cell system. It was found that voltage gradually increased with time ranging from 50 mV to 190 mV with a supply of oxygen in the anode, but it declines gradually to zero in the absence of aeration with time and depletion of nutrients.

**Key-words:** Exo-electrogenesis, Microbial fuel cell, Proton exchange membrane, *Rhizobium leguminosarum,* Sporulation

## INTRODUCTION

It is really difficult task to study metabolic activities of a single bacterial cell, hence in order to avoid this problem the culture is usually manipulated in such a way that all the cells of culture should be showing same metabolic activities. This method is very useful in physiological studies and also known as synchronous culture method [1-3]. A study of these cells would enable one to postulate the sequence of events occurring in a single cell during the process of sporulation [4]. Though this method is very effective to study the metabolic activities of *R. leguminosarum* but it fails to convey the required information when the culture is not synchronous [5].

A Microbial Fuel Cell (MFC) is a device that converts chemical energy from bio-convertible organic substrate, directly into electrical energy through the metabolic activity of microorganisms.

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A simple MFC setup contains two chambers respectively anode and cathode separated by Proton Exchange Membrane (PEM). The microorganisms are inoculated in an anodic chamber, where they oxidize the substrate and generate protons and electrons. The electrons are transferred from anode to cathode through the external circuit and the protons pass through the proton exchange membrane to cathode, where the proton meets the oxygen and electrons to form water [6].

Mechanism of electron transformation from bacterial cell to the anode is known by three ways, firstly, using exogenous mediators (those present outside the cell) such as thionine, methylene blue or neutral red and potassium ferricyanide. Secondly, using mediators produced by the bacteria and finally by direct transfer of electrons from the respiratory chain enzymes i.e. cytochromes to the outer cell membrane, which in turn is reduced and then leaving it in a reduced state to shuttle the electrons to the electrode [7]. *Geobacter sulfurreducens*, *Geobacter metallireducens,* and *Rhodoferax ferrireducens* have been shown to produce the voltage in a mediator less microbial fuel cell [8].

Hence, in order to study metabolic activities of *Rhizobium leguminosarum* in liquid broth, the microbial

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fuel cell could be used as an effective tool. This study focuses on voltage generated by the microbial fuel cell using fresh broth of *R. leguminosarum* with respect to aeration and in absence of aeration. It could be an effective method to determine the metabolic rate of cells in different physiological and nutritional conditions.

## MATERIALS AND METHODS

Pre-isolated and pre-characterized culture tube of *R. leguminosarum* from the soil samples Rajiv Gandhi Biotechnology Centre, Nagpur, India premises was considered for inoculation of fresh growth medium in the laboratory of the Department of Biotechnology on 16 December 2017.

**Preparation of fresh culture broth-** The fresh nutrient medium was prepared using Yeast Mannitol medium (YEMA medium), which included $K_2HPO_4$ 0.05%, $MgSO_4$ 0.02%, NaCl 0.01%, Mannitol 1%, $CaCO_3$ 0.3%, Yeast 0.1% and Distilled water. The contents of the medium was thoroughly mixed and allowed for autoclaving at 121$^{O}$C and 15 lb pressure. After the process of autoclaving the medium, the medium was allowed to settle down to room temperature. This process can be done more rapidly by placing the container of nutrient medium in cool stream of water. Once the content was cooled then the pH of the medium was measured using digital pH meter. The optimum pH of this medium for Rhizobium species should be 7 and hence it was necessary to confirmed it. If the pH was not neutral, then it was made neutral by adding a strong acid like HCl or strong base like NaOH. After gaining optimum pH, the broth was inoculated with 10% inoculums of an *R. leguminosarum* and the content was allowed to grow in well-aerated desktop fermentor or cotton-plugged glass flask. Culture requires oxygen to multiply and grow efficiently; hence aerobic fermentor has been always preferable. The medium was allowed to grow for next 48 hours [9].

**Microbial fuel cell construction-** The MFC was constructed using two screw-capped plastic bottles with the total working volume of 1 lit and it served as anode (anaerobic) and cathode (aerobic) chambers. Both anode and cathode chambers were connected with 1 cm in diameter and 5 cm long tube which was filled up with salt bridge made of 1M Potassium chloride (KCl) solution and 3% agar powder. The agar salt bridge acts as a barrier between the anode and cathode chambers. The reason for using agars as salt bridge is to provide an internal electrical connection between the chambers, while minimizing the transfer of ions from the electrical environment [10]. Stainless steel mesh of 500 gm and having diameter of 2 mm was used as anode and cathode. Before the MFC operation, the electrodes were soaked in 0.1 M HCl solution for a day to remove possible contamination and after the MFC operation; the electrodes were washed with 0.1 M NaOH solution to neutralize the surface contaminants [11]. The electrodes were externally connected with 12 ohm resistance using copper wire. This setup was prepared in triplicates in order to minimize the errors in voltage recorded by the volt meter. Aeration of 600 ml per hour was supplied to the cathode chamber using 1 liter injection syringe. Both the bottles are screw capped properly in order to make both the chamber air tight.

**Microbial fuel cell operation-** All the three microbial fuel cell setups are surface sterilized by wiping with 70% alcohol and UV light exposure for 20 minutes. All the three anode chambers of microbial fuel cell assemblies were filled with 400 ml freshly prepared broth culture of *R. leguminosarum* in each chamber. After inoculation, the assemblies are screw capped properly to maintain anaerobic conditions inside the chamber. In cathode chamber, 800 ml of 1 M KCl solution was filled in all the three assemblies. All the three assemblies are screw capped and aeration of 600 ml per hour is provided to the assemblies using 1 liter injection syringe. Whatman filter was attached to the syringe before aeration in order to provide sterile air flow [9].

## RESULTS

A resistance of 12 ohm was applied across cathodes and anodes of each assembly and voltage was recorded in the voltmeter after every fifteen minutes. After one hour and 45 minutes, the aeration was stopped and voltage was recorded after every 15 minutes for five more times successively. Detailed data of recorded voltage in mV is summarized in Table 1 & Fig. 1.

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**Table 1:** Voltage recorded by three microbial fuel cell assemblies after every 15 minutes

| Time (Minutes) | Voltage recorded by Set A (mV) | Voltage recorded by Set B (mV) | Voltage recorded by Set C (mV) |
|---|---|---|---|
| 0 | 90 | 50 | 70 |
| 15 | 130 | 70 | 100 |
| 30 | 130 | 90 | 120 |
| 45 | 160 | 108 | 127 |
| 60 | 185 | 130 | 135 |
| 75 | 184 | 145 | 150 |
| 90 | 184 | 180 | 182 |
| 105 | 184 | 180 | 182 |
| AERATION | | STOPPED | |
| 120 | 190 | 190 | 180 |
| 135 | 180 | 190 | 170 |
| 150 | 150 | 180 | 120 |
| 165 | 140 | 144 | 108 |
| 180 | 130 | 25 | 80 |
| 195 | 10 | 00 | 00 |

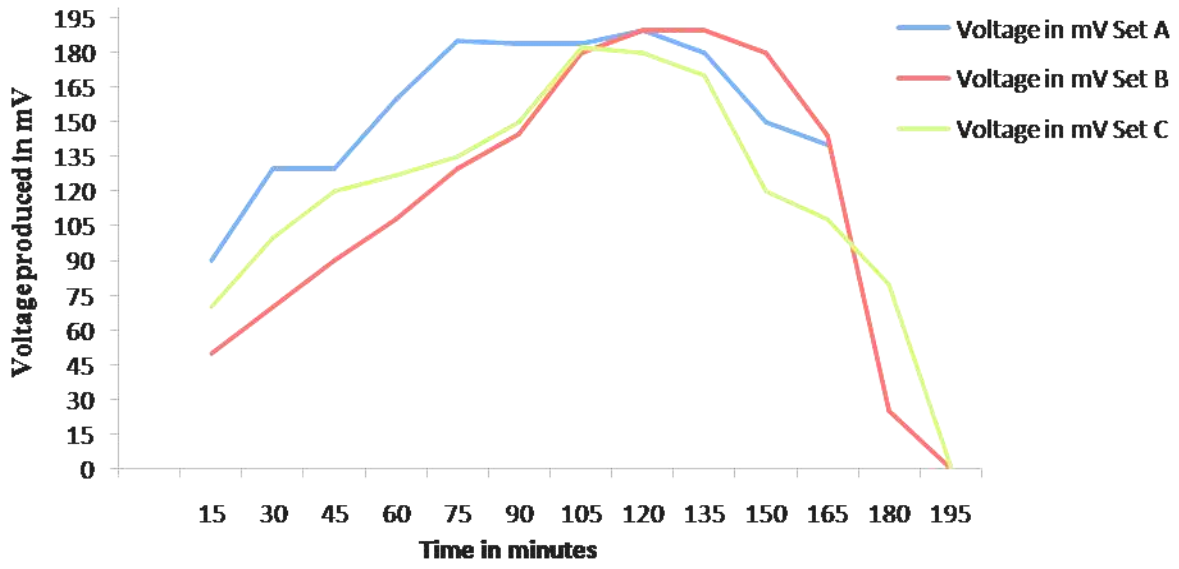


**Fig. 1:** Voltage produced by the culture of *Rhizobium* species with respect to time

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## DISCUSSION

There has been an increase in recent years in the number of reports of microorganisms that can generate electrical current in microbial fuel cells. Although many new strains have been identified the few strains individually produce power densities as high as strains from mixed communities. Enriched anodic bio-films have generated power densities as high as 6.9 W per $m^2$ (Projected anode area), and therefore are approaching theoretical limits [12].

Power density, electrode potential, coulombic efficiency, and energy recovery in single-chamber microbial fuel cells (MFCs) were examined as a function of solution ionic strength, electrode spacing and composition, and temperature. By the increasing the solution ionic strength from 100 to 400 mM by adding NaCl increased power output from 720 to 1330 $mW/m^2$. Power generation was also increased from 720 to 1210 $mW/m^2$ by decreasing the distance between the anode and cathode from 4 to 2 cm. The power increases due to ionic strength and electrode spacing resulted from a decrease in the internal resistance. Power output was also increased by 68% by replacing the cathode (purchased from a manufacturer) with carbon cloth cathode containing the Pt loading [13].

It is a surprise to many researchers that the most significant block to achieving high power densities in MFCs is the system architecture, not the composition of the bacterial community [14].

But power output by MFCs has been consistently increasing over time. Improvements in system architecture and operation have increased power densities from 1500 $mW/m^2$ using oxygen as the final electron acceptor at the cathode [15,16].

From Fig. 1 & Table 1 describes that the voltage produced by a freshly prepared broth of *R. leguminosarum* increases with time from its actual value to 180–190 mV with aeration of 600 ml per hour of sterile air in cathode chamber in 105 minutes. But when the aeration was stopped completely, the voltage produced by the culture in microbial fuel cell setup also decreases gradually and it becomes zero after 180 minutes of starting the experiment. This change in voltage with respect to time and with respect to aeration observed in very similar pattern in all the three sets, namely A, B, and C or microbial fuel cell assembly. Hence, the graph of voltage against time gradually increases with aeration and it gradually decreases from the peak when the aeration is stopped in the cathode chamber of microbial fuel cell assemblies. The graph declines to zero after 180 minutes.

The increase in the voltage generated by microbial fuel cells with aeration suggests that metabolic rate of the growing culture in the anode chamber increased with aeration, but after stoppage of aeration, the metabolic rate of the bacterial culture decreased gradually due to insufficiency of oxygen in the cathode chamber and as a result voltage produced by it also decreased and it becomes zero after 195 minutes of experiment when oxygen was completely exhausted in the cathode chamber. Zero voltage recorded by microbial fuel cell assemblies suggests that the fuel cell stops working and cells in the culture might have died.

## CONCLUSIONS

From this study, it could be concluded that pre-isolated strain of *R. leguminosarum* is highly aerobic in nature and require enough oxygen to metabolize and reproduce. The cell behavior or nutrient uptake studies of *R. leguminosarum* could be done using the voltage generated by it in microbial fuel cell assemblies. The maximum voltage generated by a freshly prepared broth of *R. leguminosarum* is in between 180 mV–190 mV in microbial fuel cell assembly.

In future, more work is required to be done on the assembly for increasing this range of voltage. In addition to this comparative study of different electrode material can also be done in order to get an efficient and steady voltage for the sample organism.

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## CONTRIBUTION OF AUTHORS

All authors equally contributed to this article.

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