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**Research Article**

# ELECTRICAL POTENTIAL OF VARIOUS MICROORGANISMS BY THE USAGE OF MICROBIAL FUEL CELL

**Sachin Kumar[1]., Aman Kaushik[2]., Surbhi Agarwal[3]., Dinesh Bhardwaj[4]., Ramanuj[5]., Shridhar Somani[6] and Ramandeep[7]**

[1]Department of Food Engineering, National Institute of Food Technology Entrepreneurship and Management
[2,5]Department of Basic and Applied Sciences, National Institute of Food Technology Entrepreneurship and Management
[3]Department of Food Science and Technology, National Institute of Food Technology Entrepreneurship and Management
[5,6,7]Carrer Institute of Technology and Management

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**ABSTRACT**

Microbial Fuel Cell is a bio-electrochemical cell wherein the bacterial metabolism aids in electron generation and hence the production of voltage. In a Microbial Fuel Cell the microorganisms are anaerobically cultured i.e. cultured in absence of terminal electron acceptor i.e. oxygen. This results in generation of electrons in free state making them available for transfer in external circuit. The process, thereby, results in development of potential difference between the two electrodes i.e. anode and cathode. This study involved the extensive study of Microbial Fuel Cell where E.coli was cultured in 3L L.B. media. The voltage recorded was 745mV. The working volume of MFC to 600ml and connected 9 of them in series for voltage upgradation was also studied. The maximum voltage then recorded was 4.5V. In series connection of MFCs also exploited the potential of E.coli cultured in L.B. media. To explore the potentials of microorganisms beyond E.coli to be used in MFC, MFCs based on S.cerevisiae (cultured in Potato Dextrose Broth) and A.niger (cultured in Saboraud's broth) were developed. These MFCs were to test the potential of other microbial species. Physical parameters like the type of electrode, electrode surface area etc. influences the electrochemical cells. So, these were tested in case of Microbial Fuel Cells by subjecting it to different operational parameters involving higher inoculation, higher cathode area, higher anode area and extra aeration facilitated at cathode. The results obtained were then comparatively analyzed with a MFC being operated under normal conditions i.e. comparison was made with default.



## INTRODUCTION

Microorganisms are ubiquitously present in nature. They hold a vast potential owing to their small size coupled to complex biological machinery inside. These microorganisms have been occupying a central position amidst researchers and are being successfully exploited for various reasons (Kim *et al.,* 2007). Their diversified usage can be summed up into following categories: Research oriented applications, Commercial applications, Healthcare applications and Environmental protection. Microbial Fuel Cells are an innovative extension of microbial strength (Aelterman *et al.*, 2008). MFCs have been gaining attention in recent years because it produce society's most widely useful energy form electricity directly without combustion (Hyung-Sool Lee *et al*., 2008). They employ applications of various disciplines including biotechnology, electronics, chemistry, physics etc. They cover 3 overwritten categories i.e. research applications, commercial significance and play a crucial role in environment protection. A microbial fuel cell (MFC) or biological fuel cell is a bio-electrochemical system that drives current by mimicking bacterial interactions found in nature (Korneel Rabaey *et al*., 2003; Byung Hong Kim *et al*., 2007; The Hai Pham *et al*., 2008). In a MFC, electron donors are oxidized at an anode with concomitant production of carbon dioxide, protons, and electrons (The Hai Pham *et al*., 2008). Several factors limit the performance of

**Corresponding author:* **Sachin Kumar**
Department of Food Engineering, National Institute of Food Technology Entrepreneurship and Management

MFCs, such as activity of the biocatalysts, electron transfer losses both at the anodes and the cathodes, and the internal resistance (The Hai Pham *et al*., 2008). Micro-organisms catabolize compounds such as glucose, acetate, butyrate, or wastewater (Uwe Schroder 2007; K. Scott *et al*., 2006; Byung Hong Kim *et al*., 2007; Booki Min *et al*., 2004; Sonal G. Chonde 2014). The electrons gained from this oxidation are transferred to an anode, where they depart through an electrical circuit before reaching the cathode (Bradley R. Ringeisen *et al*. 2007). Here they are transferred to a high potential electron acceptor such as oxygen (Zhen He *et al.,* 2006; K. Scott *et al*., 2007; Byung Hong Kim *et al*., 2007; Bernardino Virdis *et al*., 2008). As current now flows over a potential difference, power is generated directly from biofuel by the catalytic activity of bacteria. Thus MFC is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms (Logan *et al.,* 2006). A typical microbial fuel cell consists of anode and cathode compartments separated by a cation specific membrane. In the anode compartment, fuel is oxidized by microorganisms, generating electrons and protons. Electrons are transferred to the cathode compartment through an external electric circuit, and the protons are transferred to the cathode compartment through the membrane. Electrons and protons are consumed in the cathode compartment, combining with oxygen to form water. These cells are gaining in considerable significance and *huge sums are being invested in this field to explore its hidden horizons.*

## MATERIALS AND METHOD

### *Materials*

For this study, Anodic chamber, Cathodic chamber were made manually in laboratory. Salt bridge was made to maintain electrical neutrality of MFC. The entire apparatus was connected using connecting wires. L.B. Media, Sabourand Media, Potato Dextrose Broth and Nutrient media used were of analytical grade. $CuSO_4$ solution (0.1M) was used as oxidizing solution in cathodic vessel. Mutlimeter was used to measure the results.

In anodic chamber, microorganisms are cultured under anaerobic environment using growth media which facilitates the production of electrons in free state. Electrons available in free state are then transferred to anode made up of Graphite rods (K. Scott *et al.,* 2007; Peter Aelterman *et al*., 2008). On receiving electrons, the anode becomes electronegative, thereby allowing electron transfer in external circuit. Graphite rods forming anodes were made of leads from 2-B pencils (Korneel Rabaey *et al*., 2003) which were then broken down into equal pieces and joined together using fevitite. The resultant joint was wrapped around by copper wire to maintain electrical neutrality. Whereas, Cathodic chamber was made up of 1.5% copper sulphate solution as oxidizing salt in which a cathode wire, made up of copper and helically coiled, was dipped. Presence of oxidizing salt solution allows the development of potential difference across the two electrodes and helical coiling of wire increases the surface area and hence the efficiency of the MFC. Volume of broth and oxidizing solution were kept same.

Salt bridge was made up of 1.125% KCl as conducting salt and 1.2% Agar. It is crucial for transfer of protons preventing building up of $H^+$ ions in anodic compartment. Conducting salt facilitated the cation movement while agar has provided semi-solid matrix for transfer. After pouring in the solution, salt bridge was allowed to solidify. After setting the apparatus, surface was sterilized by 15 minutes exposure to UV-radiation followed by pouring of autoclaved media with inoculation of microorganism (Booki Min *et al*., 2005). Anaerobic conditions were maintained by sealing the vessel with parafilm.

### *Microorganisms*

*Escherichia coli, Saccharomyces cerevisiae* and *Aspergillus niger* were used to produce electrons in the external media in free state hence, development of a potential difference between the two electrodes.

### *MFC based on microorganisms*

**MFC based on E. Coli (**Byung Hong Kim *et al*., 2007**)**

### *Demonstration of MFC in 3L culture broth*

Successful operation of a Microbial Fuel Cell was first demonstrated in a vessel with 3L of nutrient media. 48 hours old culture of *E.coli* was inoculated in L.B. media, used as the nutrient source for *E.coli* and the inoculum size was kept at 2%. The inoculation was immediately followed by sealing of the containers with parafilm so as to maintain anaerobic environment for the microorganism. Then, readings were taken at regular intervals of time during the course of culturing to study the pattern of potential difference development across the two electrodes.

**Figure 1** Simple model of a Microbial fuel Cell

### *Connecting MFCs in series for voltage upgradation*

Anaerobic respiration of bacteria result in electron production in free state which later drives current in external circuit. The development of potential difference across the two electrodes guided us to study if this developed voltage can be increased. So, voltage upgradation was studied by connecting these microbial fuel cells in series (Byung Hong Kim *et al*., 2007).

**Figure 2** Arrangement of MFCs in series

***For voltage upgradation in MFCs 9 electrochemical cells were connected in series***

Each of the MFC (600ml vessel) containing L.B. broth as the nutrient source was inoculated with 24 hours old active culture of *E.coli* at inoculum size of 5% to observe voltage development. Each of the vessel was individually inoculated. Once the inoculation process was done successfully, in series connection was established between the individual vessels, so as to record cumulative effect of 9 in-series connected vessels. After the set-up was completed, readings were taken at regular intervals.

During the course of culturing, once a substantial voltage was recorded, the MFCs in-series were used to lit Light emitting diodes (LED). Initially one of the LED was lit followed by two the very next day. The LEDs were arranged in series with the set-up and the connections were provided using a breadboard. Nine MFCs were connected in series and their combined influence on potential difference development was recorded.

***Influence of operating parameters on MFC***

It has been shown by the researches that the physical parameters such as different materials making up anode and cathode, varying surface areas of the electrodes etc. have a direct effect on the electron transfer and hence, the voltage development (Uwe Schroder 2007).

Similarly it was anticipated that varying the working parameters for MFC will influence the electron generation and, hence, the developing potential difference across the two electrodes. So, the parameters that were altered to observe the effect are: Increased cathode and anode areas, Higher inoculum size and Extra aeration to cathodic vessel. All these parameters were assessed by comparative analysis with a MFC working under normal conditions.

20 hours old active culture of E.coli was used to inoculate anode compartment of MFC containing 600ml of L.B. broth as the nutrient source. 5% inoculum was considered for MFC based on normal, higher cathode area, higher anode area and extra aeration whereas 10% was considered in case of higher inoculation.

The inoculation was done at an interval of 3 minutes in different MFC's i.e., normal MFC followed by high inoculation, higher cathode area MFC, higher anode area MFC and finally MFC with extra aeration. Normal MFC was the MFC operating under the normal parameters i.e. no change in the physical or operating parameters. It has a standardized or normal cathode/anode area, 5% inoculation density and no forced aeration. In the MFC with higher inoculation density, the inoculation size was increased to 10% since it was observed that increasing the inoculation density in comparison to normal will yield higher voltage development in shorter span of time. MFC with higher cathode area is similar to the normal in all terms except the cathodic area. Higher cathode area is provided by incorporating 2 instead of usual one cathode where as in MFC with higher anode area, 2 anodes were used instead of one. In MFC with provision for extra aeration, higher diffusion of air or provision for extra aeration was provided by creating a hole in the closing lid of the cathode chamber. It was thought to have direct influence on the electron transfer.


**Figure 3** MFCs under varying parameters placed inside incubators

**MFC based on *S. cerevisiae***

*S.cerevisiae* is significant when grown in higher sugar concentration and, hence, its successful exploitation would mean its fruitful application to fermentation units with higher residual waste sugar such as sugar mills. Therefore, 8.68% of 4 days old culture of *S.cerevisiae* (grown in anaerobic environment) was inoculated in 3285 ml of Potato Dextrose Broth. The vessel was parafilmed after inoculation and the readings were taken at regular intervals.

**Figure 4** MFC based on *S. cerevisiae*

***MFC based on A. niger***

*A.niger* is significant when grown in higher sugar concentration and, hence, 3 days old culture of *A.niger* (grown in anaerobic conditions) was inoculated at density of 10% to 600ml of Saborauds dextrose broth. The anode compartment of the MFC was parafilmed soon after inoculation followed by recording developing potential difference.

**Figure 5** MFC based on A.niger is placed in incubator

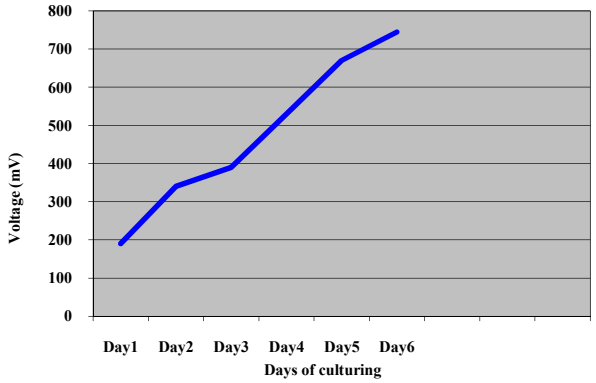
***Statistical Analysis***

All experiments were conducted in duplicate and data was analyzed using EXCEL. Differences were considered significant at a level of $p<0.05$.

## RESULTS AND DISCUSSION

### *MFC based on E. coli.*

### *Demonstration of MFC in 3L culture broth*

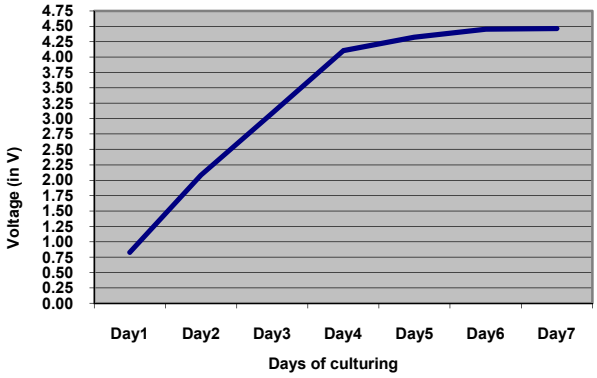



**Graph 1** Voltage production with respect to days of culturing for 3L culture broth

From the graph 1 it can be clearly visualized that the potential difference across the two electrodes has risen with passage of time. Availability of nutrient media facilitated the voltage development even after a week of culturing. The growth observed during initial days was rapid owing to cellular multiplication, however, the curve starts tapering with increasing days of culturing with no significant changes in the readings observed during last day of culturing which could be due to media exhaustion and/or microbial growth around the anode preventing the electron access to the anode for transfer. The results shown that the final voltage obtained after a week of culturing was 745 mV.

The smooth operation of the microbial fuel cell accompanied with voltage rise reported with each passing day clearly demonstrated the underlying potential of the bio-electrochemical cell and paved the path for future studies on the subject. It clearly demonstrated the fact that subjecting microbial flora to anaerobic conditions holds vast potential for meeting energy crises. It directed future experimentations on the concerned subject and laid the truth of vast microbial potential.

### *Connecting MFCs in series for voltage upgradation*




**Graph 2** Voltage production with respect to days of culturing for MFC's connected in series

It can be seen from the curve that the maximum voltage recorded during the course of culturing was 4.5V. During the earlier days of culturing the microbial multiplication took place and hence the electron transfer was high and, thereby a certain rise in developed voltage can be seen. However, the curve tapered towards the end of culturing with no significant voltage changes observed. The exhaustion of media and the possibility of highly dense media due to microbial multiplication limited the electron transfer towards the end.

After 9 days of culturing maximum voltage obtained was 4.5V.Such a voltage also enabled to light 2 LEDs with 330**Ω** resistor connected across in series. The voltage recorded was significant in terms of lighting LEDs but was still not enough to register any other outcomes. Also it was observed that more cells arranged in series enhanced the voltage further. The results obtained were also very significant in terms of size of a microbial fuel cell since, the working volume was reduced from 3000ml to 600ml. Despite of it the results obtained were good, promoting the concept of MFC compaction.

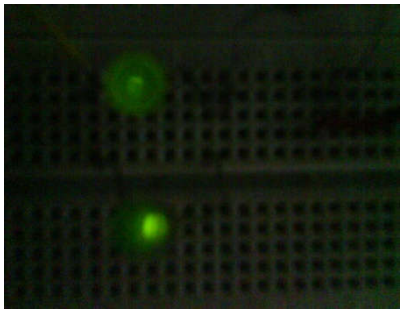
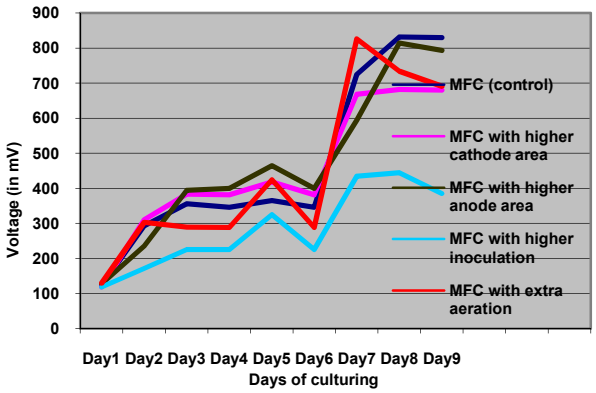


**Figure 1** Two LEDs lit using in-series MFCs

### *Influence of Operating Parameters on MFC*




**Graph 3** Voltage production with respect to different operating parameters of MFC's

After a week time of culturing, MFCs were placed in incubators because of specific temperature requirements. Graph 3 shows that different parameters affect the voltage development differently. Results shows that higher inoculation causes hindrance to higher voltage development thus not a promoting factor. The results of other four parameters were comparable in terms of maximum voltages reported. However, the only difference was the time in which it has been accomplished. For instance, the voltage rise reported in higher anode was maximum initially but normal overshot it after 4 days of culturing. Extra aeration reported highest rise in middle period were as the voltage obtained was least for higher inoculation. In case of higher anode, voltage was more than

higher inoculation. Other 3 had highly comparable outputs and registered the highest voltages.

One more deduction made was the influence of ideal temperature. When these MFCs were placed in incubators, they showed significant rise in voltages. Fall in voltages can be coupled to the access provided to terminal electron acceptor (oxygen); thereby, preventing anaerobic conditions.

Following were the maximum voltages obtained after 9 days of culturing under different conditions:

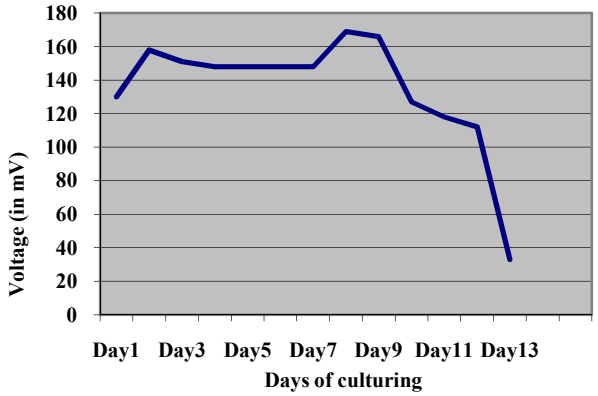
- MFC (normal) -819mV
- MFC (high inoculation) -448mV
- MFC (Higher cathode area) -835mV
- MFC (Higher anode area) -689mV
- MFC (Extra aeration) -827mV

These comparative results revealed maximum voltage can be obtained in minimum time by altering specific parameters of MFC.

The higher anode vessel didn't yield maximum voltage even though it showed promise in starting. This could be because of growth of electrons aside electrodes preventing electron transfer. Similarly, the MFC based on higher inoculation showed very poor response than anticipated. This could be due to the fact that very high density of microbial population prevents efficient electron transfer.

MFC based on higher cathode area and extra aeration enhanced the operability of cathode and thereby, positively influenced the electron transfer and hence, the development of potential difference across the two electrodes.

***MFC BASED ON S.cerevisiae***

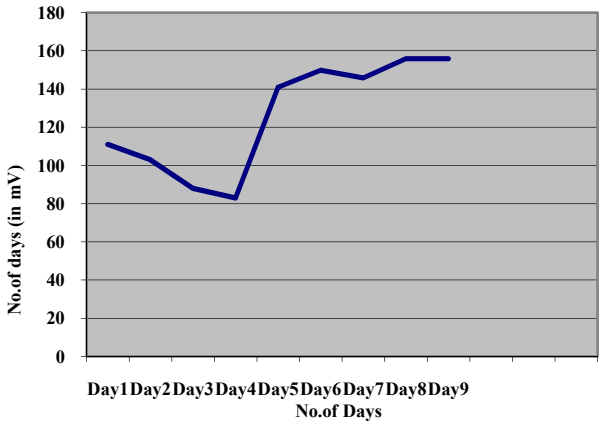



**Graph 4** Voltage production for MFC based on *S. cerevisiae*

The graph 4 shows the pattern of generation of electrons in free state. In other words, it explains the pattern of potential difference generation. It was observed that the growth of potential difference was largely limited to around 150mV. However, a slight rise in voltage was recorded when the MFC was placed in the incubator. The sharp decline in the curve towards end could be related to a free access provided to oxygen. In this case oxygen acted as terminal electron acceptor and prevented the availability of free electrons

Maximum voltage obtained during culturing of *S.cerevisiae* for 12 days was 171.2mV. It was observed that for major period of time the potential difference obtained was around 150mV. This could be because of number of reasons such as in *S.cerevisiae*, the electron transport chain runs inside the cell unlike *E.coli* where ETC runs in the membrane preventing the free access of the electrons generated in the external media in free state. Also, in *S.cerevisiae* no natural mediators like pili of *E.coli* are available for electron transfer to the anode.

***MFC BASED ON A.niger***




**Graph 5** Voltage production for MFC based on *A. niger*

The graph 5 explains the pattern in which potential difference development was observed when *A.niger* was subjected to anaerobic respiration in MFC. The drop in voltage observed initially was due to the improper sealing of the anodic chamber which allowed the access to terminal electron acceptor i.e. oxygen. The curve showed exponential growth in the middle time zone because the sealing was re-done properly and also, the MFC was placed in incubator. A stationary phase of voltage was observed towards the end period owing to exhaustion of the nutrient media supporting growth of the microbe. Maximum voltage obtained after 9 days of successful culturing of *Aspergillus niger* was 156.8 mV.

During the culturing of *A.niger* in MFC, the voltage recorded was not as high as in case of *E.coli* but the data was comparable to that of *S.cerevisiae*. This could be because, in *A.niger*, the electron transport chain runs inside the cell unlike, *E.coli* where ETC runs in the membrane. This prevents the easy and free access of the electrons generated in the external media in free state. Also, in *A.niger* no natural mediators like pili of *E.coli* were available for electron transfer to the anode.

## CONCLUSIONS

Successful operation of a Microbial Fuel Cell under varying nutritional, environmental and operating conditions yielded anticipated results. Some of them bring in accordance to the researches already done, while some being newer. From the study it can be concluded that anaerobic culturing of E. coli in a 3L broth generate a potential difference by connecting electron donating chamber (cultured microorganism in this case) and an oxidizing solution ($CuSO_4.5H_2O$ here) across a salt bridge with proper cathodes and anodes provided. Also, the potential difference obtained was not dependent on the volume of broth. Infact, the number of electrons transferred decided the fate of voltage recorded. This electron transfer in turn depended on the efficiency of anode and the number of electrons available to anode. No practical difference observed in two cases clearly implies that the electrons produced in 3L broth

case were not made available to the same extent as in 600ml broth case. So, electron transfer was restricted because of blockage provided to electron movement to anode. In addition to this, connecting a number of MFCs in series certainly resulted in voltage upgradation. Higher inoculation conditions prevented efficient electron transfer as higher inoculation increases viscosity of media which in turn hinders the electron flow to the anode and thus, lesser voltage. Also, culturing of microorganisms under varying environmental factors affected voltage recorded. In all the parameters considerably high voltages were recorded showing that these factors directly influence the potential difference recorded across the two electrodes.

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