

## Design of microbial fuel cell for power generation using milk and *Lactobacillus* bacteria

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This study investigates the use of milk as a substrate in a Microbial Fuel Cell (MFC) for power generation with *Lactobacillus* bacteria. In place of semipermeable membrane separating the anodic and cathodic chamber in MFC, salt bridge made from agar-agar gel is used as a conducting medium.  $\text{KMnO}_4$  is used as an oxidizer in cathodic chamber in MFC and open circuit voltage (OCV) is observed for various systems by changing anodic and cathodic chamber volume, bacterial concentration. Maximum OCV of 2.01 V is obtained with 300 million *Lactobacillus* sporogenes added to anode of 600 mL working volume containing 100 mL milk and 500 mL water mixture. Novel approach was used to design new structure of salt bridge which can act as conducting medium and also as a cathode. This system achieved a maximum OCV of 1.13 V. Sodium hypochlorite (0.6 w/v%) has been evaluated as an alternative oxidant to potassium permanganate, achieving a maximum OCV of 1.66 V. The obtained results depicted that the milk as a substrate in MFC along with *Lactobacillus* has considerable power generation scope which can be further improvised.

**Keywords:** *Lactobacillus* Bacteria, MFC, Microbial fuel cell, Milk substrate, Power generation

### Introduction

Industrialization and extensive use of conventional energy sources present significant challenges to sustainable and renewable power generation<sup>1</sup>. Microbial Fuel Cells (MFCs) have emerged as a promising technology in this domain, offering a clean and efficient way to convert chemical energy from waste materials or wastewater into electrical energy using electroactive bacteria through redox reactions<sup>2-3</sup>. This innovative approach provides dual benefits of energy generation and waste treatment, addressing critical environmental concerns.

Previous studies on MFCs have explored substrates like industrial wastewater, organic effluents, and dairy waste, emphasizing their potential for renewable power generation and wastewater treatment<sup>4-5</sup>. For instance, lactic acid bacteria have been utilized in MFCs for degrading lactose in dairy wastewater, showcasing their dual capability for pollutant removal and electricity generation. Despite these advances, challenges such as low power output, the high cost of materials like proton exchange membranes (PEMs), and the complexity of oxygen

reduction reactions at the cathode limit their practical applications<sup>6-7</sup>.

The use of milk as a substrate in MFCs is a relatively unexplored area. While a few studies have reported voltage generation using milk, the low power output has hampered its adoption for practical applications. Additionally, the potential of combining raw milk with specific bacterial strains such as *Lactobacillus* for enhanced energy generation has not been thoroughly investigated. To address these gaps, the present study focuses on the novel design and operation of MFCs using raw milk as the primary substrate<sup>8-9</sup>.

In this work, a dual-chambered mediator-less MFC is utilized, employing a cost-effective salt bridge made from agar-agar gel as a substitute for PEMs. Furthermore, a novel approach of integrating the salt bridge as both a conducting medium and a cathodic component has been introduced. This innovative design aims to reduce operational costs and simplify the MFC structure without compromising efficiency<sup>9-10</sup>. The performance of the system is assessed by varying key parameters, including bacterial concentration, anodic and cathodic chamber

volumes, and oxidizing agents such as potassium permanganate and sodium hypochlorite.

The findings of this study aim to highlight the feasibility of using raw milk as a substrate in MFCs, emphasizing its potential for scalable and sustainable power generation<sup>11</sup>. By addressing existing limitations and introducing novel design elements, this research contributes significantly to the field of renewable energy technologies.

## Experimental Section

### Materials

#### *Design of Salt Bridge for MFC*

The salt bridge was prepared using commercially available agar-agar gel and common salt as a low-cost electrolyte as shown in Fig. 1. The mixture was heated and then filled in U-structured PVC salt bridge and kept for cooling overnight. In the similar fashion other salt bridges were made but in place of adding salt as electrolyte into the agar-agar gel,  $\text{KMnO}_4$  was directly added to the gel to study the effect of this on voltage generation. For further experiments, paper ash was mixed with  $\text{KMnO}_4$  and agar-agar gel.

#### *Design of MFC*

A mediator less dual chambered MFC was used for this study<sup>7</sup>. The setup consisted of plastic container which was used as anode chamber<sup>13</sup>. The anode chamber of 600 mL working volume was used. For cathode rather than using similar volume container the general ceramic cups of working volume of 100 mL were used to reduce the volume of cathode chamber in order to make MFC setup more cost effective and reduce overall volume. For further experimentation, generally available ceramic cups of working volume 100 mL were used to study its effect on voltage generation.

#### *Bacterial Culture and Medium*

Bacterial culture was added externally to the substrate in the anode. Commonly available



Fig. 1 — Salt Bridge prepared using PVC pipe and fittings

*Lactobacillus* sporogenes as tablets were used for investigation. Two types of *Lactobacillus* Sporogenes were used, one used with concentration of bacterium not more than 100 million (A) and other used contained *Lactobacillus* not more than 300 million sporogenes (B).

Raw milk was added to two anodic chambers (600 mL working volume each) kept at anaerobic conditions with 100 mL of milk mixed with 500 mL of water to which *Lactobacillus* (B) were added. cathodic chamber consisted of  $\text{KMnO}_4$  as oxidant 0.5 g added to water in 100 mL working volume ceramic cups kept open to air (aerobic condition). Graphite electrodes were used for both anode and cathode having length 5 cm and diameter of 7 mm, two graphite electrodes in each chamber were used to increase the surface area<sup>14-15</sup>. This system was connected in series stacking connection by connecting cathode of one cell to anode of another cell. The voltage generation was measured continuously for this MFC (1) setup and to this LED was connected as load to conclude that system produced enough current to glow the 1.5V LED.

In further experimentation, in place of using 600 mL working volume of anodic chamber, the anodic chamber of 100 mL working volume ceramic cup similar as that of cathodic chamber were taken. As *Lactobacillus* bacterium are aero tolerant anaerobes, so the anodic chamber was kept open to air similar to that of cathodic chamber. Raw milk of volume 16 mL and 84 mL water mixture was added to two anodic chamber each and cathodic conditions were unchanged from that of earlier experiment and two MFC were connected in series similar to previous experiment and voltage generation for this system MFC (2) was measured periodically.

Rather than using 300 million (A) sporogenes of *Lactobacillus* the 100 million (B) sporogenes were used for further experimentation of effect of microbe concentration on voltage generation. For this similar setup as that of MFC (2) was used. Only in place of two MFCs in series here three MFCs units were used in series having 100 million *Lactobacillus* Sporogenes in each MFC anode and cathodic conditions were again kept unchanged and voltage measurement was done for this MFC (3). Schematic representation of MFC (3) is shown in Fig. 2.

In place of using U tube salt bridge connecting anodic and cathodic chamber, it was decided to try up new idea of a salt bridge which itself can act as a cathode. Due to this approach cathodic chamber was

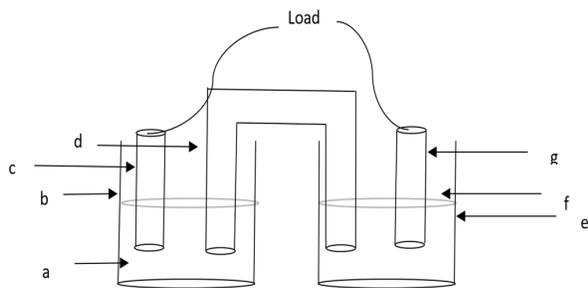


Fig. 2 — Schematic representation of MFC (3); a: water milk mixture, b: anodic chamber, c: anodic electrode, d: salt bridge, e: cathodic chamber, f: oxidizer, g: cathodic electrode

minimized. This system had one anodic chamber with working volume of 100 mL to which a salt bridge made up of simple PVC pipe piece of length 6.5 cm and diameter 15 mm was used. Various changes were made to this design and constituents in it for successive experiments to study this effect on voltage generation.

Initially, agar-agar gel was prepared without adding salt to it. This mixture was then added to the salt bridge discussed above and to this mixture  $\text{KMnO}_4$  and water mixture was added along with single graphite electrode having length 5 cm and diameter 7 mm. In this way similar system as that of cathodic chamber was made in small PVC pipe and was dipped in the anodic chamber hence to reduce the volume of cathodic chamber that were used in previous experiments in this research. Schematic diagrams of salt bridge and one cell from MFC (4) are shown in Fig. 3 and 4, respectively. This setup MFC (4) was studied by using milk as substrate in anode similar to that in case of MFC (2). In the similar way two setups were made and connected in series connection. This novel approach reduced the cathodic chamber and its subsequent volume.

Similar to setup MFC (4) which did not contain salt in Agar-Agar gel in salt bridge, a new setup MFC (5) was built with salt bridge containing Agar-Agar gel and salt mixture to which  $\text{KMnO}_4$ -water mixture was added. Similar to that of MFC (4), this setup also included two anodic chambers to which salt bridges acting as cathode were used and this setup was connected in series to check voltage generation in this setup. Another novel approach was studied in which agar-agar gel and salt mixture were added to single salt bridges and  $\text{KMnO}_4$  mixed with burned paper ash and water was added to this bridges MFC (6) setup. These bridges were used for similar conditions of anode as that of MFC setups in MFC (4)

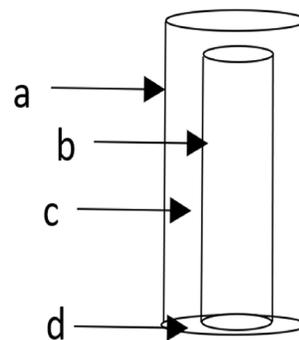


Fig. 3 — Schematic diagram of Salt bridge from MFC (4) a: PVC pipe salt bridge, b: Cathodic electrode, c: Salt bridge constituent mixture, d: Wire mesh

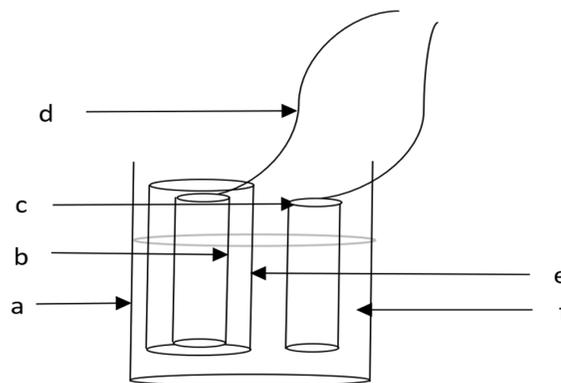


Fig. 4 — Schematic diagram of one cell from MFC (4) a: Anodic chamber, b: Cathodic electrode, c: Anodic Electrode, d: Connecting wire, e: Salt bridge acting as cathode, f: Anodic substrate

and MFC (5). In further research, in place of using milk as a substrate for the microbial fuel cell, the rice water i.e., water after washing rice in households was used. This rice water was initially fermented for 5 days and then used for MFC as substrate. 100 mL rice water was added to two MFC anodes having salt bridge cathode similar to that of MFC (6). This setup MFC (7) having two anodic chambers were connected in series and open circuit voltage was measured. In place of  $\text{KMnO}_4$  as an oxidant it was decided to use sodium hypochlorite as an oxidant for further experimentation MFC (8). For this sodium hypochlorite 0.6 w/v % was used in cathode with 0.4 mL of sodium hypochlorite added to 100 mL working volume cathodic chamber along with water. The anodic chamber had 600 mL working volume with 100 mL raw milk and 500 mL water mixture. Lactobacillus of 100 mL sporogenes were used in anodic chamber and this system was connected by U- tube salt bridge which was used for MFC (1)

system. In these systems two MFC units were connected in series and their OCV was measured.

**Results and Discussion**

**MFC (1) setup**

The MFC (1) setup had two anodic chambers of working volume 600 mL each and two cathodic chambers of working volume 100 mL each connected in series and *Lactobacillus* of 300 million sporogenes were used. This setup was studied for 5 days and had maximum OCV of 2.01 V at the end of day-4 (Fig. 5). The voltage gradually increased with fermentation of milk by the bacteria and curd formation started taking place within some time and OCV started increasing then<sup>14</sup>. After the end of 2<sup>nd</sup> day, milk was separated from milk water mixture and curd started to settle at the top with water remaining at bottom. Actual set-up of MFC (1) is shown in Fig. 6. An LED was

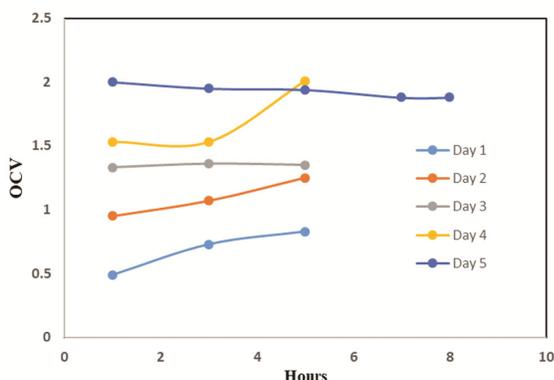


Fig. 5 — OCV vs Hours plot for MFC (1)



Fig. 6 — Actual setup of MFC (1)

connected to the system as a load, which glowed once the OCV exceeded 1.5 V, demonstrating sufficient current generation to power the LED (Fig. 7).

**MFC (2) setup**

MFC (2) setup used 100 mL working volume anodic chamber in place of 600 mL that was used for MFC (1). In the similar fashion as that of MFC (1), two anodic chambers and cathodic chambers were connected in series and OCV was recorded. OCV vs Hours plot for MFC (2) is shown in Fig. 8. This system gave 1.61 V maximum OCV which was lesser than the MFC (1) setup. This maximum OCV was obtained on day-3 and the voltage was seen quite steady during day-3 of the operation as compared to that of MFC (1).

**MFC (3) setup**

In MFC (3) setup 100 million sporogenes of *Lactobacillus* bacteria were added to three anodes of 100 mL working volume each and cathode conditions



Fig. 7 — LED as Load connected to MFC (1)

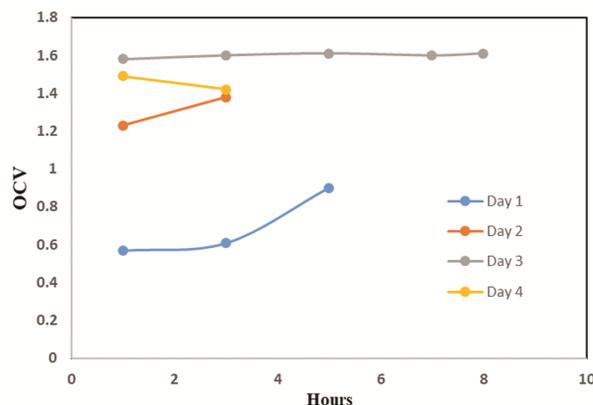


Fig. 8 — OCV vs Hours plot for MFC (2)

were unchanged and were similar to that of MFC (1) and MFC (2). These three units were connected in series and OCV was measured and this system gave maximum OCV of 2.11 V (Fig. 9). This indicates that a reduced bacterial concentration decreases the OCV in systems connected in series<sup>13-15</sup>.

#### MFC (4) setup

In MFC (4) single salt bridge which also acting as cathodic chambers were used in both anodic chambers. This system was connected in series and OCV was recorded for this with maximum OCV obtained was 1.07 V which was much lesser than the previous setups (Fig. 10).

#### MFC (5) setup

In MFC (5) setup the salt as an electrolyte was added in the agar- agar gel mixture in salt bridge to which  $\text{KMnO}_4$  and water mixture were added and this setup was running similar to that of MFC (4). Initially the OCV increased rapidly as compared to that of the MFC (4). The maximum OCV obtained was 1.13 V (Fig. 11), so, no considerable increase was obtained in maximum OCV of MFC (5) as compared to that of MFC (4).

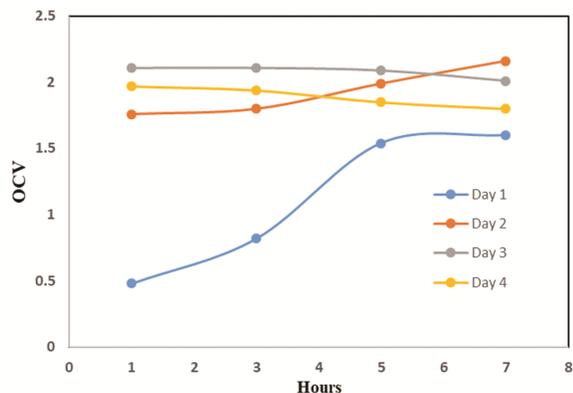


Fig. 9 — OCV vs Hours plot for MFC (3)

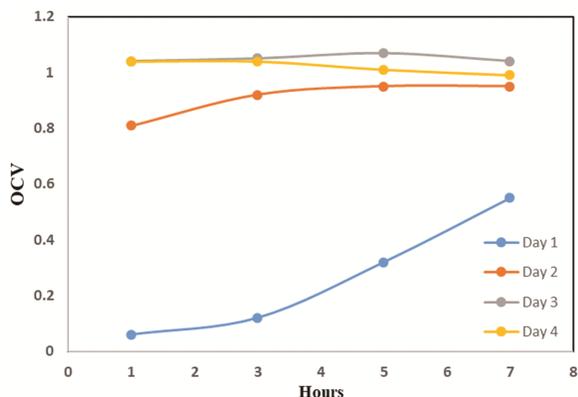


Fig. 10 — OCV vs Hours plot for MFC (4)

#### MFC (6) setup

The MFC (6) setup used burned paper ash as a constituent in MFC. But, the Maximum OCV obtained by this system was just 0.72 V (Fig. 12) which indeed concluded that use of ash drastically decreased the voltage generation.

#### MFC (7) setup

In the MFC (7) rice water was used as a substrate in place of milk. Max OCV vs Day plot for MFC (7) is shown in Fig. 13. This system gave maximum OCV of 0.92 V which is considerably good by using

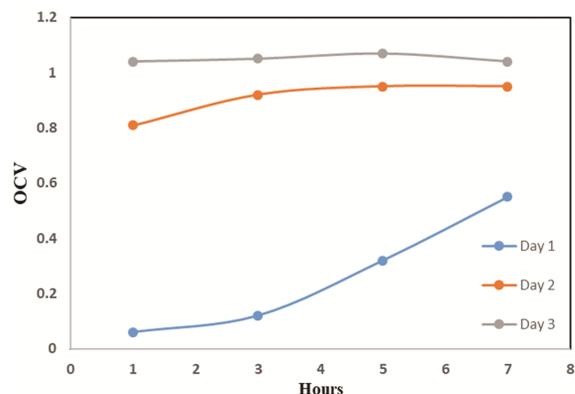


Fig. 11 — OCV vs Hours plot for MFC (5)

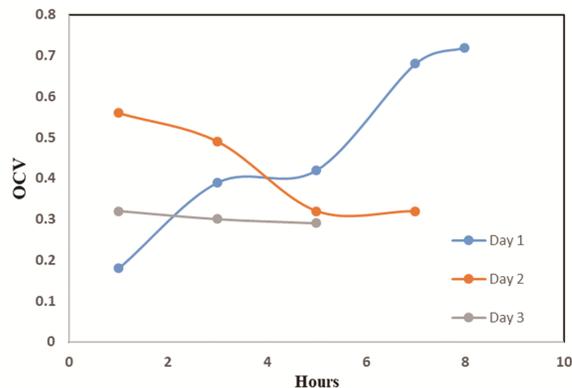


Fig. 12 — OCV vs Hours plot for MFC (6)

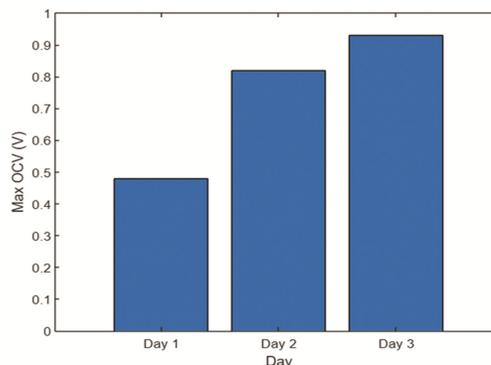


Fig. 13 — Max OCV vs Day plot for MFC (7)

the natural bacteria formed during the fermentation of this water.

#### MFC (8) setup

In this MFC, the  $\text{KMnO}_4$  was replaced by sodium hypochlorite 0.6 w/v % as an oxidant. 0.4 mL of sodium hypochlorite was added in cathodic chamber (100 mL working volume) along with water. The anodic chamber used was of working volume 600 mL similar to MFC (1), only 100 million *Lactobacillus sporogenes* were used. This system gave maximum OCV of 1.66 V (Fig 14).

#### Comparative Analysis of MFC Setups

The investigation into microbial fuel cells (MFCs) revealed significant variability in open circuit voltage (OCV) among different setups. Table 1 summarizes the maximum OCV for each configuration.

The maximum OCV of 2.01 V observed in MFC (1) aligns with the higher range of results reported in

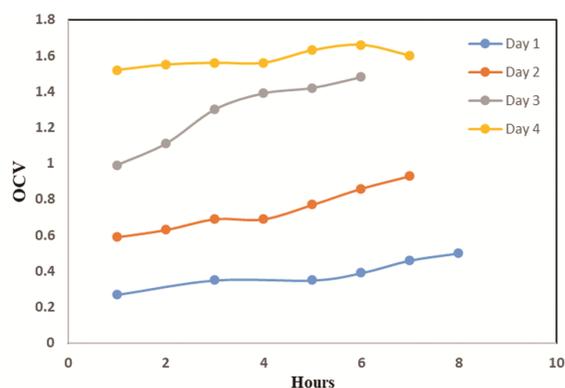


Fig. 14 — OCV vs Hours plot for MFC (8)

Table 1 — Summary of Maximum OCV in different MFCs

MFC Type	Lactobacillus concentration	Maximum OCV (V)
MFC (1), two units in series	300 million sporogenes	2.01 V
MFC (2), two units in series	300 million sporogenes	1.61 V
MFC (3), three units in series	100 million sporogenes	2.11 V
MFC (4), two units in series	100 million sporogenes	1.07 V
MFC (5), two units in series	100 million sporogenes	1.13 V
MFC (6), two units in series	100 million sporogenes	0.72 V
MFC (7), two units in series	Nil	0.93 V
MFC (8), two units in series	100 million sporogenes	1.66 V

similar studies utilizing dairy waste as a substrate. For instance, studies by Mansoorian *et al.* (2016) achieved up to 1.8 V using dairy wastewater, while Phillips *et al.* (2017) recorded lower efficiencies when using raw milk, indicating the optimization achieved in this study by modifying bacterial concentration and chamber volume. The alternative use of sodium hypochlorite as an oxidant in MFC (8) yielded a comparable performance to systems using  $\text{KMnO}_4$ . Previous research by Eliato *et al.* (2016) noted that  $\text{KMnO}_4$  is highly effective but cost-prohibitive, making sodium hypochlorite a promising alternative for practical applications.

The innovative design incorporating a salt bridge as a cathode (MFC 4–6) introduced operational simplicity but showed reduced OCV, warranting further refinement. This is consistent with findings by Silva-Palacios *et al.* (2023), who reported that cathodic material innovation often requires balancing trade-offs between cost and performance. This study highlights milk's efficacy as an MFC substrate, especially when paired with *Lactobacillus* bacteria, demonstrating considerable potential for energy generation. The scalability of setups like MFC (3) and the economic feasibility of sodium hypochlorite as an oxidant offer practical avenues for further research and commercialization.

#### Conclusion

The key approach of this study was to use milk as a substrate in microbial fuel cell and to assess its practicability. Various approaches were used to study voltage generation from raw milk as a substrate using *Lactobacillus* bacteria. This includes changing the concentration of bacteria, changing anodic and cathodic chamber volumes. Use of Salt bridge was assessed in place of semipermeable membrane which is commonly used in the process of microbial fuel cell. Novel approach of using salt bridge which acting both as conducting medium and cathodic chamber was evaluated. The  $\text{KMnO}_4$  as an oxidant gave maximum OCV of 2.01 V and this OCV varied with change in conditions such as change in bacterial concentration, volume of cathodic and anodic chamber. Sodium hypochlorite 0.6 w/v% also gave considerable high OCV of 1.66 V and depicted that it can be further studied as an oxidant with comparison of potassium permanganate. The use of salt bridge can also be further evaluated in comparison of membrane in terms of power generation. The salt bridge acting

as a cathode showed potential for further research and gave OCV of 1.13 V in MFC (5). This research demonstrates that milk, used as a substrate in MFCs with *Lactobacillus*, offers significant potential for power generation.

### Conflicts of Interest

The author(s) declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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