Optimization of Earthen Pot Microbial Fuel Cells for Sustainable Energy Recovery from Dairy Sludge Waste

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Abstract

Microbial Fuel Cells (MFCs) represent a promising technology for sustainable energy generation and waste management by harnessing the metabolic activity of microorganisms to produce electricity from organic waste. In this study, an earthen pot-based microbial fuel cell (EPMFC) was investigated as a low-cost and eco-friendly alternative to conventional MFCs. The research focused on optimizing key parameters such as substrate concentration, catholyte composition, and electrogens selection to enhance the performance of EPMFCs.
Experiments were conducted using dairy waste-activated sludge as the substrate, varying solid concentrations (5%, 10%, and 15%), and different catholytes (Water, NaCl, and KCl). The study also examined the impact of electrogenic bacteria (*Bacillus subtilis* and *Escherichia coli*) on power generation. Voltage, power density, and current density were measured over multiple batch cycles, and the reuse of electrodes and chambers was evaluated.
The results demonstrated that a 10% solid concentration yielded the highest voltage output, particularly with KCl as the catholyte. Bacillus subtilis showed superior electrogenic capabilities compared to Escherichia coli, with a maximum recorded voltage of 452.66 mV, power density of 382.29 mW/m², and current density of 717.68 mA/m². Reuse of electrodes and chambers initially improved performance but led to a slight decline in voltage over successive cycles due to biofilm accumulation and substrate depletion.
Overall, this study highlights the potential of EPMFCs as a cost-effective and efficient method for bioelectricity generation and wastewater treatment. The findings provide valuable insights for optimizing MFC performance, paving the way for scalable and sustainable energy solutions.

Key Words: Earthen Pot MFC, Catholyte, Electrogens, Solid Concentration

1. Introduction

Microbial Fuel cells (MFCs) have been studied as a technology for sustainable development due to their ability to produce electricity from the organic matter containing waste. Thus, MFC provide eco-friendly option for energy production and waste management. This technology harnesses the metabolic activity of microbes. In anaerobic condition microbes generate protons, electrons and Carbon dioxide. Without available external electron acceptors, these electrons are moved towards electrodes, which enables production of electricity while reducing the organic matter in waste. The proton passes through a membrane to the cathode where they combine with electrons to reduce an electron acceptor present. Despite its potential, the cost, operational parameters, anode electrode, cathode electrode , substrate utilised, substrate concentration, ,catholyte power output are key area of ongoing research aimed at enhancing energy efficiency and overall performance (Ghadge et al., 2015; Khilari & Pradhan, 2017; Sugumar & Dharmalingam, 2020).

Many researchers have shown that the use of porous membrane can be used instead of ion exchange membranes due to their excellent ability to transfer protons (Daud et al., 2019, 2020; Lee et al., 2015; Ortiz-Martínez et al., 2015; Salar-García et al., 2019)

Bahera et. al., demonstrated the competitive performance of low-cost earthen pot without employing the expensive Nafion membrane. They have also concluded that that the internal resistance of earthen pot MFC was lower than the MFC employing Proton exchange membrane (Bagchi & Behera, 2020; Behera et al., 2010).

Earthen pot microbial Fuel cells (EPMFCs) are a very good combination of traditional pottery and modern microbiological technology to produce electricity from high organic matter containing waste. Because of their special qualities- such as ease of use, affordability and cultural significance they provide an attractive option for waste to energy generation and environmental remediation. The earthen pot wall acts as ion exchange membrane separating two compartments. The anode chamber, usually enclosed in an earthen pot, is filled with organic substrate and electrogenic bacteria. These electrogenic bacteria oxidises organic matter and release electrons, which are transmitted to anode electrode. A cathode electrode in the cathode chamber makes it easier accepts the electrons and reduces the catholyte completing the circuit and produce electricity (Daud et al., 2020).

The substrate concentration is crucial in the electricity generation of microbial fuel cells (MFCs). It influences metabolism of microbes by making organic matter available. The efficient conversion of organic matter is possible only optimum substrate concentration. Increase in substrate concentration boosts the electron flow towards the anode, generating more electricity. However, increasing the substrate concentration to excessive levels reduces the microbial activity causing inhibition of electricity generation. Thus, balanced concentration ensures maximum efficiency of MFCs (Ullah & Zeshan, 2020).

Another important thing which affects the efficiency of MFCs is catholyte. The Voltage generated in Microbial Fuel cells (MFCs) is significantly influenced by the type and concentration of catholyte. Oxidising agents such as Potassium ferricyanide, Potassium chromate, Potassium permanganate and salts such as NaCl and KCl improves the ability of cathode to accept the electrons. The reduction reaction at cathode is significantly influenced by catholyte which directly affects the potential difference between anode and cathode. Moreover, the ionic conductivity in the catholyte also reduces internal resistance , further improving the overall power generation (Pandit et al., 2011; Singh & Dharmendra, 2020).

In this study, a low-cost earthen pot Microbial Fuel Cell (MFC) is analyzed, focusing on its performance under varying conditions. The aspect in the study includes the following.

1. Substrate Concentration: The earthen pot MFC is studied using various substrate concentrations, which acts as organic matter used as the food source for the microorganisms in MFC. The variation of substrate concentrations aims to study how change in availability of organics affect the output and efficiency of MFCs.
2. Catholyte : The use of different catholytes in cathode chamber of MFC , helps to how the type of electrolyte affects the MFC. The overall voltage and current produced in MFC is affected by the catholyte as it facilitates the reduction reactions at cathode.
3. Electrogens: The electrogens (microbes that generates electricity) can enhance electron transfer process, potentially enhancing the efficiency of MFC. By using different electrogens the research seeks to identify most effective elctrogens boosting the output of earthen pot MFC.

Overall, the goal of the study is to optimize the MFC's design for low-cost energy generation by understanding how changes in substrate concentration, catholyte composition, and electrogens selection affect its performance.

2. Material and Methods

2.1 Collection of Waste

The Waste Activated Sludge from Dairy (WASD) was procured from Dudhsagar Dairy, Mahesana, and subsequently stored at a controlled temperature of 4°C to prevent microbial degradation and maintain its physicochemical properties. This preservation ensured the stability of the sludge composition, preventing any significant biochemical changes before its utilization in experimental procedures. The sludge was maintained under these conditions until further analysis and experimental applications to ensure reproducibility and accuracy of results.

2.2 Design of Experimental set ups

The study was performed on MFC constructed with earthen pot of 400mL capacity. The wall of the earthen pot was nearly 4-5 mm thick, itself acts as a proton exchange membrane. The earthen pot itself was the anodic chamber, which was placed in a plastic container working as cathodic chamber. The graphite rods (1.5 cm diameter and 15 cm length were used as electrodes. The electrodes were connected externally by insulated copper wire through external resistance of 100 ohm.

2.3 MFC Operation

A set of three EMFC was labelled as EMFC1 were filled with 300 ml 5% solid, other set of three EMFC 2 300 mL 10% solid further another set of three more EMFCS were labelled EMFC 3 and filled with 300 mL of 15% solid

Initially the EPMFC was filled with the substrate at varying solid concentration (5%, 10% ,15%) of Waste Activated Sludge of Dairy (WASD) industry. The EMFCs were then placed in different catholytes (water, NaCl and KCl) as shown in table below.

S.No	Solid Concentration	Catholyte
1	5%	Water
2	5%	NaCl
3	5%	KCl
4	10%	Water
5	10%	NaCl
6	10%	KCl
7	15%	Water
8	15%	NaCl
9	15%	KCl

The best solid concentration was used for the next experiment in which different electrogens were used in the anodic chamber. In experimental setup Anodic chamber was inoculated with 10% of active culture.

In the experiment, catholyte solutions were prepared in three different sets based on their chemical composition. The first set used water as the catholyte and included three conditions: WC (Water and Control), WB (Water and *Bacillus subtilis*), and WE (Water and *Escherichia coli*). The second set contained NaCl as the catholyte with three conditions: NC (NaCl and Control), NB (NaCl and *Bacillus subtilis*), and NE (NaCl and *Escherichia coli*). The third set utilized KCl as the catholyte, consisting of KC (KCl and Control), KB (KCl and *Bacillus subtilis*), and KE (KCl and *Escherichia coli*).

The experiment with electrogens was conducted for three Batch cycles of 15 days (every time the substrate in anodic chamber was replaced with fresh substrate and inoculum without washing the chamber and anode, so that the effect of reuse of biofilm on voltage generation can be studied. Catholyte was replaced in each cycle.

All the experiments were performed in triplicates.

2.4 Analysis and Calculations

The Characterization of waste was done using standard methods

The characteristic such as pH, Total Solid (TS), Volatile Solid (VS), Suspended Solid (SS) , Chemical Oxygen Demand (COD) were analysed by standard protocols mention in American Public Health Association (APHA).

The Open Circuit Voltage (OCV) was recorded using a Digital multimeter after connecting the resistance (100 Ω) few minutes after obtaining the stabilization of voltage.
The Current I was calculated by using the formula
I=V/R, where V is Voltage and R is resistance
Power (W) was calculated using relation
P=I*V, where I and V represents current (I) and voltage (V), respectively.
Power density (mW/m^2) and current density (mA/m^2) were calculated by dividing the obtained power and current by the anode surface area (m^2) (Surface area of anode = 0.007422m^2).

3. Result and Discussion

3.1 Characteristic of waste

The characteristic was observed as pH: 7.2 , Total Solid (TS): 10500 mg/L, Volatile Solid (VS): 8085 mg/L , Suspended Solid (SS) : 5800 mg/L and Chemical oxygen Demand (COD) : 25708 mg/L were analysed by standard protocols mention in American Public Health Association (APHA).

3.2 Optimization of Solid Concentration

The Solid concentration is crucial for generation of electricity.
Three different concentrations of WASD were used as mentioned. Initially all the sets of EMFC were operated under OCV. The Voltage was recorded every 24 hours. The results in Fig. 1, indicate that an increase in concentration from 5% to 10% led to a significant rise in voltage. However, a further increase in solid concentration to 15% resulted in a decline in voltage.

Highest voltage was generated in 10% solid using KCl, 284.64±3.8 mV. The voltage generated in 10% solid in NaCl and Water was 199.30±3.3 mV and 123.96±2.2 mV.

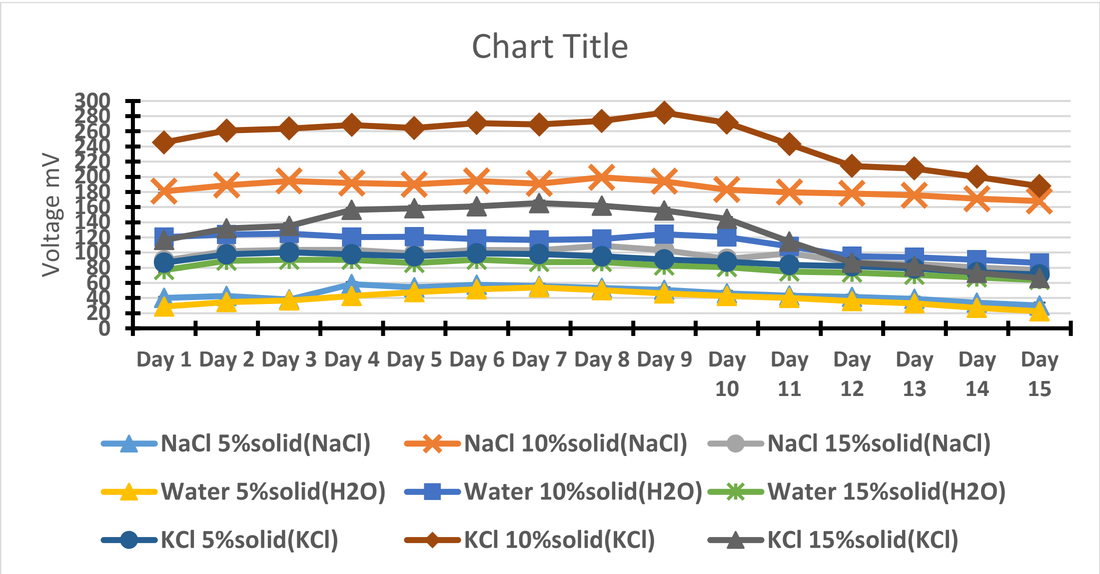

Fig. 1 Effect of Solid Concentration on Voltage generation of EMFC

Fig 2 Shows the effect of solid concentrations on different electrolytes. It was observed that 10% solid concentration was giving best voltage generation in catholytes.

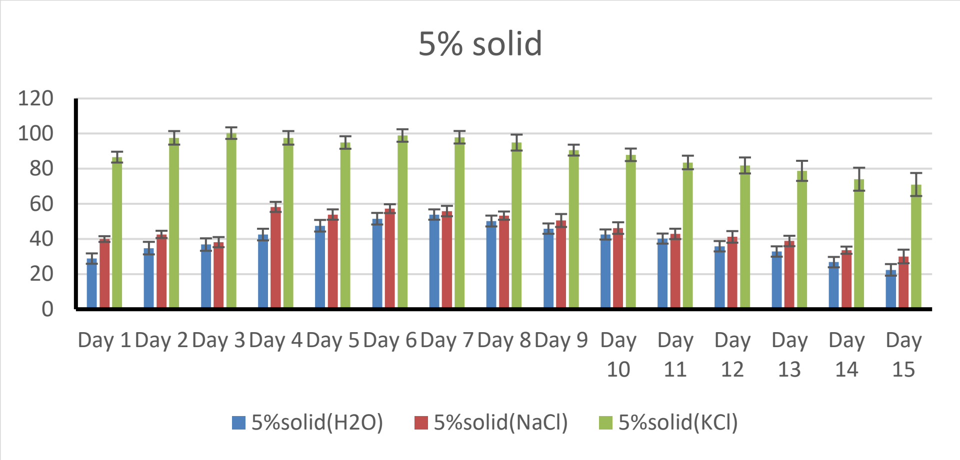

Fig 2 a: Effect of 5% solid concentration on Voltage generation in EMFC

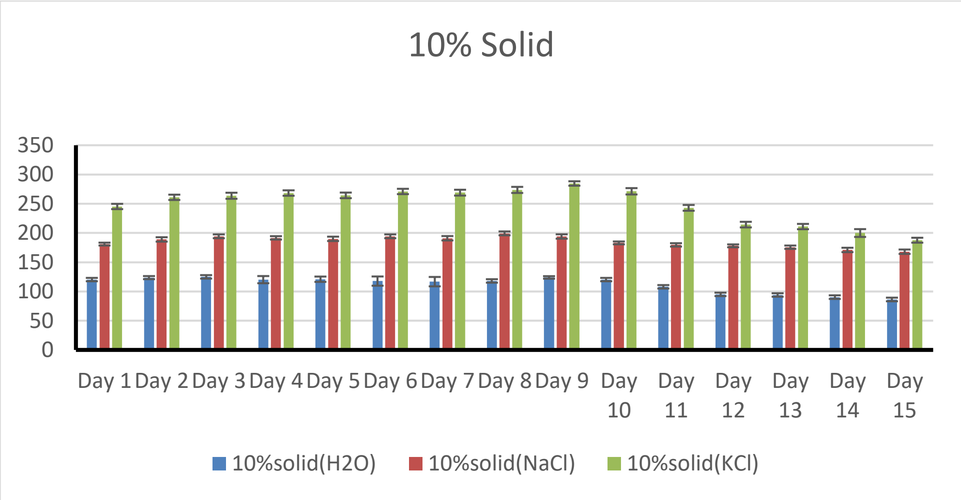

Fig 2 b: Effect of 10% solid concentration on Voltage generation in EMFC

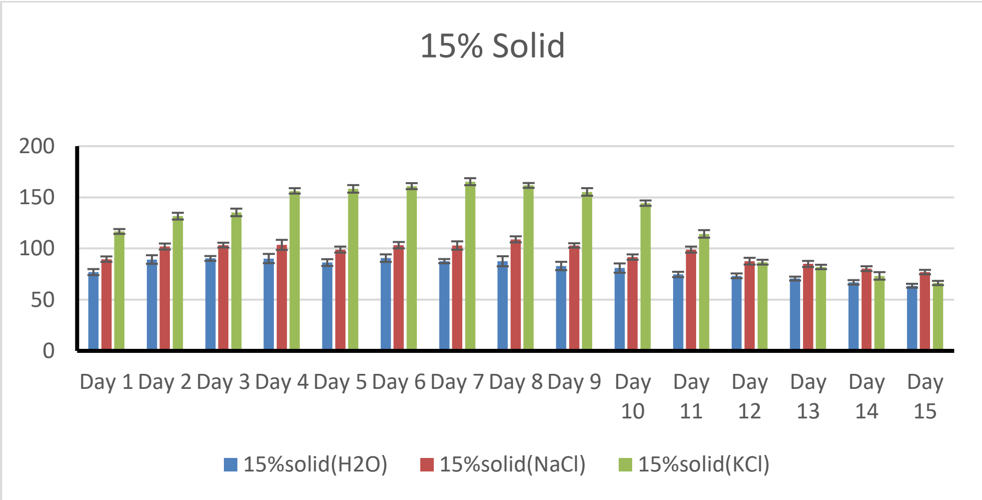

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Fig 2 c: Effect of 15% solid concentration on Voltage generation in EMFC

Thus, maintaining optimal solid concentration in microbial fuel cells (MFCs) is crucial for efficient operation. The voltage output is significantly influenced by the concentration of solid substrates, as they serve as the primary source of organic matter for microbial metabolism.

Initially, an increase in solid concentration enhances microbial activity by providing more nutrients, leading to a higher rate of bio electrochemical reactions and improved electron transfer to the anode. This results in a rise in voltage output due to the efficient breakdown of organic matter and increased ion conductivity within the system. However, beyond a certain threshold, excessive solid concentration can lead to substrate overloading, causing metabolic stress on microbial communities due to the accumulation of toxic byproducts such as volatile fatty acids and ammonia. (Sugumar & Dharmalingam, 2020; Ullah & Zeshan, 2020)
Additionally, high solid concentrations can impair mass transfer processes, restricting oxygen and nutrient diffusion, thereby limiting microbial growth and reducing the efficiency of electron transport. Furthermore, excessive solids can lead to clogging and increased internal resistance, further diminishing voltage output. Therefore, maintaining an optimal solid concentration is crucial for ensuring efficient microbial activity, proper mass transfer, and sustained electrochemical performance in an EMFC (Ismail & Radeef, 2019; Liu et al., 2005; Zhang et al., 2013)

3.3 Optimization of Catholyte.

In microbial fuel cells (MFCs), the choice of catholyte significantly influences the system's voltage output. This study shows that KCl was the catholyte giving highest voltage generation in at all substrate concentrations. At 5% solid concentration 98.93±3.56 mV at 10% solid concentration highest Voltage recorded was 284.64±3.77mV and at 15% solid concentration 165.30±3.49mV.Fig. 3 depicts the voltage generation at different catholytes.

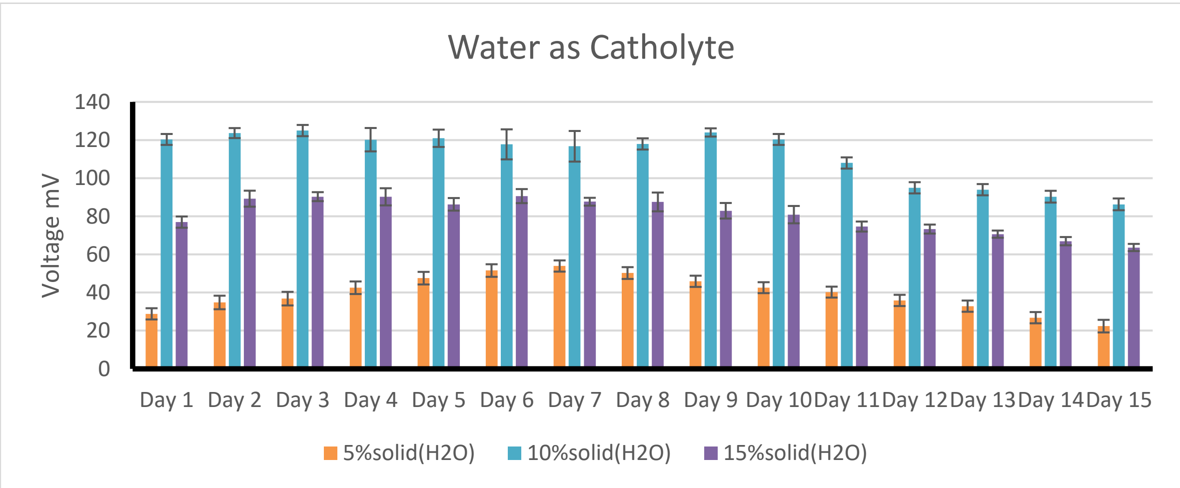

Fig. 3 a : Water as Catholyte

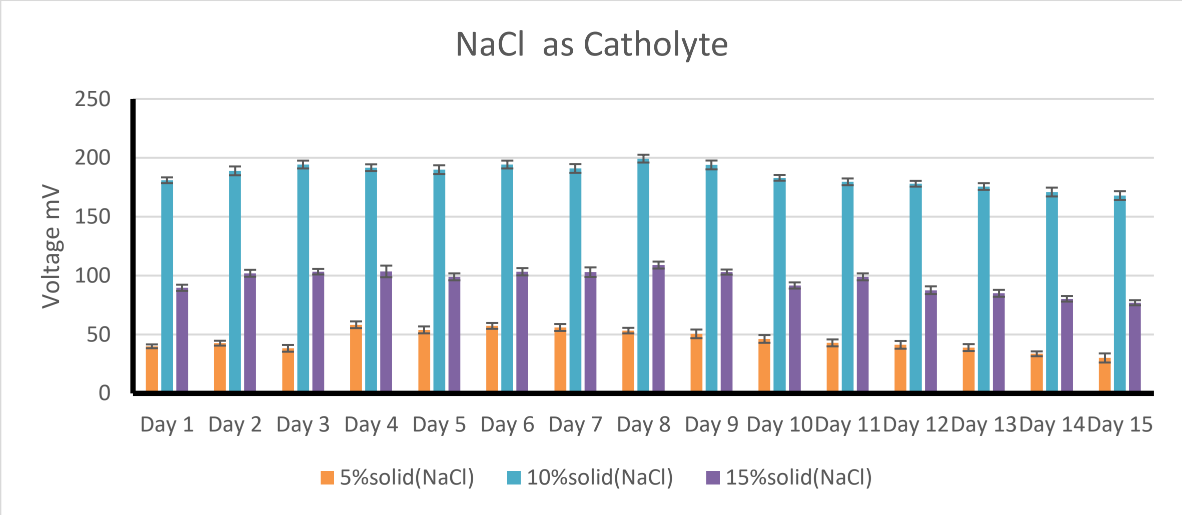

Fig. 3 b : NaCl as Catholyte

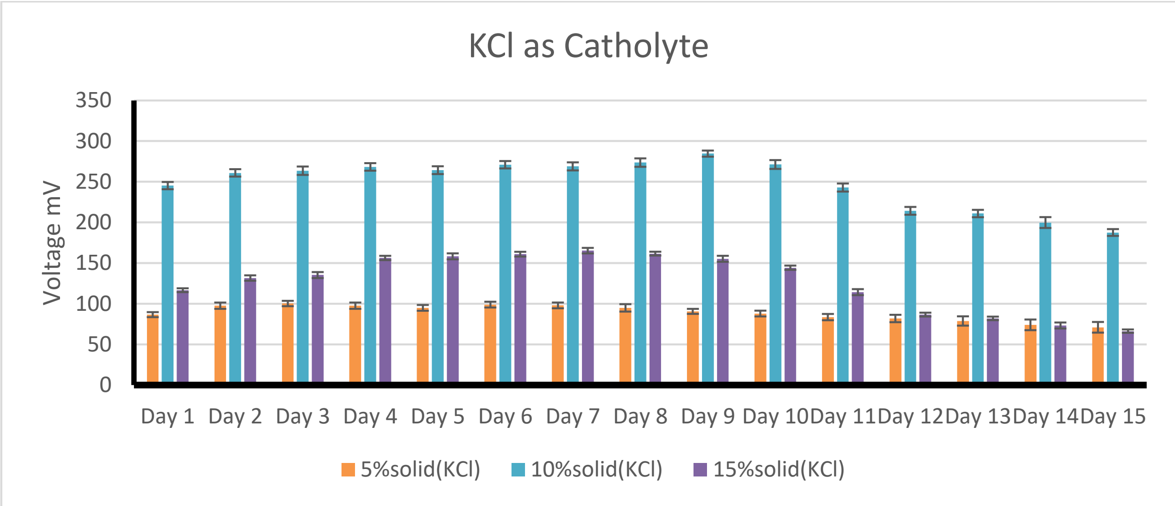

Fig. 3 c : KCl as Catholyte

Studies have demonstrated that using potassium chloride (KCl) as the catholyte yields higher voltage outputs compared to sodium chloride (NaCl). For instance, research comparing different salt bridges found that MFCs utilizing KCl achieved a maximum voltage of 447 mV and a power density of 10.12 mW/m², whereas those with NaCl recorded lower values. Another study reported that with a 1M concentration of KCl, the MFC generated 823 mV, surpassing the 713mV produced with 1M NaCl under similar conditions. These findings suggest that the superior ionic mobility and conductivity of potassium ions (K^+) enhance charge transfer processes within the MFC, thereby reducing internal resistance and improving overall efficiency. Additionally, K^+ ions play a crucial role in microbial physiology, supporting enzymatic activities and maintaining cellular osmotic balance, which can further enhance electron transfer processes at the anode. Therefore, selecting KCl as the catholyte can lead to improved electrochemical performance and increased energy generation in MFC systems (Ali et al., 2018; Muhammad et al., 2021).

3.4 Optimization of electrogens

Using 10 % solid concentration and different electrolytes the result obtained by different electrogens is shown in Fig. 4

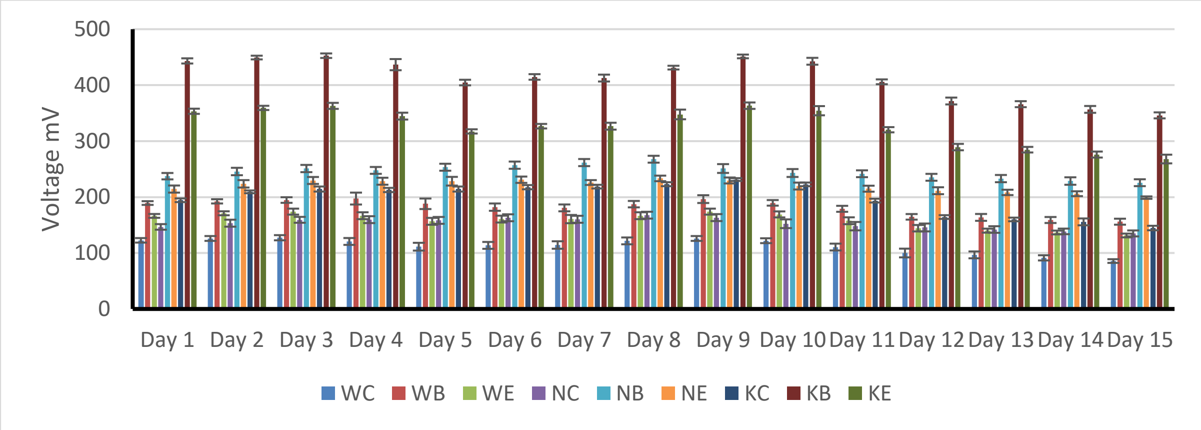

Fig. 4 Effect of Electrogens on Voltage generation of EMFC

WC: Water as catholyte and no added electrogen, WB: Water as catholyte and *Bacillus subtilis* electrogen WE: Water as catholyte and *Escherichia coli* as electrogen ,NC: NaCl as catholyte and no added electrogen ,NB: NaCl as catholyte and *Bacillus subtilis* electrogen ,NE: NaCl as catholyte and *Escherichia coli* as electrogen ,KC: KCl as catholyte and no added electrogen ,KB: KCl as catholyte and *Bacillus subtilis* electrogen ,KE: KCl as catholyte and *Escherichia coli* as electrogen

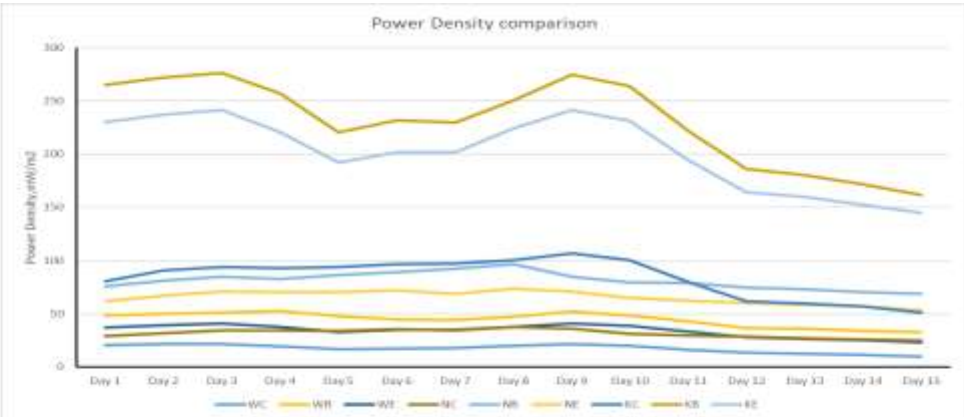

Fig. 5 Effect of Electrogens on Power Density of EMFC

WC: Water as catholyte and no added electrogen, WB: Water as catholyte and *Bacillus subtilis* electrogen WE: Water as catholyte and *Escherichia coli* as electrogen ,NC: NaCl as catholyte and no added electrogen ,NB: NaCl as catholyte and *Bacillus subtilis* electrogen ,NE: NaCl as catholyte and *Escherichia coli* as electrogen ,KC: KCl as catholyte and no added electrogen ,KB: KCl as catholyte and *Bacillus subtilis* electrogen ,KE: KCl as catholyte and *Escherichia coli* as electrogen

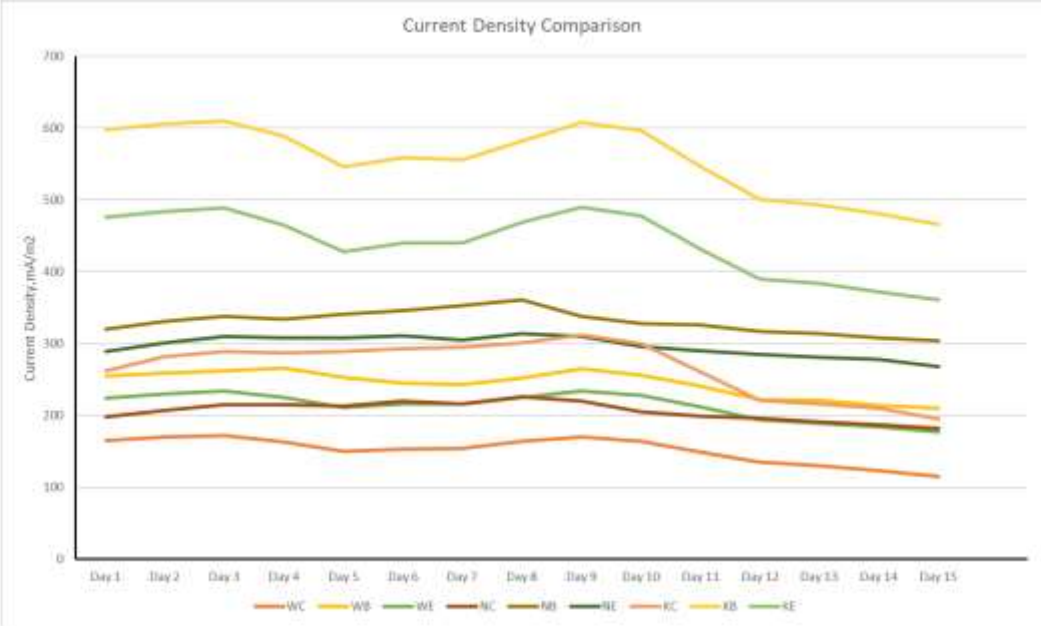

Fig. 6 Effect of Electrogens on Current Density of EMFC

WC: Water as catholyte and no added electrogen, WB: Water as catholyte and *Bacillus subtilis* electrogen WE: Water as catholyte and *Escherichia coli* as electrogen ,NC: NaCl as catholyte and no added electrogen ,NB: NaCl as catholyte and *Bacillus subtilis* electrogen ,NE: NaCl as catholyte and *Escherichia coli* as electrogen ,KC: KCl as catholyte and no added electrogen ,KB: KCl as catholyte and *Bacillus subtilis* electrogen ,KE: KCl as catholyte and *Escherichia coli* as electrogen

From the result it was observed that the highest voltage, power density and Current density are 452.66 $\pm$ 4.0mV , 382.29 mW/m^2 and 717.68 mA/m^{2}respectively. in all the three sets of Organisms was in *Bacillus subtilis* with KCl as catholyte.

The power density generated by *Bacillus subtilis* using NaCl and KCl as catholytes was 96.53 mW/m^2 and 382.29 mW/m^2, respectively, representing an increase of 1.83-fold and 7.28-fold compared to the control. The corresponding current densities for *Bacillus subtilis* with NaCl and KCl as catholytes were 369.64 mA/m^2 and 717.68 mA/m^2, which were 1.35 and 2.69 times higher than the control, respectively.

Similarly, the power density for *Escherichia coli* with NaCl and KCl as catholytes was 73.35 mW/m^2 and 241.45 mW/m^2, showing an increase of 1.79-fold and 5.92-fold over the control. The current densities in the same conditions were 314.38 mA/m^2 and 570.37 mA/m^2, which were 1.34 and 2.50 times higher than the control, respectively.s

Thus, the choice of bacterial strains significantly influences voltage generation. Many studies have demonstrated that utilizing *Bacillus* species can lead to higher voltage outputs compared to *Escherichia coli* (*E. coli*). For instance, research indicated that MFCs employing *Bacillus* sp. achieved a peak voltage of 0.65 volts over five days, with optimal pH and conductivity parameters of 7 and 24 mS/cm, respectively. In contrast, *E. coli* has been observed to generate

lower voltage outputs when used as the sole inoculum in MFCs (Páez et al., 2019; Yoganathan & Ganesh, 2015)
These findings suggest that while *Bacillus* species may inherently possess more efficient electron transfer mechanisms conducive to higher voltage generation in Earthen Pot MFCs.

3.5 Reuse of Electrodes and Chambers

Fig. 7 clearly shows that the second cycle was giving higher voltage generation. It was also noted that, in third cycle there was decrease in voltage generation in every set , but decrease was very less in KE compared to other sets.

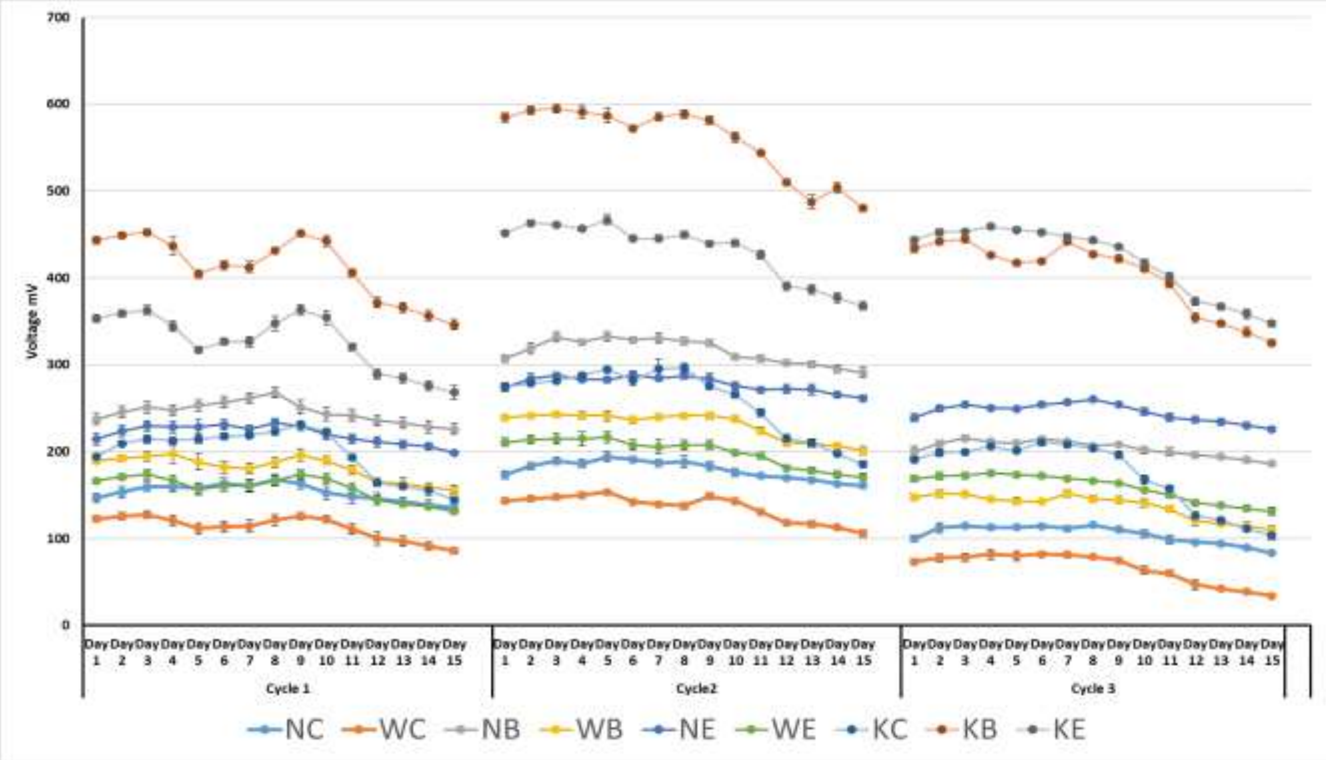

Fig. 7 Effect of reuse of Anode and Chambers

WC: Water as catholyte and no added electrogen, WB: Water as catholyte and *Bacillus subtilis* electrogen WE: Water as catholyte and *Escherichia coli* as electrogen ,NC: NaCl as catholyte and no added electrogen ,NB: NaCl as catholyte and *Bacillus subtilis* electrogen ,NE: NaCl as catholyte and *Escherichia coli* as electrogen ,KC: KCl as catholyte and no added electrogen ,KB: KCl as catholyte and *Bacillus subtilis* electrogen ,KE: KCl as catholyte and *Escherichia coli* as electrogen

Fig,8 shows the comparison of three cycles with respect to voltage power density and current density.

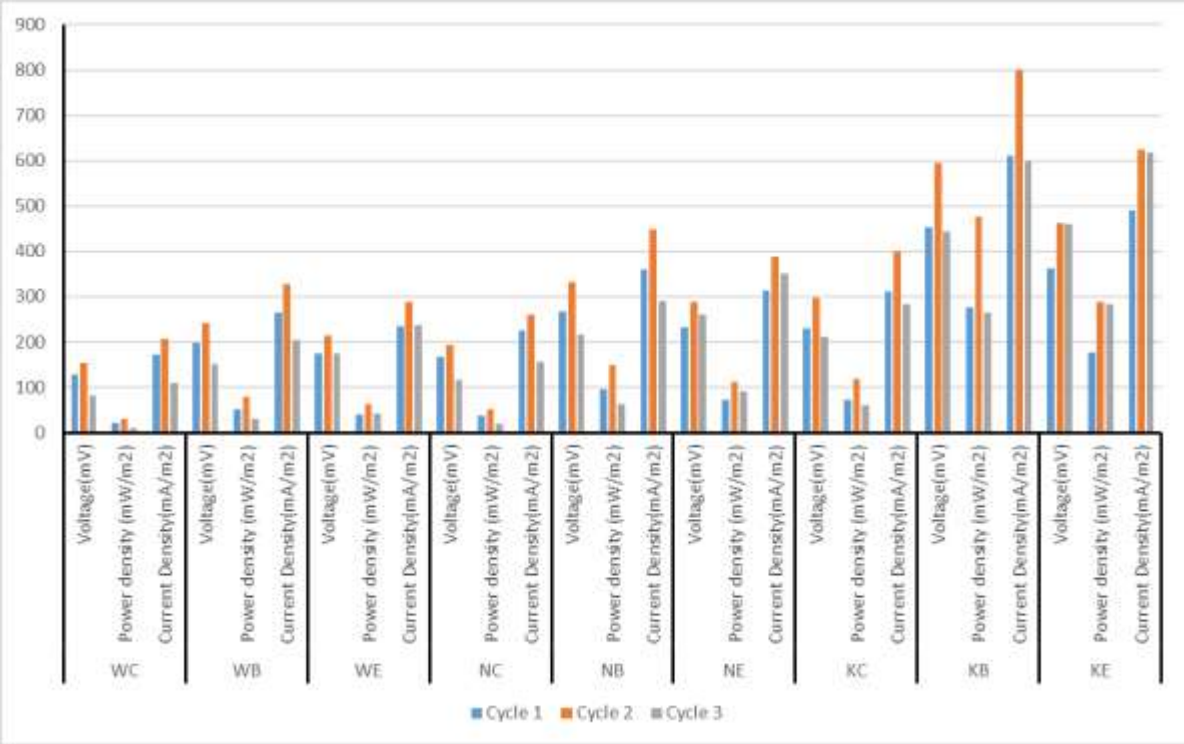

Fig. 8 Comparison of Voltage, Power Density and Current Density in three Cycles

WC: Water as catholyte and no added electrogen, WB: Water as catholyte and *Bacillus subtilis* electrogen WE: Water as catholyte and *Escherichia coli* as electrogen ,NC: NaCl as catholyte and no added electrogen ,NB: NaCl as catholyte and *Bacillus subtilis* electrogen ,NE: NaCl as catholyte and *Escherichia coli* as electrogen ,KC: KCl as catholyte and no added electrogen ,KB: KCl as catholyte and *Bacillus subtilis* electrogen ,KE: KCl as catholyte and *Escherichia coli* as electrogen

The best result was obtained in cycle 2 in the set with KCl as catholyte and *Bacillus subtilis* as electrogens in anode chamber.

The reuse of electrodes and anode chambers in EMFC initially enhances voltage generation due to the retention of electroactive biofilms, which accelerate microbial acclimatization and improve electron transfer efficiency. These pre-established biofilms reduce the start-up time and contribute to a stable increase in voltage output. However, over time, excessive biofilm growth can lead to mass transfer limitations, increased internal resistance, and electrode fouling, ultimately causing a decline in voltage generation. Additionally, prolonged operation without adequate substrate replenishment results in nutrient depletion, limiting microbial metabolism and reducing electron production. Material degradation, such as corrosion or loss of conductivity in electrodes, further impacts performance, especially in metal-based electrodes compared to carbon-based ones. Studies suggest that optimized electrode spacing and routine maintenance, such as cleaning or replacing electrodes, can help sustain voltage output over multiple cycles .Therefore, while electrode and chamber reuse initially improves voltage generation, long-term efficiency depends on managing biofilm accumulation, preventing electrode degradation, and ensuring continuous substrate availability (Greenman et al., 2021; Wang et al., 2009; Xing et al., 2024) .

Conclusion

The results clearly show that the maximum power density and current density were obtained with a 10% solid concentration, KCl as the catholyte, and *Bacillus subtilis* as the electrogen. In particular, the system showed a peak current density of 801.22 mA/m^2 and a maximum power density of 476.46 mW/m^2 during the second cycle of reuse.

The results indicate that the microbial fuel cell's (MFC) electron transfer efficiency was improved by the metabolic activity of *Bacillus subtilis*. It is possible that the use of KCl as a catholyte further enhanced ionic conductivity, which in turn facilitated charge transport and reduced internal resistance. The system's overall performance was improved by the notable rise in power and current density during the second reuse cycle, which suggests that either biofilm formation or microbial community adaption produced an ideal electrochemical environment.

The present study offers a novel and sustainable approach to bioelectricity generation by optimizing earthen pot microbial fuel cells (MFCs) using secondary dairy sludge waste as a substrate. Unlike conventional MFC designs, the use of low-cost, locally available earthen pots provides an eco-friendly and affordable alternative for scalable energy systems, especially in rural and resource-limited settings. This research is significant in its dual focus on waste valorisation and clean energy production, addressing key environmental challenges associated with dairy industry effluents. Through the systematic optimization of operational parameters such as substrate concentration, electrode configuration, and external resistance, the study enhances the performance and efficiency of MFCs for bioelectricity generation. By integrating waste management with renewable energy recovery, the work contributes to the development of decentralized, sustainable energy solutions aligned with global environmental goals and the principles of circular economy.

The practical usefulness of this system for sustainable bioelectricity generation may be further understood with additional research into its long-term stability, substrate degradation efficiency, and possible scale-up.

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