

# **EXPERIMENTAL INVESTIGATION OF A BIOFUEL CELL**

by

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## THESIS EXAMINATION INFORMATION

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## ABSTRACT

This thesis presents an investigation of bioenergy generation through a soil-based microbial fuel cell (MFC). The MFC utilized soil samples of 100 g and various environmental and operational conditions to assess the generation of voltage. The parameters of interest included temperature, pH, glucose concentration, sodium chloride, and volume of water. The temperature experiment showed that moderate temperatures of 35 °C and 45 °C yielded stable voltage outputs. The maximum voltage obtained was approximately 55 mV at a temperature of 45 °C and 50 mV at a temperature of 35 °C. On the other hand, higher temperatures of 50 °C decreased the voltage from approximately 30 mV to as low as 16 mV. The experiment on glucose concentration showed the optimal concentration of glucose, as a voltage of approximately 66 mV was obtained at a glucose concentration of 1.0 g. The experiment on sodium chloride showed improved performance at moderate salinity. The addition of 1.5 g of sodium chloride at a temperature of 25 °C yielded a peak voltage of approximately 70 mV. On the other hand, the addition of 1.0 g of sodium chloride at a temperature of 40 °C yielded a peak voltage of approximately 100 mV. The experiment on pH and temperature showed exceptional performance at a pH of 7 and a temperature of 40 °C. The voltage was as high as approximately 950 mV. Soil moisture was also seen to greatly influence performance, as the addition of 300 mL of water yielded an initial voltage of approximately 427 mV. The voltage gradually decreased over time to approximately 230 mV due to mass transfer limitations. The results show that bioenergy generation through soil-based MFC improves at optimized environmental conditions. The scientific literature consistently shows that soil-based microbial fuel cells generate more bioenergy when key environmental conditions are optimized.

**Keywords:** Microbial Fuel Cell; Bioenergy Production; Soil-Based Bioelectrochemical System; Sustainability.

## **AUTHOR'S DECLARATION**

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Unspoken contributions sometimes roar the loudest; for the lion-hearted presence behind the scenes, I am sincerely grateful.

## **STATEMENT OF CONTRIBUTIONS**

I hereby certify that I am the sole author of this thesis and that no part of this thesis has been published or submitted for publication. I have used standard referencing practices to acknowledge ideas, research techniques, or other materials that belong to others. Furthermore, I hereby certify that I am the sole source of the creative works and/or inventive knowledge described in this thesis

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## NOMENCLATURE

|       |                                       |
|-------|---------------------------------------|
| AEL   | Alkaline electrolysis                 |
| ATR   | Autothermal reforming                 |
| CCS   | Carbon capture and storage            |
| EC    | Electrical conductivity               |
| EDS   | Energy-dispersive X-ray spectroscopy  |
| h     | Specific enthalpy (J/kg)              |
| HEBM  | High-energy ball milling              |
| HGR   | Hydrogen generation rate              |
| HHV   | Higher heating value (kJ/mol)         |
| kJ    | Kilo Joule                            |
| LCA   | Life cycle assessment                 |
| LHV   | Lower heating value (kJ/mol)          |
| mHGR  | Maximum hydrogen generation rate      |
| n     | Number of moles                       |
| PEM   | Proton exchange membrane              |
| PEMEL | Proton exchange membrane electrolysis |
| POM   | Partial oxidation of methanol         |
| POX   | Partial oxidation                     |
| ppm   | Part per million                      |
| RMF   | Resistance melting furnace            |
| SEM   | Scanning electron microscopy          |
| SOEL  | Solid oxide electrolysis              |
| SMR   | Steam methane reforming               |
| SR    | Steam reforming                       |
| TDS   | Total Dissolved Solids                |
| TEM   | Transmission electron microscopy      |
| XPS   | X-ray photoelectron spectroscopy      |
| XRD   | X-ray diffraction                     |

### Symbols

|                    |  |
|--------------------|--|
| $E_a$              | Activation energy (J/mol)                                    |
| $E_X^{Q_{in}}$     | Thermal exergy (J)   |
| $\Delta_f H^\circ$ | Standard molar enthalpy of formation at 298.15K (kJ/mol)     |
| $\Delta_f G^\circ$ | Standard molar Gibbs energy of formation at 298.15K (kJ/mol) |
| $C_p$              | Molar heat capacity at 298.15K (kJ/molK)                     |
| $S^\circ$          | Standard molar entropy at 298.15K (J/molK)                   |
| wt%                | Weight percent   |
| $\mu\text{s/cm}$   | Micro siemens/centimeters                                    |
| $\eta_{con}$       | Conversion efficiency  |
| $\eta$             | Energy efficiency  |
| $\eta_{ex}$        | Exergy efficiency  |

### Chemical Compounds

|                         |                          |
|-------------------------|--------------------------|
| $\text{AlCl}_3$         | Aluminum chloride        |
| $\text{Al}_2\text{O}_3$ | Aluminum oxide (alumina) |

|                                       |                                  |
|---------------------------------------|----------------------------------|
| C <sub>2</sub> H <sub>5</sub> OH      | Ethanol                          |
| CH <sub>3</sub> COOH                  | Acetic acid                      |
| (CH <sub>3</sub> COO) <sub>2</sub> Mg | Magnesium acetate                |
| Ce                                    | Cerium                           |
| CeO <sub>2</sub>                      | Cerium oxide (ceria)             |
| Co                                    | Cobalt                           |
| CoCl <sub>2</sub>                     | Cobalt(II) chloride              |
| CO <sub>2</sub>                       | Carbon dioxide                   |
| Ga                                    | Gallium                          |
| Ir                                    | Iridium                          |
| KOH                                   | Potassium hydroxide              |
| Li                                    | Lithium                          |
| LiBH <sub>4</sub>                     | Lithium borohydride              |
| La                                    | Lanthanum                        |
| Mg                                    | Magnesium                        |
| MgCl <sub>2</sub>                     | Magnesium chloride               |
| Mg <sub>2</sub> Cu                    | Magnesium cupride                |
| MgH <sub>2</sub>                      | Magnesium hydride                |
| MgO                                   | Magnesium oxide                  |
| Mg(OH) <sub>2</sub>                   | Magnesium hydroxide              |
| Mg <sub>2</sub> Ni                    | Magnesium nickelide              |
| NaCl                                  | Sodium chloride                  |
| NaOH                                  | Sodium hydroxide                 |
| Na <sub>2</sub> Mg(OH) <sub>4</sub>   | Sodium tetrahydroxomagnesate(II) |
| Ni                                    | Nickel                           |
| NiCl <sub>2</sub>                     | Nickel(II) chloride              |
| Pd                                    | Palladium                        |
| Ru                                    | Ruthenium                        |
| SnO <sub>2</sub>                      | Tin(IV) oxide                    |
| ZnO                                   | Zinc oxide                       |
| Zr                                    | Zirconiu                         |

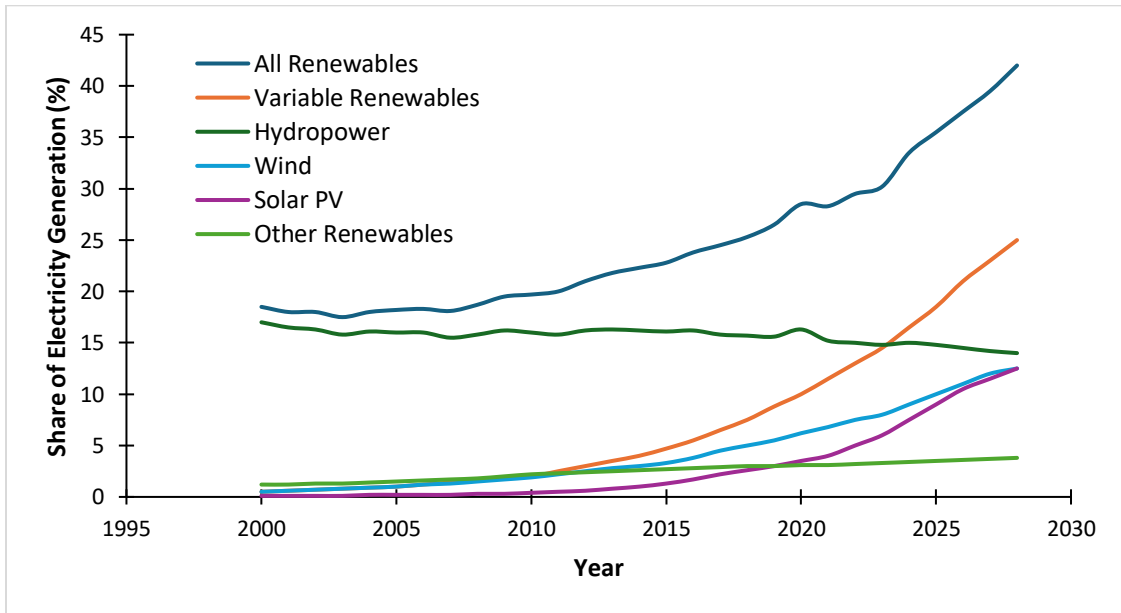
### Acronyms

|      |                                      |
|------|--------------------------------------|
| AEL  | Alkaline Electrolysis                |
| ATR  | Autothermal Reforming                |
| CCS  | Carbon Capture and Storage           |
| CE   | Coulombic Efficiency                 |
| COD  | Chemical Oxygen Demand               |
| EC   | Electrical Conductivity              |
| EDS  | Energy-Dispersive X-ray Spectroscopy |
| HGR  | Hydrogen Generation Rate             |
| HEBM | High-Energy Ball Milling             |
| HHV  | Higher Heating Value                 |
| LCA  | Life Cycle Assessment                |
| LHV  | Lower Heating Value                  |
| mHGR | Maximum Hydrogen Generation Rate     |

|       |                                       |
|-------|---------------------------------------|
| MFC   | Microbial Fuel Cell                   |
| MEC   | Microbial Electrolysis Cell           |
| MDC   | Microbial Desalination Cell           |
| PEM   | Proton Exchange Membrane              |
| PEMEL | Proton Exchange Membrane Electrolysis |
| POM   | Partial Oxidation of Methanol         |
| POX   | Partial Oxidation                     |
| PD    | Power Density                         |
| CD    | Current Density                       |
| ppm   | Parts Per Million                     |
| RMF   | Resistance Melting Furnace            |
| SEM   | Scanning Electron Microscopy          |
| SOEL  | Solid Oxide Electrolysis              |
| SMR   | Steam Methane Reforming               |
| SR    | Steam Reforming                       |
| TDS   | Total Dissolved Solids                |
| TEM   | Transmission Electron Microscopy      |
| XPS   | X-ray Photoelectron Spectroscopy      |
| XRD   | X-ray Diffraction                     |

# 1. INTRODUCTION

Renewable energy plays a vital role in addressing global energy and environmental concerns. The increasing dependence on fossil fuels has caused depletion of resources and increased levels of greenhouse gases, thereby increasing concerns about global warming. In response to these concerns, many countries worldwide are moving towards the adoption and implementation of sustainable and green energy. Renewable energy includes solar, wind, hydro, biomass, geothermal, and ocean energy. These are sustainable and environmentally friendly resources. These resources provide long-term energy security without depleting fossil fuel resources. Among these energy resources, solar photovoltaic and wind energy technologies are growing at the fastest rates globally. This is due to the rapid advancement in these technologies, as depicted in Figure 1.1. The integration of renewable energy systems in modern power systems requires efficient power electronics and intelligent control strategies. Smart grids and energy storage systems further enhance reliability and grid stability. Additionally, renewable technologies support economic development and promote sustainable infrastructure. Therefore, renewable energy plays a crucial role in achieving environmental sustainability and ensuring a resilient energy future.



**Figure 1.1** Electricity generation by technology of renewable energy (data from [1]).

Perhaps the main advantage of using a renewable energy resource is its ability to sustain itself when used properly. There are numerous other additional advantages:

Renewable energy extraction depends on meteorological and environmental conditions when extreme weather conditions exist such as polar regions or deserts etc. The use of renewable resources will not cause serious harm to the environment. The use of renewables provides diversity, balancing the harmful effects of using just a single energy or power source. Wide ranges of products can now be derived from renewables, and some renewable energy technologies do not require sophisticated components, parts, and materials [2].

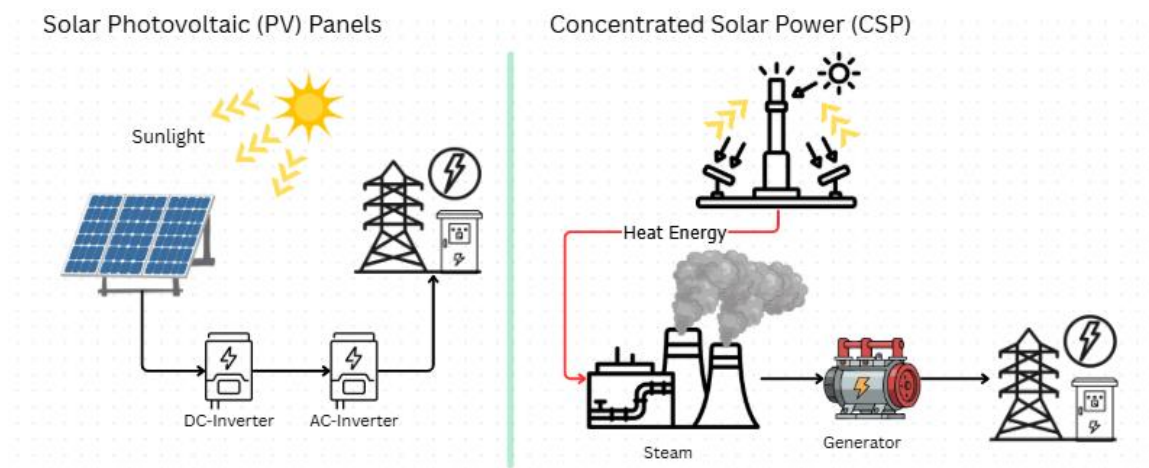
Renewables usually require large tracks of land, Most renewable energy resources are not consistently available, and timeliness of production and use may be a challenge, Storage of energy and power will also be a challenge for most renewables some are available in large amounts for short periods, and this necessitates expenses on energy storage facilities, Some renewables require special, costly materials (e.g., solar photovoltaic (PV) cells) with substantial carbon footprints during manufacturing and Economics for example, while the price of the solar PV cell has gone down below \$1/watt peak (Wp), the balance-of-systems costs (i.e., wirings, transmission, battery banks, etc.) are still relatively high [2].

### **1.1 Solar Energy Systems**

Solar energy is mainly converted to electricity using PV technology. Emission is related to absorption; sun doesn't emit but releases solar radiation based on its physical/chemical processes. Earth emits IR radiation because it absorbs solar radiation. With growing environmental concerns and the depletion of fossil fuels, solar energy has become a key technology for achieving sustainable development and reducing greenhouse gas emissions [3].

Solar energy mainly converts to electricity through the PV effect. The sunlight hits a semiconductor material, causing the photons to transfer energy to the electrons, creating electricity. The electricity produced is DC, and an inverter converts it to AC for practical purposes [4]. The other significant way to convert solar energy to electricity is through solar thermal energy. This form of energy converts sunlight to heat. Concentrated Solar Power systems make use of mirrors and lenses to focus sunlight to create heat, which then converts to electricity through steam produced in turbines [5].

Solar energy refers to solar panels, which convert sunlight into electricity in the form of DC. An inverter converts DC electricity to AC electricity, as depicted in Figure 1.2. Battery storage refers to batteries, which store energy for use at a later date. Charge controllers protect batteries from overcharging, mounting structures hold all components in place, wiring connects all components.



**Figure 1.2** Schematic illustration of power generation via solar energy

The advantages of solar energy provide a range of benefits, including being a renewable source of energy, low operational costs, minimal impact on the environment, and being scalable in nature. Once installed, solar systems require low maintenance, and savings are made on energy costs in the long run [6]. However, despite the advantages of solar energy, it also has certain limitations, including being intermittent in nature due to weather conditions, high installation costs, and the need for efficient storage systems to ensure continuous supply of energy [7].

The applications of solar energy include residential rooftops, commercial establishments, solar farms, water pumping systems, street lighting, and electrification of remote locations, along with powering satellites and other space technology. Solar energy is a clean and sustainable source of energy, and with the evolution of technology, it is likely to become a major source of energy in the near future, along with other sources of conventional energy.

## 1.2 Wind Energy Systems

Wind energy is considered to be one of the fastest-growing renewable energy technologies globally, and it plays a vital role in the transition to low-carbon power systems globally. Wind turbines harness the kinetic energy of moving air, converting it into mechanical rotational energy, which is then transformed into electrical energy by a generator. Wind power has become a significant contributor to renewable electricity globally, according to the International Energy Agency [8]. The Intergovernmental Panel on Climate Change [9] highlights the importance of the large-scale deployment of wind energy to achieve net-zero emission targets globally.

### 1.2.1 Fundamental Principles of Wind Energy

Wind turbines work on the basis of the aerodynamic lift principle, similar to an airplane wing. When wind passes through the turbine's blades, the pressure difference causes a lift force to rotate the turbine. This rotation causes the generation of electricity. The theoretical maximum efficiency limit for a wind turbine is given by the Betz limit, which states that the maximum energy extracted from the wind is 59.3% [10].

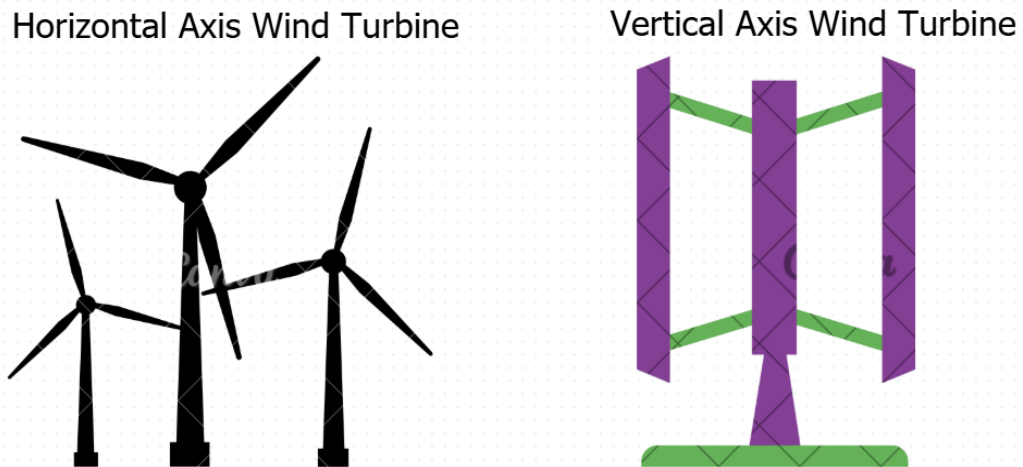
The power extracted from the wind can be given by the equation  $P = 1/2 \rho A v^3 C_p$ , where  $\rho$  is the density of the air,  $A$  is the swept rotor area,  $v$  is the wind speed, and  $C_p$  is the power coefficient. This equation shows the importance of the selection of the site for the wind farm [11].

The equation demonstrates that wind power output is highly sensitive to wind speed ( $V^3$ ), air density, and flow conditions, meaning that selecting a site with high, stable wind speeds and low turbulence is critical to maximizing energy production and system efficiency.

### 1.2.2 Types of Wind Turbines

Wind turbines are generally classified as horizontal axis wind turbines (HAWTs) and vertical axis wind turbines (VAWTs), as depicted in Figure 1.3. HAWTs are dominant in commercial wind farms due to higher efficiency compared to VAWTs, which have lower efficiency and are used for special purposes only [10].

VAWTs exhibit lower efficiency than HAWTs due to non-uniform aerodynamic loading, negative torque during rotation, wake interference, and lower achievable tip speed ratios, all of which reduce their power coefficient and overall energy extraction capability.



**Figure 1.3** The types of wind turbines (adapted from [12]).

### **1.2.3 Onshore and Offshore Wind Energy**

Onshore wind farms are built on land, and they have lower installation and maintenance costs. Offshore wind farms are built in the sea or ocean, where wind speeds are higher, thus ensuring a greater yield from the wind energy. However, offshore wind farms have higher capital costs and are technically challenging [13].

### **1.2.4 Environmental and Economic Aspects**

Wind energy has numerous environmental benefits, such as no fuel usage and no emission of carbon dioxide. Lifecycle analyses show that wind energy has one of the lowest carbon footprints of any energy source [9]. In addition, wind energy has a lower cost of electricity (LCOE) due to technological and economies of scale advancements [8].

Wind energy is a mature and economically viable source of renewable energy. Its continued deployment is critical to the realization of the sustainability development goals and climate change mitigation. Continued research is being carried out to enhance its efficiency.

## **1.3 Hydro Energy Systems**

Hydro energy, also known as hydropower, is one of the oldest and most widely used forms of renewable energy technology around the world. It harnesses the potential and kinetic energy of flowing or falling water to produce mechanical energy, which is then converted into electricity through turbines and generators. Currently, it is the largest contributor to the world's electricity production among all the renewable energy technologies [8].

The basic principle of the production of hydroelectric power is the conversion of the gravitational potential energy of the stored water into electricity. The potential energy of the stored water depends on the height of the reservoir of the dam. The released water has the potential to convert this energy into electricity by rotating the turbines connected to the generators. The generated power can be given by the formula:  $P = \rho g Q H \eta$ , where  $\rho$  is the density of the water,  $g$  is the acceleration due to gravity,  $Q$  is the rate of flowing water,  $H$  is the hydraulic head, and  $\eta$  is the efficiency of the system [14]. The current efficiencies of the hydroelectric systems are as high as 85%.

Hydropower plants are generally divided into three types: storage, run-of-river, and pumped storage hydropower plants. Storage hydropower plants are important for providing grid stability and for managing peak load demand, whereas run-of-river hydropower plants have relatively smaller environmental footprints, depending on the natural river run-off.

Pumped storage hydropower plays a vital role in the storage of energy, particularly in systems with high levels of intermittent renewable power sources, including wind and solar power (International Renewable Energy Agency [13]).

From the environmental point of view, hydropower has the least impact on the environment in terms of the emission of greenhouse gases during operation. Lifecycle analysis of the carbon intensity of different electricity generation systems reveals that hydropower has the least carbon intensity among different electricity generation systems [9]. Large-scale hydropower projects, however, have the potential to cause environmental and social impacts, including ecological and social displacement.

Hydro energy is the backbone of the world's renewable electricity systems, thanks to the high efficiencies, long lifespans, and flexibility of hydropower plants to provide grid flexibility. As the world moves toward the decarbonization of the energy sector, pumped storage hydropower will continue to play an important role in the integration of intermittent renewable energy sources into the energy sector.

#### **1.4 Biomass Energy**

Biomass energy is a renewable energy source that is produced from organic materials, which include wood, agricultural wastes, municipal wastes, and energy crops. It is based on the conversion of chemical energy contained in biological materials into heat, electricity, or biofuels through a series of processes that include combustion, gasification, pyrolysis,

and anaerobic digestion. It is a very important component of the global energy supply, especially in rural and developing areas, and is also used for renewable electricity and biofuel production [8].

The principle of biomass energy conversion is based on the process of photosynthesis, where plants convert carbon dioxide from the atmosphere into organic compounds. When this organic material is used for energy, the amount of carbon dioxide that is released is balanced by the amount that is absorbed by the plant, thus providing a carbon-neutral source of energy [9]. The overall process of conversion is based on the type of material used and the conversion process.

Biomass systems include solid biomass systems, biogas systems, and liquid biofuel systems. Solid biomass is used for combined heat and power generation. Anaerobic digestion is used for biogas production, which is a mixture of methane and carbon dioxide, used for electricity generation. Advanced biofuels, such as bioethanol and biodiesel, are also produced from biomass, which is used for decarbonizing transport fuels [13].

From an environmental point of view, biomass energy has a lower carbon footprint compared to fossil fuels, but there are also some issues regarding the sustainability of this source of energy, which include issues of deforestation and food vs. fuel [9].

### **1.5 Geothermal Energy Systems**

Geothermal energy is a renewable energy source that utilizes heat from the Earth's interior for electricity generation and direct utilization for heating purposes. It is different from intermittent renewable sources since geothermal power plants have the capability for dispatchable, high capacity factor electricity generation, given the continuous availability of heat from the Earth's interior, apart from plant availability [8]. Most geothermal systems make use of hydrothermal reservoirs that are permeable, or where permeability is low, enhanced geothermal systems (EGS) that make use of hot rock formations [15].

Three types of geothermal plant technologies are commonly used for electricity generation, namely, dry steam plants where steam is used for electricity generation, flash steam plants where hot water is used for generation, and binary cycle plants where heat is transferred to a secondary fluid that has a lower boiling point. The binary cycle plant is of critical importance for moderate temperature resources, where non-condensable gases are reduced through heat exchange systems [16]. The electrical power output is a function of

the mass flow rate, thermodynamic properties of the produced fluid, and temperature difference available for the conversion process, whereas sustainable production is a function of the balance between fluid extraction and reinjection, taking into account reservoir recharge and injection mechanisms [17].

Environmental and system considerations are of critical importance for geothermal resources, given that, based on lifecycle assessments, greenhouse gas emissions are considerably lower for geothermal compared to fossil fuel power generation, although some factors may affect the intensity of emissions for different resources and systems [9]. The environmental factors that need critical attention include induced seismic risks, water issues, and potential impacts of geothermal resources, including the presence of minerals in the brine fluid [15].

## **1.6 Nuclear Energy Systems**

Nuclear energy is an electricity production method based on a low-carbon technology. It generates power by means of controlled nuclear fission. In a nuclear power plant, heavy atomic nuclei of elements like Uranium-235 split to emit a lot of heat energy when struck by neutrons. This heat energy is used to produce steam, which drives turbines connected to generators. It is an important source of global low-carbon electricity today. [8]

### **1.6.1 Principles and Reactor Technology**

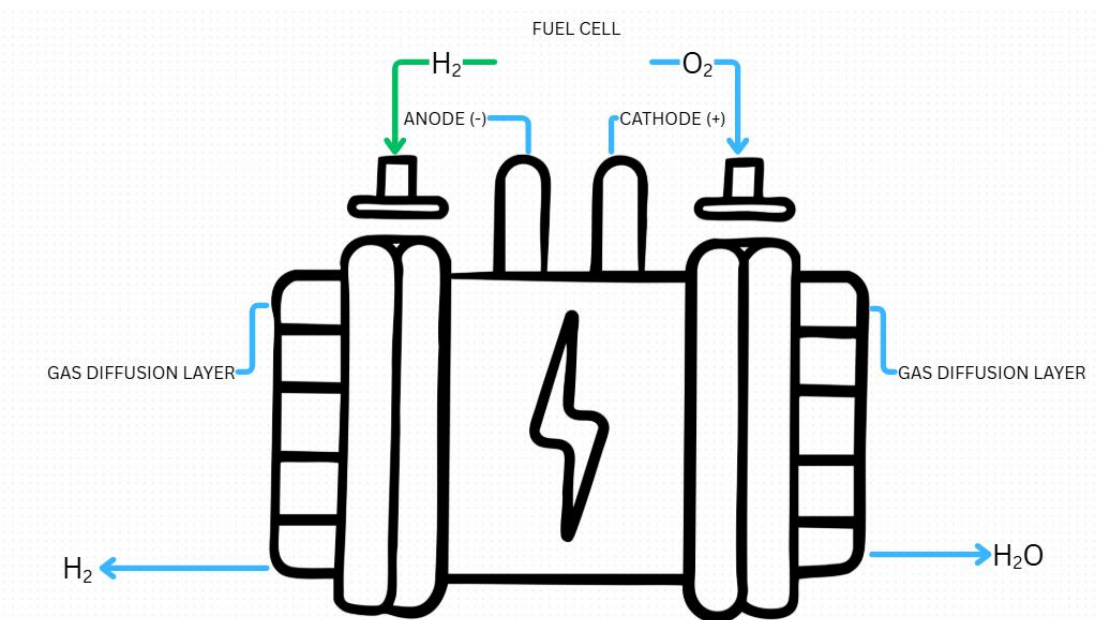
The basic idea of nuclear power production is based on the controlled chain reaction. Control rods control neutron flow, and moderator slows down neutrons to maintain fission. The most commonly used reactors are Pressurized Water Reactors (PWR) and Boiling Water Reactors (BWR) [18]. The thermal efficiency of conventional nuclear power plants varies between 30% and 37% due to thermodynamic cycle limits.

### **1.6.2 Environmental and Safety Considerations**

Lifecycle assessments show that nuclear power generates greenhouse gases comparable to wind power and much fewer than fossil fuels [9]. The drawbacks of nuclear power are waste management, cost, and safety concerns, especially after major accidents at Chernobyl and Fukushima. Advanced reactor designs, such as small modular reactors and Generation IV reactors, are being developed for improved safety, waste management, and cost-effectiveness [8].

## 1.7 Fuel Cell

The general structure of a fuel cell is illustrated in Figure 1.4. Fuel cells are electrochemical conversion systems. They convert chemical energy from a fuel into electricity. Fuel cells do not have any combustion in their operation. Unlike other power generation systems, fuel cells produce electric power due to an electrochemical reaction between hydrogen and oxygen, which are the reactants, and produce water and heat as by-products. Fuel cells are quite comparable to batteries. The main difference is that fuel cells do not require recharging, and they can produce electric power as long as fuel is supplied [19]. Fuel cells are regarded as a major component of future hydrogen-based energy systems and strategies for sustainable development due to their efficiency, environmental friendliness, and quiet operation [20].



**Figure 1.4** Schematic illustration of a fuel cell (modified from [21]).

At the anode, hydrogen gas is oxidized to release electrons and protons. The electrons flow externally to provide power, while the protons react with oxygen at the cathode to form water. This process of oxidation and reduction is continuous if there is a constant flow of fuel and oxidizer.

### 1.7.1 Types of Fuel Cells

Generally, fuel cells can be divided according to the electrolyte material and the fuel cell operating temperature. The main types of fuel cells are: Proton Exchange Membrane Fuel

Cells (PEMFC), Phosphoric Acid Fuel Cells (PAFC), Alkaline Fuel Cells (AFC), Molten Carbonate Fuel Cells (MCFC), and Solid Oxide Fuel Cells (SOFC). Low-temperature fuel cells include PEMFC and AFC. They operate between 60 and 250°C. They can be used for transportation purposes. High-temperature fuel cells include MCFC and SOFC. They operate between 600 and 1000°C. They can be effectively used for stationary power generation and cogeneration plants. This is because high-temperature fuel cells can provide high electrical efficiencies and high-grade waste heat [20]. Advances in fuel cell technologies include the use of direct fuel cells, which include Direct Methanol Fuel Cells (DMFC), and the use of urea-based fuel cells [22].

### **1.7.2 Fuel Cell Applications**

Fuel cells have a wide range of applications in transportation, stationary, and portable power supplies. In transportation, PEM fuel cells are used in FCEVs, which provide zero-emission solutions for electric vehicles. In stationary power supplies, fuel cells are used for decentralized power generation, combined heat and power generation, and backup power supplies. High-temperature fuel cells are also used for industrial cogeneration due to high overall efficiency. Moreover, fuel cells are used in portable electric devices, remote electric power supplies, and even in hybrid renewable energy systems with solar and wind power technologies [19].

Fuel cell technology offers numerous advantages in terms of environmental benefits and efficiency improvement over conventional fossil-fuel-based power generation technologies. When renewable energy-based hydrogen production technologies are used, fuel cells can be used for sustainable electric power generation with zero emissions, which can be a long-term solution for energy security with environmental benefits.

### **1.8 Biofuel Cells**

Biofuel cells (BFCs) are bio-electrochemical systems that convert chemical energy contained in biological substrates into electrical energy through catalytic biological reactions. Unlike conventional fuel cells that use precious metal catalysts and pure hydrogen gas, biofuel cells use enzymes or microorganisms as catalysts to convert organic substrates such as glucose, alcohol, lactate, or other biodegradable substances [23]. The invention of biofuel cell technology was a spin-off from the development of conventional

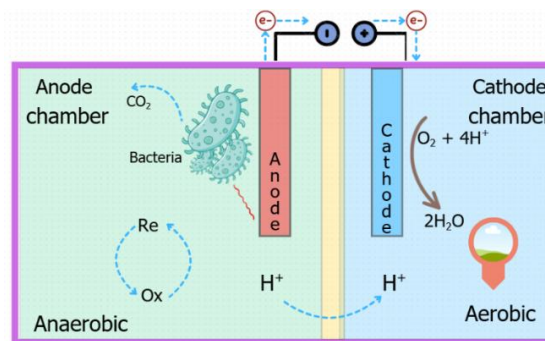
fuel cell technology that showed the direct conversion of chemical energy to electricity in the earliest forms of electrochemistry.

The advancement in bio-electrochemistry and enzyme immobilization helped scientists replace conventional catalysts with biological ones, thereby creating the concept of enzymatic and microbial fuel cells [24]. Biofuel cell operation occurs under mild conditions with a neutral pH and room temperature, with the ability for biocompatibility with the environment [25].

### 1.8.1 Types of Biofuel Cells

Biofuel cells may also be divided into various types based on the type of biological catalyst that is used for the conversion of biochemicals to electricity. The main types are as follows:

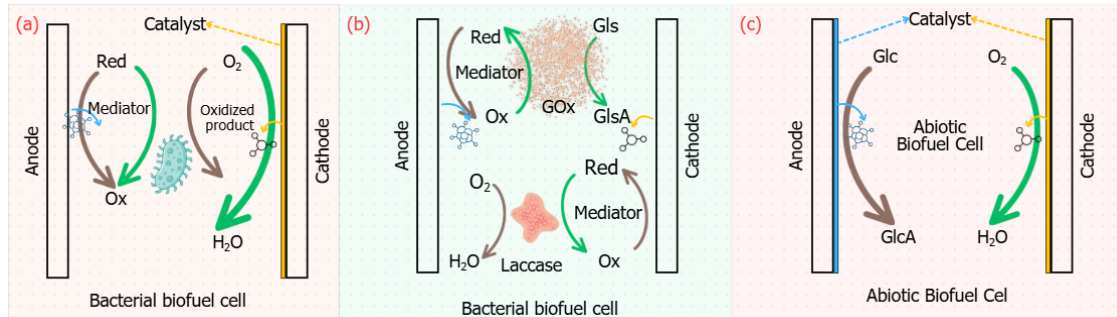
- Microbial Biofuel Cells (MFCs) use whole microorganisms as a biological catalyst for electricity generation. In MFCs, bacteria consume substrates that are organic in nature and release electrons during the respiratory processes. MFCs may employ substrates that are complex or simple in nature; also, the catalytic system may be self-regenerated, as depicted in Figure 1.5. Nevertheless, the transfer of electrons may limit the efficiency of MFCs using cell membranes and biofilms; therefore, a mediator may have to be employed for enhancing the rate of transfer [23].



**Figure 1.5** The schematic illustration of microbial fuel cell (adapted from [24]).

- Enzymatic biofuel cells (EBFCs) use isolated enzymes for oxidation and reduction reactions in the anodic and cathodic regions, respectively. Oxidoreductases, such as glucose oxidase, laccase, bilirubin oxidase, alcohol dehydrogenase, and heme enzymes, are used. However, they have limitations, such as instability, short lifespan, and difficulties in electron transfer between the active sites of the enzymes and the electrodes [26].

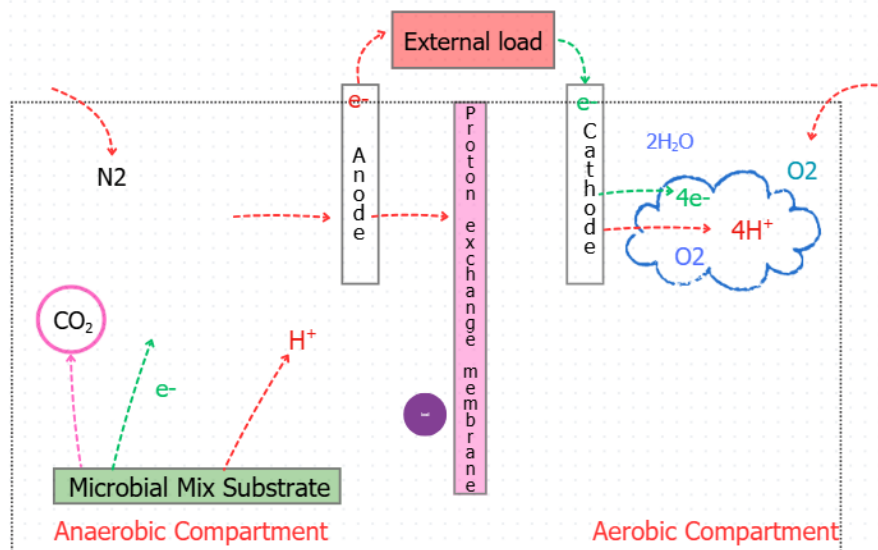
- Non-enzymatic biofuel cells use nanomaterials that have the capability for catalytic activity, similar to enzymes [23]. However, they have lower selectivity and lower efficiency compared to enzymatic systems, as depicted in Figure 1.6.



**Figure 1.6** Schematic illustrations of (a) microbial fuel cell, (b) An enzyme biofuel cell. (c) Non enzyme biofuel cell (adapted from [24]).

### 1.8.2 Working Principles of Biofuel Cells

A biofuel cell has two compartments, namely the anode and cathode compartments. In the anode compartment, biological catalysts are responsible for the oxidation of organic matter, resulting in the release of electrons and protons. In this case, enzymes act as biological catalysts. The electrons and protons released at the anode compartment are transferred through an external circuit to the cathode compartment, thus generating current. The released protons are transferred through the electrolyte or membrane to the cathode compartment. At this compartment, oxygen reduction occurs to produce water, as shown in Figure 1.7 [23].



**Figure 1.7** Microbial fuel cell working scheme (adapted from [23]).

Electron transfer is one of the factors affecting the efficiency of biofuel cells. Two electron transfer mechanisms have been identified as follows:

- Direct Electron Transfer (DET): In this case, electrons are transferred directly from the redox centers of the enzymes and the electrode surface.
- Mediated Electron Transfer (MET): In this case, redox mediators act as electron transfer agents between biological catalysts and the electrode surface [24].

DET is advantageous since it does not involve the use of mediators. However, this mechanism can only be supported by redox enzymes such as hemoproteins [26].

### **1.8.3 Applications of Biofuel Cells**

Biofuel cells have a wide variety of applications owing to their ability to harness energy from biodegradable organic matter by the use of biological catalysts. Biofuel cells can be applied in the following ways:

- Implantable medical devices: Biofuel cells can be miniaturized to implant medical sensors. They can harness energy from glucose and oxygen present in body fluids for powering implantable medical devices [25]. This application can be used for powering biosensors for various medical applications.
- Self-powered biofuel cells: Biofuel cells can be designed for self-powering applications. This means the biofuel cell can be designed to perform the functions of both a sensor and a power source. This is possible since the current produced by the biofuel cell is a function of the substrate concentration [26].
- Wastewater treatment: Biofuel cells can be designed for the treatment of wastewater. This application can be achieved by the use of microbial fuel cells for the treatment of wastewater while harnessing energy from the organic matter present in the wastewater [23].
- Low-power electronics: Biofuel cells can be applied in the powering of low-power devices. This application can be attributed to the use of renewable fuels and the mild conditions required for the operation of the biofuel cell [25].

### **1.8.4 Importance of Biofuel Cells**

Biofuel cells play an important role in the development of sustainable energy because they employ green fuels and minimize the emission of greenhouse gases into the environment. The biofuel cell is also biocompatible, thus applicable in biomedical fields [25]. The

biofuel cell also offers the advantage of combining energy production with environmental cleanup, including the treatment of wastewater [23]. The improvement of nanomaterials and bioelectronic interfaces is also likely to enhance the performance of biofuel cells [26].

### **1.9 Motivation**

The increased need for sustainable and decentralized energy systems has increased interest in bioelectrochemical technologies, such as microbial fuel cells (MFCs). Soil-based microbial fuel cells (SMFCs) provide a potential solution for developing low-cost, environmentally friendly electrical power generation technologies that use naturally occurring microorganisms to convert organic matter into electrical power. Unlike conventional fuel cells, which use expensive catalysts and pure fuels, SMFCs use naturally available substrates in the soil matrices, which is beneficial for remote sensing, environmental monitoring, and renewable energy generation.

However, the SMFC systems also have major challenges, such as low voltage output, high internal resistance, and instability in different environmental conditions. The voltage output is highly dependent on different operating parameters, such as temperature, pH, substrate concentration, ionic strength, and soil moisture content. These factors directly affect metabolism, extracellular electron transfer, internal resistance, and electrochemical reaction kinetics. However, the combined and comparative effect of different operating parameters on soil-based MFC systems is not sufficiently studied, particularly in a laboratory environment with a consistent mass and water volume.

The motivation for this research is based on the need to assess and understand how different environmental and operational parameters affect voltage generation in a soil-based MFC. Understanding the relationship between temperature (25-50°C), glucose concentration (0.5-3.0g), sodium chloride concentration (0.5-3.0g), pH (5, 7, and 9), and water volume (200 mL and 300 mL) is critical for understanding how to find the optimal conditions and how to understand the mechanisms of voltage stability and fluctuations.

This research was therefore motivated by the following key considerations:

- The need to improve sustainable bioelectricity production using simple soil-based systems.

- Lack of comprehensive experimental investigations on the effects of substrate, electrolyte, pH, and temperature on MFC performance under a single experimental framework. The need to find an optimal operational window for MFCs.
- The need to improve voltage stability for practical applications. By experimentally investigating these parameters, this research contributes to optimization of soil-based MFCs and contributes to the development of renewable energy using bioelectrochemical systems.

### **1.10 Objectives**

The main objective of this thesis was to experimentally examine voltage production in a soil-based microbial fuel cell under different environmental and operating conditions. To fulfill this purpose, specific objectives were set as follows:

- a. To design and develop a sustainable soil-based microbial fuel cell using natural soil and flexible carbon electrodes, operated under natural environmental conditions (ambient temperature, inherent soil pH, and native microbial communities without external inoculation).
- b. To examine short-term bioelectricity generation behavior ( $\leq 24$  hours) in soil-based microbial fuel cells, addressing the limited research on rapid performance dynamics in solid-phase MFC systems.
- c. To investigate the effect of glucose addition on microbial metabolic activity and extracellular electron transfer (EET) mechanisms, distinguishing between biocatalytic enhancement and electrochemical contributions.
- d. To evaluate the effect of NaCl addition on ionic conductivity and internal resistance, while also assessing its impact on microbial activity and osmotic stress in soil-based bioelectrochemical systems.
- e. To study the influence of pH and temperature variations on proton transfer, microbial activity, and voltage stability in soil-based microbial fuel cells.
- f. To analyze the effect of moisture content and degree of soil saturation (water content, RHw) on pore connectivity, ion transport pathways, and internal resistance within the system.
- g. To compare voltage enhancement mechanisms arising from organic substrate addition (glucose) and electrolyte addition (NaCl) in soil-based microbial fuel cells.

- h. To perform characterization and optimization of operating conditions for achieving maximum and stable voltage production in soil-based microbial fuel cells.

### **1.11 Novelties**

The novelty of this research is based on an integrated and systematically controlled approach for advancing the understanding of bioelectricity generation in soil-based MFC systems, unlike fragmented approaches often adopted in earlier research, as reflected in the literature.

The major novelty of this research is based on the introduction of a multi-parameter approach, whereby temperature, organic substrate, electrolyte concentration, pH, and moisture levels are evaluated. The evaluation of all these parameters is often done separately in earlier research, making it difficult for cross-comparison. The uniform approach adopted in this research facilitates reliable evaluation of parameter sensitivity.

The novelty of this research is also based on the introduction of a clear mechanistic differentiation of biologically driven and electrochemically driven performance improvement. The evaluation of both glucose and NaCl addition helps in differentiating microbial stimulation from improvement in ionic conductivity, which is often not clearly differentiated in earlier research, particularly in solid-phase MFC systems. This is a major novelty because it helps in understanding voltage generation pathways.

The second major novelty of this research is based on demonstrating system performance within optimum operating ranges, unlike linear improvement trends. The determination of threshold levels of substrate and electrolyte addition helps in understanding system performance, unlike earlier research, which often assumed improved performance based on increased substrate and electrolyte addition.

Furthermore, the pH-Temperature analysis reveals significant coupling effects that are critical to electrochemical stability and microbial activity. The evidence of unstable but patterned fluctuations in voltage behavior under neutral pH and high-temperature conditions offers fresh perspectives on biofilm dynamics, proton transport fluctuations, and transient internal resistances, which are relatively unexplored in soil-based systems.

Soil moisture has also been emphasized as another significant, though often ignored, design parameters in solid-phase bioelectrochemical systems. The significant enhancement in voltage observed at optimal moisture levels indicates that the effect of

substrate concentration may be overcome by the role of pore network connectivity and ion transport.

Finally, this thesis offers the soil-based MFC community a complete quantitative voltage characterization tool that incorporates initial, peak, minimum, and final voltage values, along with the analysis of the voltage stability trend. Quantitative voltage characterization of soil-MFC systems has rarely been reported in the literature.

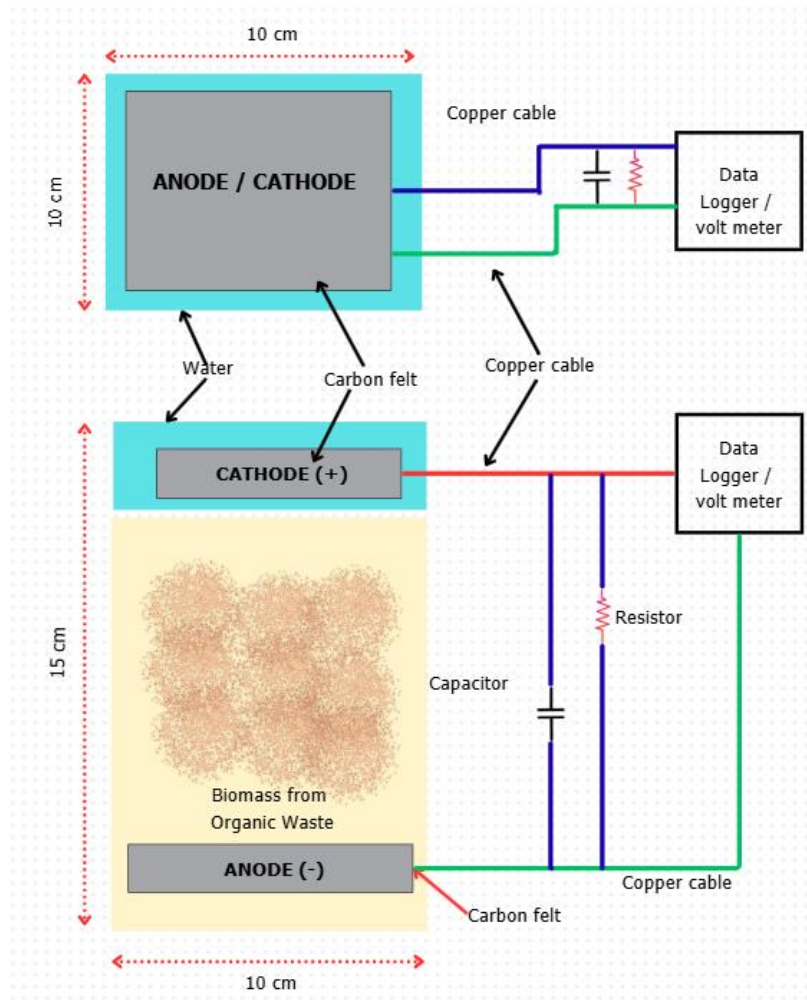
It is, therefore, the novelty of the comparative, mechanism-based, and quantitatively structured approach of this work that offers fresh perspectives on the complex interplay between biological and electrochemical mechanisms that control voltage production in natural soil environments.

## 2. LITERATURE REVIEW

This section expresses the fundamental mechanism of microbial fuel cells, electrochemical reactions, types and microbial communities. Moreover, it also shows its application area and finishes with its challenges and limitations.

### 2.1 Principles and Mechanisms of Microbial Fuel Cells

MFCs are bio-electrochemical systems in which the chemical energy contained in the substrates can be directly converted into electrical energy with the help of microbes as catalysts [27]. Although the idea of bio-electricity generation was proposed in the early research on microbes, significant progress in this direction was made in the last quarter of the 20th century with the advent of the combination of electrochemistry and microbiology, as depicted in Figure 2.1. MFC technology has attracted considerable interest due to its ability for simultaneous wastewater treatment and electricity generation.

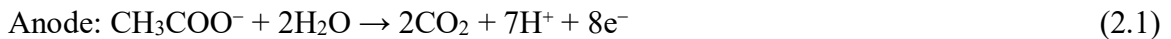


**Figure 2.1** Schematic diagram of the MFC showing its different parts (adapted from [28]).

### 2.1.1 Electrochemical Reactions

In a typical MFC system, the anaerobic oxidation of biodegradable organic matter by the microbes present at the anode releases electrons and hydrogen ions. The released electrons travel through an external circuit to the cathode, producing an electric current. At the same time, the hydrogen ions move through a membrane from the anode to the cathode to complete the circuit [29].

In the anodic chamber of a typical MFC system, electroactive microbes oxidize organic matter such as acetate[30]. According to the following equation.



In the cathode chamber, oxygen is commonly used as the final electron acceptor. Oxygen is reduced [29]. According to the following equation.



The overall reaction produces electrical energy from chemical energy. The performance of the system depends on the electrode and membrane properties, as well as the microbes present in the system [28].

### 2.1.2 Electron Transfer Mechanisms

Electron transfer from microorganisms to the anode is an important process that determines the overall efficiency of MFCs. So far, two main mechanisms have been reported in the literature for this process:

- Direct Electron Transfer (DET): Electrons are transferred from the outer membrane cytochromes of the microorganisms or conductive pili (nanowires) to the anode surface.
- Mediated Electron Transfer (MET): Soluble redox compounds, also known as redox mediators, are used for transferring electrons from microorganisms to the anode surface.

According to Schroder [31], the energy efficiency of electron transfer is closely related to the redox potentials of the intracellular electron carriers and the electrodes. Direct mechanisms are considered sustainable since they do not involve any artificial compounds.

### 2.1.3 Types of Microbial Fuel Cells

MFC configurations differ based on design and application requirements [29]. The common configurations for MFCs are:

- Double-Chamber MFC: This MFC has a separate anodic and cathodic compartment with a proton exchange membrane (PEM) separating the two.
- Single-Chamber MFC: This type of MFC usually comprises air-cathode configurations without a separate compartment for the cathode.
- Air-Cathode MFC with Membrane-less: This design enhances oxygen availability and reduces the cost of construction.
- Hybrid Bioelectrochemical Systems: MFCs for hydrogen production using MECs and freshwater generation using MDCs [27].

#### **2.1.4 Applications and Advancements**

MFCs have been explored for wastewater treatment, biosensing, heavy metals removal, biohydrogen production, and carbon sequestration, among other applications [30]. The recent developments in MFCs are nanostructured electrodes, graphene-based anodes, and reactor configurations for scaling up MFCs.

#### **2.2 Microbial Communities in MFCS**

However, the MFCs fundamentally depend on the complex microbial population that facilitates the conversion of the organic matter into electrical energy. It has also been emphasized in several studies that understanding the microbial population is critical for the optimization of MFCs [32].

Electrochemically active bacteria (EAB), also termed "exaelectrons," have the capability to transfer electrons, which are produced from the oxidation of the substrates, onto the anode surface either directly or indirectly. However, in a real MFC, electrogenic bacteria co-exist with fermentative bacteria, which facilitates the degradation of complex organic matter through a complex community [33].

##### **2.2.1 Electrogenic Bacteria in MFCs**

Of the electrogenic microorganisms that have been isolated in MFC systems, the most common ones are members of the general *Geobacter*, *Shewanella*, and *Clostridium* groups [34].

*Geobacter* species, especially *Geobacter sulfurreducens*, have been widely studied and are known to be the dominant anode-respiring bacteria in acetate-fed MFCs. These organisms have conductive pili that enable them to transfer electrons to the electrode

surface [33]. Researchers have shown that MFCs with *Geobacter*-dominated biofilms have a high coulombic efficiency.

*Shewanella* species have also been widely studied and are known to be electrogenic microorganisms that have the ability to transfer electrons both through direct contact and soluble redox mediators. *Shewanella oneidensis* has been known to produce flavins that serve as endogenous electron shuttles[34].

*Clostridium* species have also been known to exist in MFC systems. *Clostridium* is a Gram-positive fermentative bacterium that has traditionally been known to exist solely as a fermenter; however, some *Clostridium* species have also been known to exist in MFCs (figure 2.3), where they either transfer electrons to the electrode surface or produce metabolites that are further used for electricity generation by strict exoelectrogens such as *Geobacter* [35].

### **2.2.2 Biofilm Formation and Electrode Colonization**

Biofilm formation on the surface of the anode is considered to be one of the most important factors in the maintenance of electricity production in MFCs. Current production at a stable rate is achieved by the effective colonization of microbes, biofilm formation, and the development of conductive structures in the biofilm [32].

Biofilm formation on the electrodes allows for the intimate contact of microbial cells with the electrode, resulting in reduced electron transfer resistance. It has been observed that the formation of thick biofilms with high conductivity results in high current density.

Biofilm structure plays a significant role in the transfer of substrates, protons, and electrons. Operational conditions and materials of the electrodes are considered to be significant factors for biofilm formation.

### **2.2.3 Mixed Versus Pure Cultures**

MFC systems can use both pure cultures and mixed cultures of microbes. Although pure cultures are helpful in understanding the mechanism of electron transfer, they are not as feasible for wastewater treatment as mixed cultures [33].

The use of mixed cultures of microbes is more feasible for wastewater treatment because it can degrade complex organic matter such as domestic sewage and industrial wastewater.

In wastewater-fed MFCs, microbial community composition was studied, showing dominance of Deltaproteobacteria, Bacteroidetes, and Firmicutes under electricity-producing conditions [31].

#### **2.2.4 Substrate–Microbe Interactions and Power Output**

The substrate supplied to the MFC has a great impact on the microbial community and the performance of the MFC. Simple substrates such as acetate can result in the enrichment of Geobacter-dominated communities [33]. Substrates such as glucose, which can be fermented by microbes, can result in the enrichment of fermentative bacteria such as Clostridium and Bacillus. They ferment the glucose and produce intermediate products, which are further utilized by electrogens.

Experiments where the substrate is switched have shown that the microbial community can change and adapt to the new substrate. This shows the influence of the substrate on the microbes and the performance of the MFC.

In a complex substrate such as industrial wastewater containing phenolic pollutants, the microbes show synergistic effects. The microbes degrade the pollutants, while the electrogenic microbes maintain the flow of electrons to the anode [35].

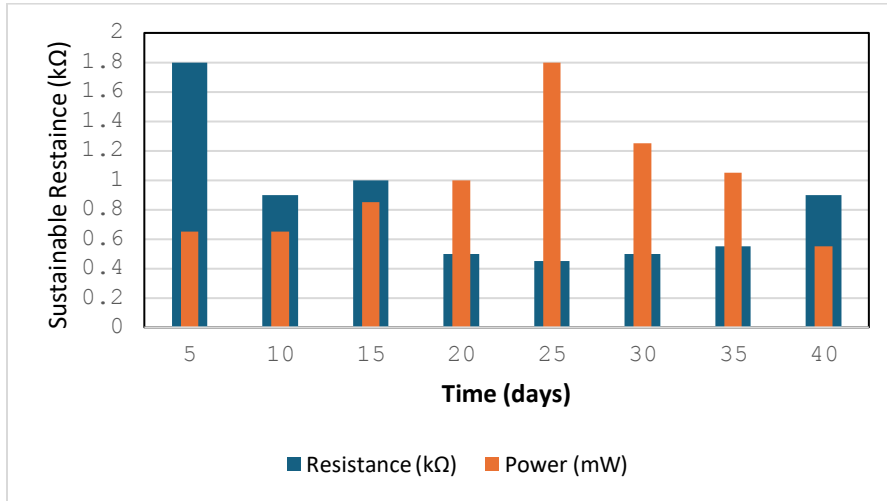
From the literature review above, it is evident that the performance of the MFC is greatly influenced by the microbial community composition, the biofilm formed by the microbes, the substrate supplied to the MFC, and the electrogens present in the system. The electrogens present are Geobacter and Shewanella.

### **2.3 Substrates and Wastewater Applications**

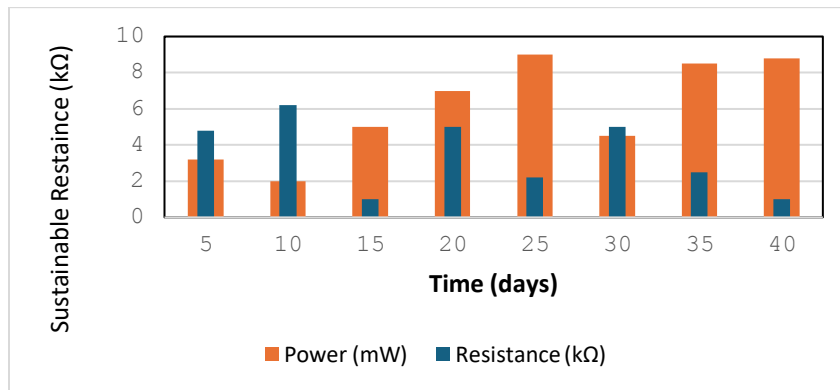
Substrate selection is one of the most important factors affecting MFC performance. The substrates provide a source of carbon and energy for the electrochemically active microorganisms and impact current density, power density, coulombic efficiency, and COD removal efficiency [36]. Glucose and other simple sugars are frequently used as MFC substrates, as depicted in Figure 2.2. However, they can lead to fermentative metabolism and the formation of intermediate products.

Acetate is considered a preferred substrate because it can be readily utilized by electrogenic bacteria, and it does not favor the development of other metabolic pathways significantly under controlled conditions [38]. Complex substrates, which include lignocellulosic biomass, agricultural residues, molasses, and food waste, have also been

used as renewable substrates in MFCs [36]. These substrates, however, might affect the power density and electron transfer efficiency due to slow hydrolysis rates.



(a)



(b)

**Figure 2.2** Sustainable power and resistance variation during two MFCS operations with the function of operating time. (a) Air Cathode, (b) Full Cell (modified from [37]).

### 2.3.1 Wastewater as Substrate

Wastewater has the advantage of allowing the simultaneous generation of electricity and treatment of waste in MFC systems. Domestic, industrial, agricultural, pharmaceutical, dairy, and chemical wastewater have been investigated as possible substrates [39]. Domestic wastewater has been observed to have the potential to support mixed microbial consortia that are capable of electricity generation as well as COD removal.

High-strength wastewater has the potential to increase the rate of electricity generation, but at the same time, substrate inhibition may also occur if the wastewater is not optimized. Synthetic wastewater has been widely used to mimic real wastewater under controlled conditions in the laboratory [38].

### **2.3.2 Substrate Influence on Performance Parameters**

Substrate type also influences the power density (PD), current density (CD), coulombic efficiency (CE), and removal efficiency for COD. Acetic acid-based systems tend to have high CE due to efficient electron transfer mechanisms; however, complex substrates may consume electrons for biomass growth.

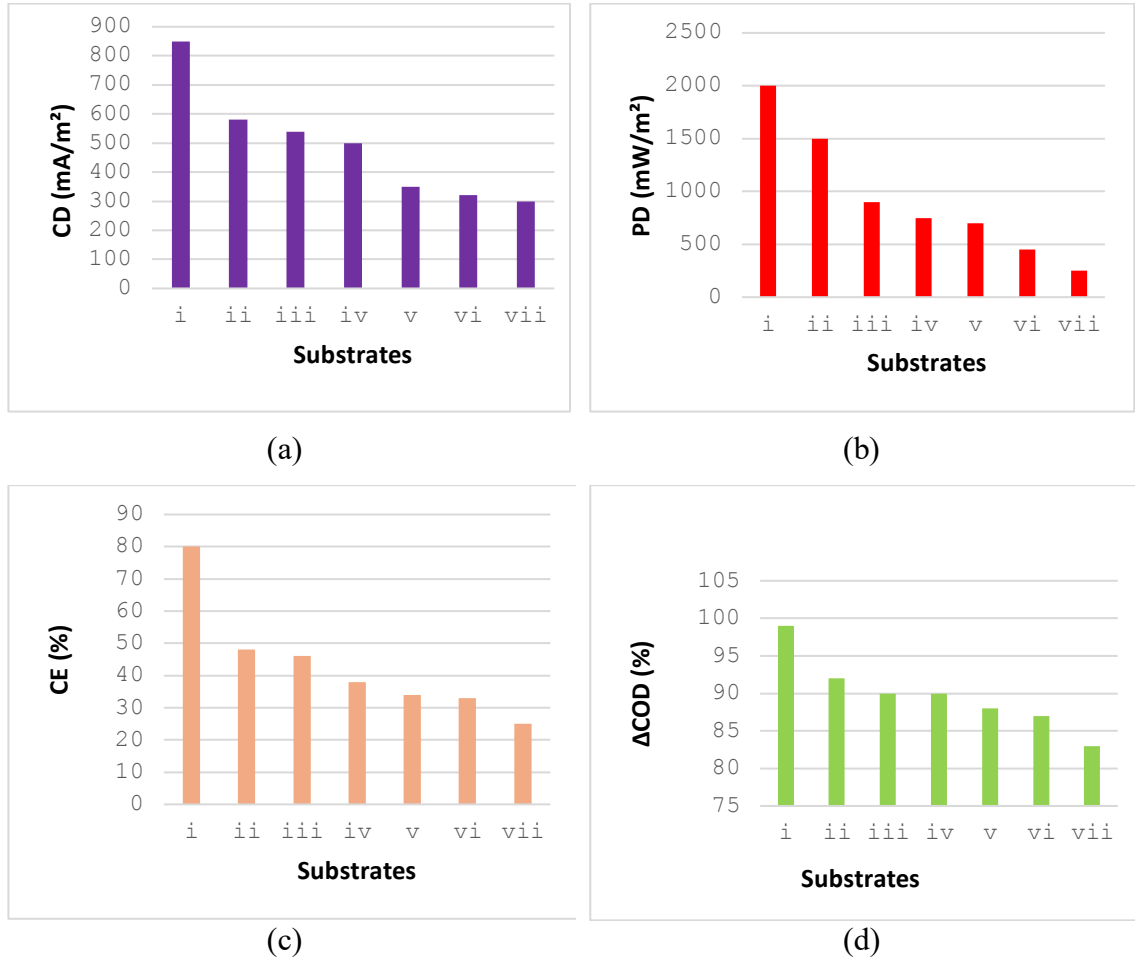
### **2.3.3 Integration with Other Systems**

MFCs have also been coupled with microbial desalination cells, constructed wetlands, and hydroponic systems for enhanced wastewater treatment and water reuse. MFCs coupled with hydroponic systems can be used for nutrient removal and water reuse for plant growth. Microbial electrolysis cells, which are a derivative of MFCs, can be used for hydrogen production with the application of an external voltage [40].

As depicted in Figure 2.3, substrate selection plays a critical role in determining electrochemical performance. Simple, readily biodegradable substrates (e.g., acetate or glucose) exhibit superior performance across all evaluated metrics, including current density (CD), power density (PD), and coulombic efficiency (CE), as shown in Figures 2.3(a–c). These substrates are more easily metabolized by electroactive microorganisms, promoting efficient electron transfer and higher energy recovery. In contrast, complex substrates or wastewater streams generally yield lower CD, PD, and CE values due to the presence of refractory compounds and competing microbial pathways. However, as illustrated in Figure 2.3(d), complex substrates tend to achieve higher  $\Delta$ COD removal efficiencies, highlighting their advantage in terms of organic matter degradation and environmental sustainability.

The figure demonstrates a clear trade-off between maximizing electrochemical performance and achieving effective wastewater treatment. While simple substrates are preferable for maximizing power generation, complex wastewater streams are more suitable for sustainable applications focused on pollutant removal and resource recovery.

This balance is essential when designing MFC systems for practical, real-world implementations.



**Figure 2.3** Seven best results organized by metric from best (I) worst (VII): (a) best current densities (CD), (b) PD, (c) CE, and (d)  $\Delta$ COD values (modified from [38])

## 2.4 Electrode Materials and Modifications

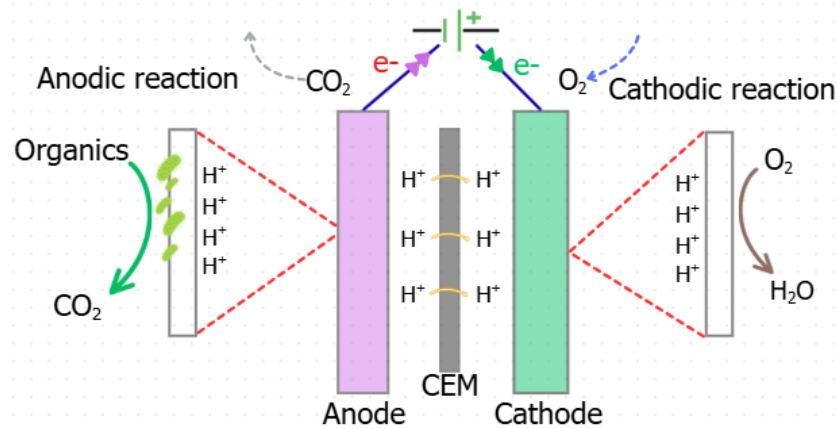
The materials used for electrodes in MFCs have a decisive role to play in terms of their performance, cost, and scale of operation. The choice of anode and cathode materials has a direct impact on power density, coulombic efficiency, hydrogen evolution, and wastewater treatment efficiency.

### 2.4.1 Anode Materials

The site for microbial oxidation occurs at the anode where a biofilm forms. The requirements for good anode material are that it should have high electrical conductivity,

low internal resistance, high biocompatibility, chemical stability, and a high surface area for microbial attachment [41].

Carbon-based materials such as a graphite rod, carbon cloth, carbon paper, carbon felt, reticulated vitreous carbon (RVC), and a graphite brush are common electrode materials that have been employed for MFCs, as depicted in Figure 2.4, due to their high resistance to corrosion and good surface properties [42].



**Figure 2.4** Schematic and working principle of the typical microbial fuel cell (adapted from [42])

Advanced electrode materials such as carbon nanotube (CNT) electrodes and graphene-modified electrodes have also been found to have high efficiency in the rate of extracellular electron transfer [43]. Modification of the surface using ammonia treatment, acid treatment, and chemical functionalization has also been found to have high efficiency in enhancing microbial attachment [44].

### 2.4.2 Cathode Materials

The cathode controls the oxygen reduction reaction (ORR) or hydrogen evolution reaction (HER), as shown in Figure 2.4. Platinum-coated carbon is known for having a high catalytic activity but is expensive. Other alternatives, such as stainless steel, aluminum, activated carbon, manganese oxides, iron-based catalysts, etc., are also explored for cost-effectiveness [44].

### 2.4.3 Impact on Performance

It has been observed that the selection of the electrode material has a direct impact on the power density, coulombic efficiency, hydrogen evolution rate, and wastewater treatment efficiency [45]. Graphene-modified electrodes are known to increase the conductivity of

the electrodes, thereby increasing the voltage generated, as opposed to the use of carbon electrodes [43]. In the case of wastewater treatment, the conductivity of the anode increases COD removal, while the catalyst of the cathode reduces the overpotential losses, thereby increasing the overall stability of the cell.

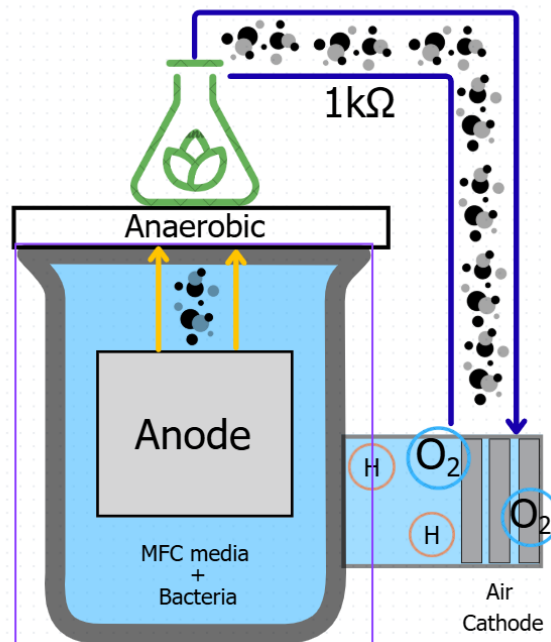
## 2.5 Reactor Designs and Membrane Considerations

Among the key engineering factors affecting the performance and feasibility of MFC is the reactor configuration and membrane selection. As emphasized by the comprehensive review by Flimban et al [46], the advancement in the development of MFC has been from the basic dual-chamber configuration to more advanced configurations for the improvement of performance and feasibility. However, scaling up the system poses other challenges pertaining to the system's hydrodynamics and membrane cost [47].

### 2.5.1 Chamber Configurations

Chamber configurations in MFCMFC systems, also known as microbial reactors, refer to the arrangement of the anodic and cathodic compartments that facilitate maximum power generation and wastewater treatment. The main types include the following:

This is shown in Figure 2.5. Self-stratifying membrane-less MFCs (s-MFCs) have shown enhanced power density with shorter hydraulic retention times (HRT), achieving:  $69.7 \pm 0.4 \text{ W m}^{-3}$  at 3 h HRT, as shown in the research by Walter et al. [48].



**Figure 2.5** Single-chamber microbial fuel cell (adapted from [46]).

Single-chamber systems provide the following advantages:

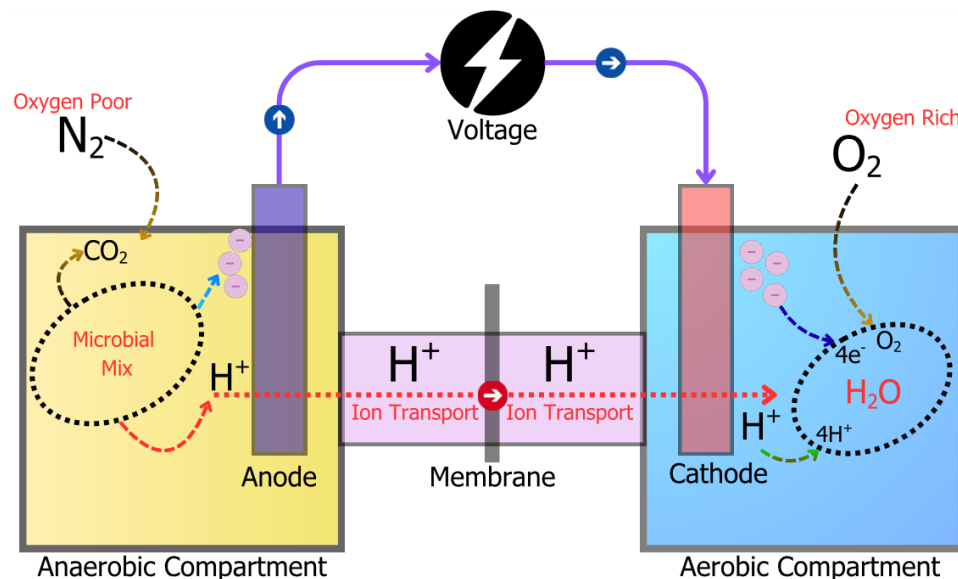
- Reduced internal resistance
- Lower complexity in construction
- Improved scalability

However, membrane-less systems also experience oxygen permeation through the electrodes, which reduces coulombic efficiency [49].

The double-chamber MFCs are considered the classical MFCs. As discussed in detail in [46], the MFCs are made up of physically separated anodic and cathodic compartments, which are connected with a proton exchange membrane (PEM) as depicted in Figure 2.6. The anaerobic conditions in the anode compartment are maintained for the oxidation of substrates, while oxygen reduction occurs in the cathode compartment.

This is due to the strict separation of oxidation and reduction, electrochemical conditions, and higher coulombic efficiencies.

However, as discussed in [48], the membrane also increases the internal resistance ( $R_{int}$ ) and is a major contributor to the overall cost, which is up to 60% of the total reactor cost.



**Figure 2.6** Major elements of MFC reactor of two-chamber MFC (modified from [46])

- Three-chamber and advanced reactor configurations, such as microbial desalination cells (MDCs) and hybrid bioelectrochemical systems, include additional reactor chambers for ion separation and recovery of resources. As discussed in [49], an anion exchange membrane (AEM) and a cation exchange membrane (CEM) are used for enhanced ion transport in the advanced reactor systems.

Three-chamber systems provide the following capabilities: Simultaneous desalination, nutrient recovery, and ionic control. However, increased membrane surface area also increases costs and ohmic losses. This section will critically discuss the different reactor chamber configurations, membranes, and reactor scale issues based on the selected sources.

### **2.5.2 Membrane Use in MFCs**

Proton exchange membranes (PEMs), particularly Nafion, have been commonly used as the standard membrane in the MFC systems [49]. The membrane has the advantages of high proton conductivity, excellent chemical stability, and low gas permeability. The most significant drawbacks of this membrane are its high cost, membrane fouling, and increased internal resistance with membrane thickness.

Demonstrated that the membrane thickness plays a critical role in proton conductivity and internal resistance. The thicker the membrane, the greater the internal resistance and the lower the oxygen crossover. Cation exchange membrane (CEM) allows the transfer of protons, whereas the anion exchange membrane (AEM) allows the transfer of negatively charged species. AEM has attracted significant attention, as explained in the following points by [47]: High selectivity, reduced oxygen transfer, and reduced internal resistance.

Improved durability and reduced biofouling of the membrane are possible with the use of tailored polymeric membranes, as explained in [50].

Ceramic membranes are high-performance membrane systems, typically used as industrial membrane systems, in high-temperature applications, and in harsh environments. The membrane materials are ceramic, including SiC, Al<sub>2</sub>O<sub>3</sub>, and ZrO<sub>2</sub>. Walter et al. [48] investigated ceramic MFC (c-MFC) systems, where ceramic membrane separators were used instead of Nafion membrane systems. The ceramic membrane showed the following

characteristics: Stable performance at different HRT values, power density of  $32.2 \pm 3.9 \text{ W m}^{-3}$ , and high energy conversion efficiency of  $2.092 \pm 0.119 \text{ kWh kgCOD}^{-1}$  at 24 h HRT. Ceramic membrane has the advantage of reduced cost, mechanical durability, and the disadvantage of reduced ionic conductivity.

In the membrane-less systems, the capital cost and ohmic resistance are reduced; however, the absence of the membrane has the disadvantage of reduced coulombic efficiency, increased substrate transfer, and oxygen intrusion, as explained by Nava et al. [47]. The membrane-less systems are applicable to low-cost systems, whereas the membrane systems are applicable to high-efficiency energy recovery systems.

### **2.5.3 Reactor Scaling**

The scaling of microbial fuel cell reactors is defined as scaling up or increasing the size of the microbial fuel cell systems. The major techniques of scaling up are as follows:

**Lab Scale Systems:** The size of the microbial fuel cell system at the laboratory scale ranges from milliliter to liter. According to Janicek et al. [49], at this scale, the system is optimized for short electrode distances, substrate feeding, and a high surface area-to-volume ratio. Flat plate and tube configurations are common at this scale.

**Pilot Scale MFCs:** The scale-up of microbial fuel cell systems, as discussed in several studies reviewed in [49], is characterized by significant challenges, including increased internal resistance, substrate distribution, hydraulic efficiency, and membrane cost scaling.

Field trials of urine-fed pilot MFCs, as discussed in [48], revealed that ceramic MFCs perform better at longer HRT, self-stratifying systems perform better at shorter HRT, and the overall energy recovery is highly dependent on hydraulic efficiency.

**Scale-up Challenges:** The major challenges of scaling up, as discussed in all five studies, are:

1. Membrane cost and durability [50]
2. Oxygen transfer and biofouling [47]
3. Increase in internal resistance with increased distance between electrodes [49]
4. Hydraulic mixing limitations [48]
5. Voltage loss in modular stacks [49]

According to Janicek et al. [49], improvements in MFC reactor design are difficult to scale up because of changes in mass transfer and current distribution.

From the literature, it can be concluded that MFC reactor design and membrane type are two important, interdependent parameters for MFC performance and scaling up. Double-chamber MFCs are easy to control in terms of electrochemical performance, which, in turn, increases cost and resistance. Single-chamber MFCs are easy to operate, scalable, and efficient, though they are less efficient.

New membrane materials, i.e., polymer, composite, and ceramic membranes, are also considered efficient in reducing cost and improving ionic conductivity in MFCs. However, scaling up MFCs with these membranes is still a challenge due to increased internal resistance, membrane durability, and hydrodynamic limitations. Future research should be conducted on cost-effective membranes with high ionic conductivity, optimization of modular stacking, minimizing distance between electrodes, and wastewater treatment along with power generation.

## **2.6 Performance Metrics**

Microbial fuel cells are bio electrochemical systems that convert chemical energy contained in organic substrates into electrical energy, with wastewater treatment being a simultaneous process. It is necessary that the assessment of the MFC process is based on an integrated evaluation of electrical and chemical properties, as wastewater treatment is a simultaneous process. Recent studies and meta-analytical results [51] have shown that voltage output, current density, power density, coulombic efficiency (CE), chemical oxygen demand (COD) removal, and energy conversion efficiency (ECE) are the key factors that need to be considered for the assessment of MFC performance,

### **2.6.1 Electrical Performance**

The common characteristics of electrical performance are voltage (V), current (I), and power density (P). Voltage is a measure of the electrochemical potential difference between the anode and cathode, whereas current is a measure of the rate of electron transfer. The power density, calculated as  $P=V \times I$  normalized per unit area or volume, is still the most reported parameter for comparison. A comparative study of different reactor configurations showed considerable differences in power output. The performance of dual-chamber MFCs treating complex food waste, as depicted in Figure 2.9, was substantially better in terms of coulombic efficiency and showed 73% greater stability in comparison to single-chamber systems [52]. In another comparative pilot-scale study, ceramic membrane MFCs showed

a power density of  $32.2 \pm 3.9 \text{ W/m}^3$ , whereas membrane-less self-stratifying systems showed a power density of  $69.7 \pm 0.4 \text{ W/m}^3$  under optimized hydraulic retention times [53]. The CE is a measure of the proportion of electrons transferred as electric current compared to the maximum theoretical electrons available from substrate oxidation. A higher CE indicates more efficient transfer of electrons to the anode, with minimal loss of electrons for biomass growth, methane production, or soluble compounds. In a comparative study, Lee et al. [51] established electron balances for MFCs. The results showed that MFCs using acetate as a substrate showed an electron recovery of about 71%, whereas MFCs using glucose showed only 49% electron recovery because of increased biomass growth and methane production. The overall efficiency of energy conversion, calculated as  $\text{ECE} = \text{CE} * \text{PE}$ , was 42% for acetate-fed MFCs, whereas it was only 3% for glucose-fed MFCs.

### **2.6.2 Chemical Performance**

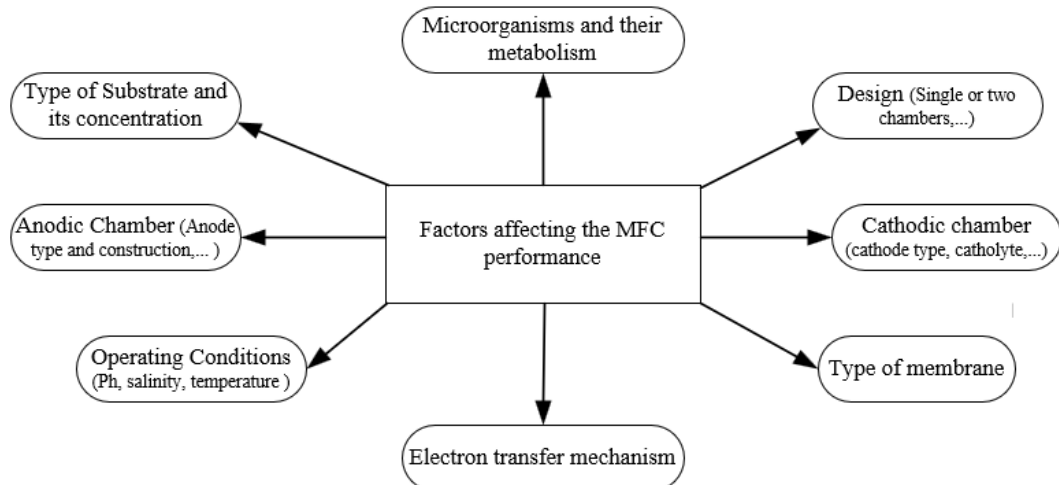
The performance of MFCs is mainly measured by assessing the COD removal efficiency. The efficiency of COD removal is a quantitative measure of the extent of degradation of organic compounds. The efficiency of COD removal by MFCs is a result of the metabolic activities of microorganisms and the efficiency of electron transfer. In a study by Zafar et al. [52], it was noted that dual-chamber MFC configurations were able to attain 18% higher efficiency in COD removal compared to single-chamber configurations. Walter et al. [53] noted that ceramic MFC configurations were able to attain a maximum normalized energy recovery per COD (NERCOD) of up to  $2.092 \text{ kWh kg COD}^{-1}$  at extended hydraulic retention times.

### **2.6.3 Factors Influencing Performance**

The operational factors have a major impact on electrical as well as chemical performance. The substrate type and concentration impact the microbial composition, electron transport pathways, and biofilm thickness. Fermentable substrates such as glucose induce competing microbial reactions, resulting in a loss of electrons and thus a decrease in CE and power density [54]. On the other hand, non-fermentable substrates such as acetate favor anode-respiring microorganisms and thus enhance electron transfer efficiency. pH affects enzymes and proton transport, while temperature affects microorganisms and internal resistance, as shown in Figure 2.7. Woodward et al. [51] showed that changes in substrate

concentration and temperature have a major impact on maximum power output and internal resistance. Therefore, it is important to optimize the load dynamically.

Conductivity is also a critical issue in MFC performance. The higher the ionic strength of the solution, the lower the internal resistance and higher the voltage stability. The choice of electrode materials affects performance by impacting microorganisms and electron transfer kinetics. Analysis of MFC performance has shown that brush anodes produce up to 130% higher power densities compared to flat-surface electrodes. This is due to the larger surface area of the brush anode, allowing biofilm formation. The addition of a platinum-coated cathode enhances power density by improving oxygen kinetics. However, it has little impact on coulombic efficiency compared to membrane selection [52].



**Figure 2.7** Factors affecting the performance of the MFC (adapted from [36])

#### 2.6.4 Comparative Studies

Furthermore, comparative reactor studies have indicated the trade-offs that exist between power density, treatment efficiency, and reactor stability. The membrane-assisted systems have indicated improved CE and COD removal efficiencies because of reduced oxygen transfer and improved proton transfer mechanisms, respectively [53]. The membrane-assisted systems, however, have the disadvantage of increased capital costs and internal resistances, while the membrane-less systems have the advantage of reduced capital costs and internal resistances, although they may have the disadvantage of reduced long-term reactor stability. The scale-up reactor studies have indicated that the optimal external

resistance is critical to maximizing the power density, and the real-time optimization techniques have the potential to eliminate power losses of up to 50% of the maximum possible power by adjusting the external load to match the internal resistance of the MFC [51].

Biosensing applications have also indicated the potential of the performance parameters of the MFC, as Feng et al. [55] have indicated that the area under the peaks of the current responses is directly related to the influent COD concentration, indicating that the MFC has the potential to be used as a tool for water quality analysis, as indicated by the improved prediction of influent COD concentrations by incorporating the use of artificial neural networks.

Therefore, the literature has indicated that the performance of the MFC is governed by the complex relationship between the electrical and chemical parameters, as indicated by the importance of high power density, which requires the optimization of the internal resistances, electron transfer mechanisms, and substrate properties, as well as high COD treatment efficiencies, which require the optimization of the microbial metabolism mechanisms.

## **2.7 Challenges and Limitations**

However, microbial fuel cells have been recognized as a promising green energy source for the future. This is because the fuel cell can harness chemical energy from organic matter to generate electricity while treating wastewater. Despite the fact that the field is progressing rapidly from a scientific point of view, various challenges and limitations still exist and are hindering the commercialization and application of the MFC system. Various reviews and comprehensive analyses have shown that the main hindrances to the application of the MFC system include low power densities, high internal resistance, membrane fouling, cost of the materials used, and the unstable nature of the microbes.

Low electric output has been cited as the main hindrance to the application of the MFC system. Although various experiments have shown an improvement in the electric output under optimal conditions, the power densities are still low for the application of the system for energy production purposes. In their work, [61] emphasized the need to minimize internal resistance and maximize the electrode surface area for the achievement of high-power densities. This would, however, complicate the system. In addition, [57]

observed that the application of the system is limited by the low electric output compared to other green energy sources.

Internal resistance is a factor that affects the efficiency of the system. In the fuel cell system, ohmic losses occur in the electrodes, electrolyte, and separators. In addition, activation losses are attributed to the electrodes. This is mainly attributed to the slow electrochemical reactions at the electrode surfaces. Moreover, the system may also experience concentration polarization. In their work, [58] emphasized the fact that high internal resistance may cause fluctuations in the electric current and a reduction in the output voltage. The electrode space may be minimized to improve the system's performance. This may, however, cause oxygen leakage into the anode chamber.

Moreover, the membrane-related issues face other technical challenges. Although proton exchange membranes (PEMs), e.g., Nafion, are commonly used for fuel cell applications owing to their high proton conductivity, their high cost and fouling are the main drawbacks. In this regard, other separators such as cation exchange membranes, anion exchange membranes, and ceramic membranes have been proposed to minimize the cost factor. However, according to [59], the trade-off between the membrane's properties still needs to be resolved for membrane-based fuel cell applications. In the case of membrane-less fuel cell configurations, the cost factor is minimized; however, the oxygen crossover and coulombic efficiencies are compromised.

On the other hand, the cost factor related to the membrane/electrode material is another challenge for fuel cell applications. Although the catalyst material for the cathode side, platinum, enhances the oxygen reduction reaction, the cost factor is impractical for large-scale applications. In this regard, the use of carbon-based catalysts is cost-effective; however, the efficiency and stability of the catalyst may be compromised [60]. In this regard, it has been reported that the high cost of electrode and membrane fabrication is a major drawback for fuel cell applications, especially for developing countries where cost-effectiveness is a key factor.

Moreover, microbial and biological factors are also responsible for the performance limitations of fuel cell applications. In this regard, the electroactive bacteria are responsible for the extracellular electron transfer. Although the growth rate is relatively slower compared to other biological treatment processes, the competition between exoelectrogens

and methanogens may cause a loss in coulombic efficiency by the production of methane gas instead of electrical energy. In this regard, [57] discussed the effects of various environmental factors on the stability and activity of the microbes. Moreover, the fluctuations in the pH levels, temperature, and substrate concentration may cause unstable output from the fuel cell.

On the other hand, the unstable performance may be attributed to the long-term effects of the fuel cell application. In this regard, the fouling and overgrowth on the electrodes may cause an increase in the resistance and a decrease in the voltage stability. Moreover, the optimal hydraulic retention time and organic loading rate need to be continuously monitored for the optimal performance of the fuel cell application. In this regard, [56] discussed the need for the design of the fuel cell architecture for addressing the various technical challenges.

The scaling up of MFCs is also a problem since it poses both engineering and economic challenges. The use of optimized electrode separation for MFCs at a laboratory scale is also a problem, but at a pilot scale, substrate distribution, voltage loss, and stacked configurations are also major problems, as described in [59]. The achievement of energy recovery comparable to anaerobic digestion is also a problem. The economic feasibility of MFCs is also a major problem. Although MFCs have many environmental benefits, including wastewater treatment and power generation, power generation is not economically viable. [58] also highlights the need for system integration and durable materials for cost-effectiveness. Renewable energy policies are also likely to promote MFCs, but more improvement is needed.

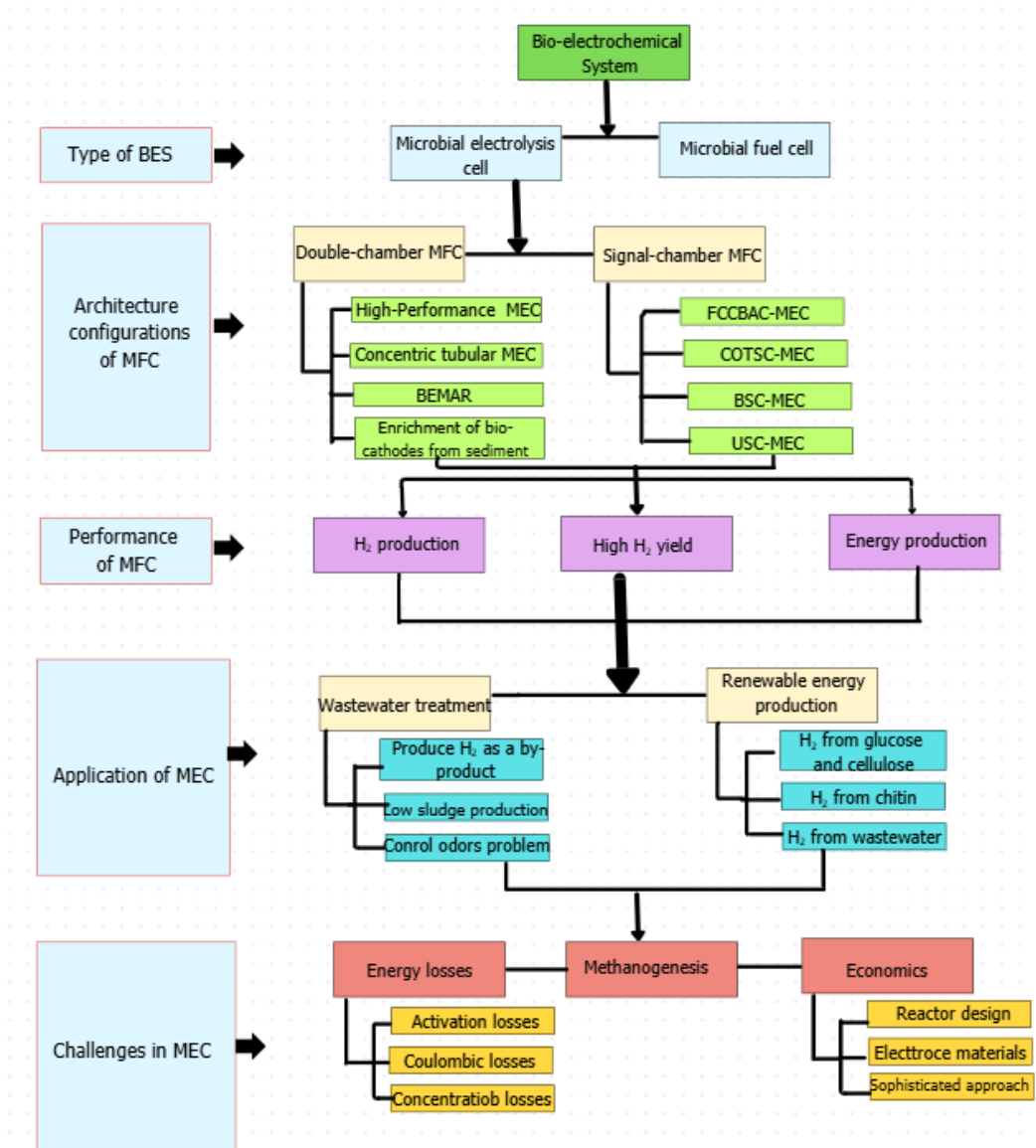
In conclusion, it is evident that microbial fuel cell technology is facing many challenges, including both technical and biological challenges. The major challenges facing MFCs are power density, internal resistance, membrane fouling, cost, microbial stability, and scaling up. Therefore, future research should be focused on addressing these issues. Overcoming these challenges is critical for moving from the laboratory to practical applications for sustainable energy and wastewater treatment.

## **2.8 Recent Trends and Innovations**

It has been seen that microbial fuel cells have developed greatly over the last few years, transforming from a laboratory-scale experimental device to a multi-functional

bioelectrochemical device, capable of performing functions such as wastewater treatment, renewable energy harvesting, desalination, and valorization.

Recent comparative studies have shown that innovative MFC designs can attain substrate removal efficiencies of over 90% and power densities of up to  $800 \text{ mW m}^{-2}$  at optimized conditions [61]. These developments show a clear transition from simple parameter optimization to structural and material innovation.



**Figure 2.8** Flow diagram of the type, applications, and challenges in MEC systems (modified from [63]).

### **2.8.1 Bioelectrode Development from Waste or Renewable Materials**

One of the innovations is the development of bioelectrodes from renewable and waste resources (Figure 2.8). Most electrochemical systems use graphite plates, carbon cloths, or platinum-coated cathodes, which are expensive. Recent studies have shown that sustainable alternatives include biochar-based anodes, carbon electrodes produced from agricultural residues, and activated carbon produced from industrial by-products [61].

These materials offer high surface area, porosity, and microbial adhesion properties while reducing the cost of fabrication. Bharagava and Saxena [62] proposed various bioremediation schemes where the fabricated electrode materials from waste follow the circular economy approach. In this approach, the wastes are converted to high-performance electrochemical devices.

Advanced methods for bioelectrode fabrication have been proposed to improve the performance of the fabricated bioelectrodes by increasing the roughness of the electrode surfaces and facilitating the formation of biofilms. Porous carbon substrates and biodegradable scaffolds have been proposed for this purpose [64].

The use of flexible electrodes for the fabrication of bioelectrodes has been proposed for the construction of modular reactors for decentralized wastewater treatment systems. This shows the impact of innovation in electrode materials on the performance of the bioelectrochemical devices by increasing the coulombic efficiency and power output.

### **2.8.2 Hybrid Systems Combining MFCs with Hydroponics MECs or Hydrogen Evolution**

Another revolutionizing factor observed in the field of MFC research is the use of nanomaterial-enriched electrodes. The use of carbon nanotubes, graphene oxide, rGO, nanofibers, and conductive polymers has been shown to improve the electrical conductivity and activity of the catalyst [63]. Based on the innovation path through patents, the integration of nanomaterials is a major evolutionary path for the development of MFC technology, especially for the electrodes and separators [65]. The use of nanofibers for the construction of the electrodes has shown enhanced mechanical strength and biofilm thickness for the electrodes [56]. Moreover, the use of composite electrodes for the construction of the MFC has shown enhanced oxygen reduction reaction activity at the cathode [63]. Although the use of nanomaterials has shown promising results for the

improvement of the performance of the MFC, the cost and safety factors should also be considered for the large-scale implementation of the system.

One of the major innovation paths for the improvement of the performance of the MFC system is the hybridization of the system with other technologies. The use of microbial electrolysis cells (MECs), which are a direct extension of the MFC system, has been shown to improve the performance of the system for the production of hydrogen through microbial electrohydrogenesis under a low applied potential of 0.2 V [64]. Moreover, the use of a hybrid system comprising both the MFC and the MEC has been shown to improve energy efficiency for the simultaneous production of electricity and hydrogen gas. The use of microbial electrolysis and desalination cells has been shown to be the major innovation path for the extension of the MFC system [61].

Hydroponic and constructed wetland integration also adds another dimension in the enhancement of MFCs. In constructed wetland-microbial fuel cells (CW-MFCs), constructed wetlands and MFCs are combined for better nutrient removal, especially for nitrogen and phosphorus, while retaining the electrochemical activity of MFCs [63]. This type of MFC configuration can be considered suitable for sustainable wastewater management practices. Effluent from MFC treatment units can be used for hydroponic cultivation, which creates a closed-loop water, energy, and food nexus system. This type of MFC configuration can be considered a good example of MFC versatility, not just for electricity production.

Innovative reactor designs also contribute to the innovation in MFCs. Three-dimensional porous scaffolds, brush anodes, stacked modular, and multi-chamber MDCs are examples of innovative reactor designs that enhance mass transfer and ion transport in MFCs [61]. MDCs are a type of MFC configuration where additional chambers are added for better desalination and electricity production. Stacked modular MFCs are used for better scalability, though voltage balancing and resistance management are also concerns in these MFCs. Innovative designs in MFCs are significantly better than conventional flat-plate MFCs in terms of performance and efficiency.

### **2.8.3 Nanomaterial Enhanced Electrodes and Advanced Electrode Architectures**

Comparative studies of traditional and innovative MFC technologies show improved performance indicators. The innovative MFCs show improved flexibility, substrate

removal, power density, and decreased material dependence [61]. The addition of nanomaterials improves electron transfer rates, while the use of renewable bioelectrodes improves capital cost. Hybrid MFCs show improved multifunctionality and resource utilization, thus suitable for incorporation into biorefineries [64]. Patent mapping of MFC literature also shows improved structural optimization and material innovation as major drivers of MFC technological evolution [63].

Despite the improved MFC performance and sustainability, several limitations exist. The addition of nanomaterials increases the cost of MFC production. The environmental impact of nanomaterials over a long period of time also requires further evaluation. The use of hybrid MFCs complicates the operation of MFCs and may result in higher maintenance costs. However, the emerging trends show a promising future of MFCs and other innovative technologies capable of overcoming the performance limitations of traditional MFCs.

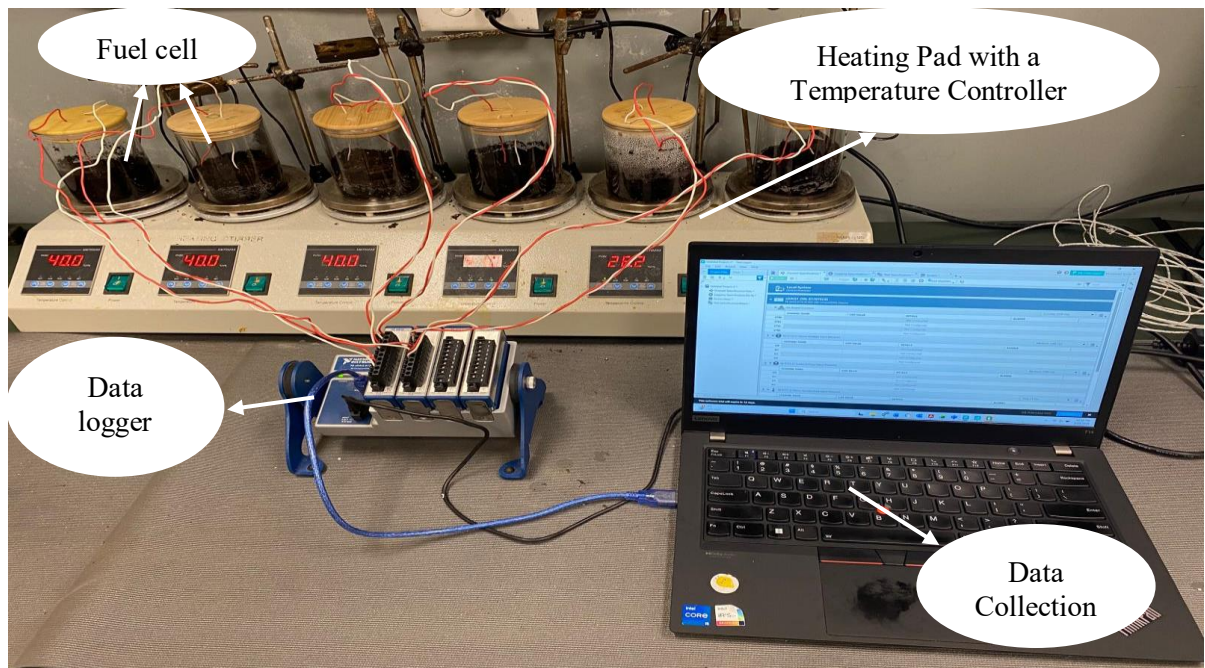
The emerging trends of MFC research and development include the use of renewable bioelectrodes, nanomaterial-enhanced MFCs, and the use of MECs, hydroponics, and desalination systems. The emerging trends show improved electron transfer efficiency, reduced internal resistance, improved substrate removal, and multifunctionality. The emerging trends show a promising future of MFCs and other innovative technologies capable of overcoming the performance limitations of traditional MFCs. The evolution of MFC technologies shows a promising future and a possible contribution to a more sustainable environment and generation of renewable energy within a circular economy.

### 3. EXPERIMENTAL APPARATUS AND PROCEDURE

The chapter entails a description of the procedures adopted during the experiment and the equipment employed. A detailed description of all the equipment employed during the experiment is provided. The methodology adopted during the experiment is also discussed, including the procedures adopted for the evaluation of biofuel cells.

#### 3.1 Experimental Setup

This section provides an in-depth description of the experimental setup developed for the assessment of voltage generation in a soil-based biofuel cell (BFC) system. A comprehensive overview of the experimental setup used is provided in Figure 3.1. The experimental setup was designed in a way that allowed the assessment of the impact of factors such as moisture content, temperature variation, carbon substrate concentration, ionic strength, and pH conditions on bioelectricity generation. A total of eight experiments were conducted, utilizing six cylindrical plastic containers as independent reactor units.



**Figure 3.1** Actual view of the experimental setup of solid-based fuel cell

Each experiment employed 100 g of natural soil as a bio-electrochemical medium, but variables such as water volume, temperature, concentration of glucose, concentration of sodium chloride, and pH were all varied. This method provided an organized way of varying variables so as to obtain optimum operating conditions for maximum voltage generation.

The experiments employed six cylindrical-shaped plastic containers, each having a volume of 500 mL. The containers were chosen because they are inert, non-conductive, and can withstand temperature fluctuations within a range of 25-50°C. The cylindrical shape was chosen so as to ensure uniform distribution of the soil. Each container was clearly marked according to the specific condition of use. The inner configuration of each container was a single chamber, membrane-less system, whereby both electrodes, i.e., anode and cathode, are immersed in the same matrix of soil.

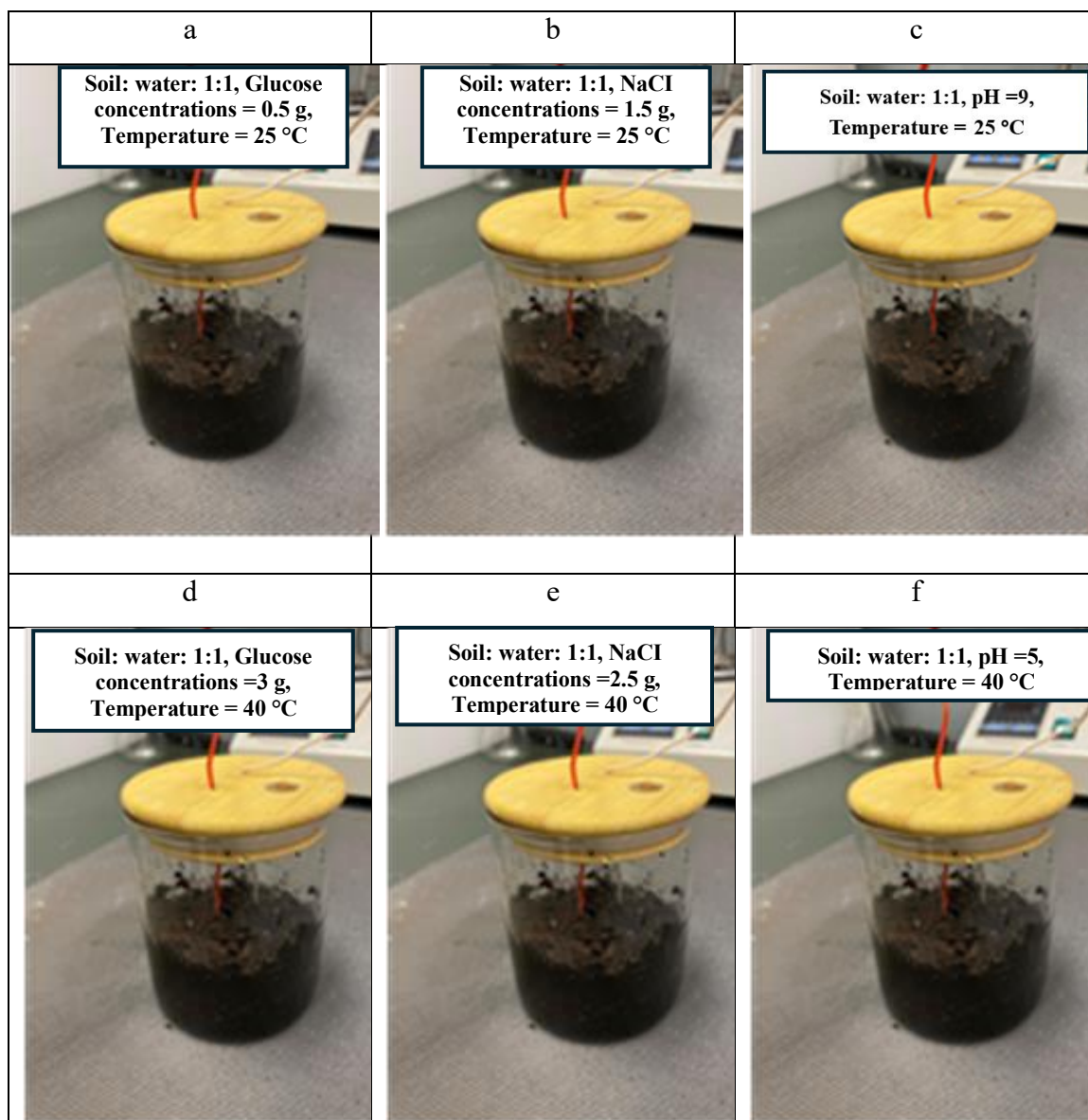
In each experiment, precisely 100 g of soil was measured using a digital balance. This was done so as to ensure that any variation in voltage was a result of variation of other variables, not density of microbes. The soil serves as a habitat for microbes, as well as an electrolyte, which facilitates oxidation reactions so as to produce electrons at the anode surface.

The volume of water is a critical parameter since it facilitates proton transport as well as ionic conductivity within the matrix of soil. The experiments employed three different volumes of water, i.e., 100 mL, 200 mL, and 300 mL. Distilled water was employed so as to avoid any interference from external ions. The water was gradually added to the soil, mixing it thoroughly. Low volumes of water might impede proton conductivity, whereas excessive water might impede oxygen transfer.

The volume of water is a critical parameter since it facilitates proton transport as well as ionic conductivity within the matrix of soil. The experiments employed three different volumes of water, i.e., 100 mL, 200 mL, and 300 mL.

Figure 3.2 presents different soil-based MFC configurations under varying glucose concentration, NaCl concentration, pH, and temperature at a constant soil-to-water ratio (1:1), where moderate glucose enhances electron transfer but excess causes instability, NaCl improves conductivity but may induce osmotic stress, higher temperature (40 °C) accelerates reactions but can reduce biofilm stability, and non-neutral pH (5 and 9) limits proton transport and overall performance.

Temperature is a factor that greatly affects the metabolism of microbes and the activity of enzymes. The temperature used in the experiment was maintained at the following levels: 25°C, 30°C, 35°C, 40°C, 45°C, and 50°C. This was done by the use of an incubator that maintained a temperature range of  $\pm 1^\circ\text{C}$ .



**Figure 3.2** Experimental setups for different operating parameters: (a) Soil: water: 1:1, Glucose concentrations = 0.5 g, Temperature = 25 °C, (b) Soil: water: 1:1, NaCl concentrations =1.5 g, Temperature = 25°C, (c) Soil water: 1:1, pH=7, Temperature =25°C, (d) Soil: water: 1:1, Glucose concentrations =3 g, Temperature = 40°C, (e) Soil: water: 1:1, NaCl concentrations =2.5 g, Temperature = 40°C, (f) Soil water: 1:1, pH=5, Temperature = 40°C

Carbon rods were used for the electrodes, which served as cathode and anode. Carbon was used for electrodes due to its high electrical conductivity, chemical stability, and low price. The anode was embedded in the lower part of the soil, which helped in creating an anaerobic environment for the bacteria. This is necessary for the bacteria's oxidation process. The cathode was placed close to the soil's surface, which helped in the reduction of oxygen.

The electrodes were kept 6 cm apart, which helped in minimizing the voltage loss and prevented electrical short circuits. Copper wires were fixed properly to the electrodes and insulated properly so that they did not come in contact with the soil particles.

Glucose was used as a carbon source for the bacteria, which helped in the production of electrons. Glucose was used in different concentrations, i.e., 0.5 g, 1.0 g, 1.5 g, 2.5 g, and 3.0 g. Glucose was dissolved in water, which was used in the experiment, and was mixed with the soil. Glucose increases the voltage output, which is optimal within a certain range, and may inhibit the voltage output after a certain point is reached.

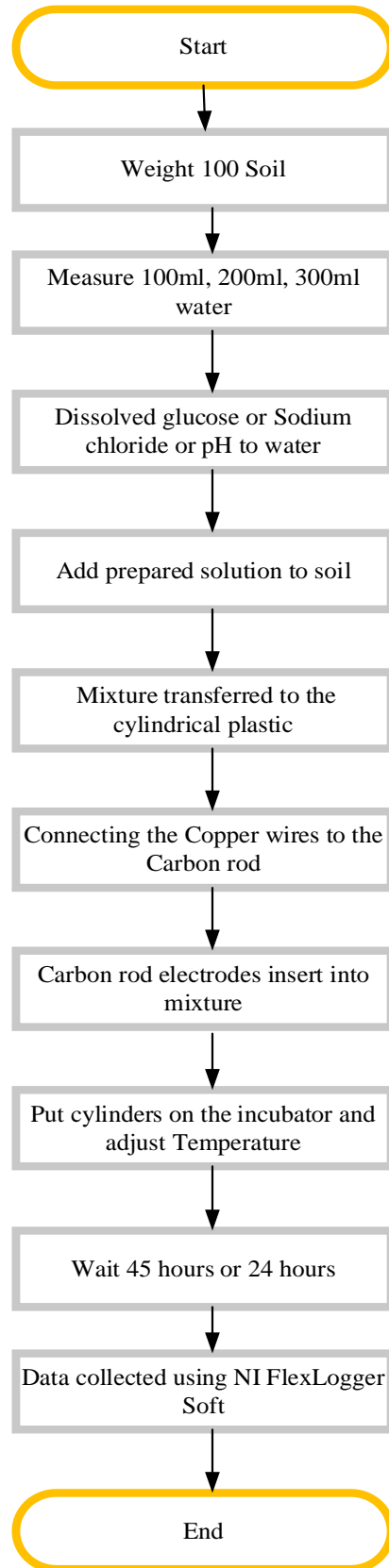
### **3.2 Experimental Procedure**

The experimental procedure was designed for the systematic evaluation of voltage production in a soil-based biofuel cell (BFC) in a laboratory setting. A total of eight experiments were conducted, utilizing six cylindrical plastic containers (500 mL capacity) used as independent bioelectrochemical reactors. A constant soil mass of 100 g was maintained, with varying water content, temperature, glucose concentration, sodium chloride concentration, and pH levels. The procedure was designed for reproducibility, parameter isolation, and data acquisition. Figure 3.3 illustrates the experimental procedure for the thesis work. Exactly 100 g of homogenized soil was accurately measured using a calibrated analytical balance for each reactor.

Distilled water was used for providing moisture and dissolving additives. Three water contents were used: 100 mL, 200 mL, and 300 mL.

Glucose was used as an external carbon source for the BFC, with concentrations of 0.5 g, 1.0 g, 1.5 g, 2.5 g, and 3.0 g. Sodium chloride was used for enhancing ionic conductivity, with concentrations of 0.5 g, 1.0 g, 1.5 g, 2.5 g, and 3.0 g. The pH levels used for this experiment were pH 5 (acidic), pH 7 (neutral), and pH 9 (alkaline).

The pH was determined using a calibrated digital pH measurement paper and was maintained within the desired range by adjusting with diluted hydrochloric acid (HCl) and/or sodium hydroxide (NaOH). A pH level of 7 is generally favorable for microbial growth, but a higher or lower pH may inhibit microbial metabolism.



**Figure 3.3** Experimental steps for voltage production flow chart from a biofuel cell.

For each experiment, the amount of glucose and NaCl that was needed was completely dissolved in a known volume of distilled water before being mixed with the soil. The prepared solution was then gradually added to the 100g sample of soil while stirring continuously to ensure that the moisture was distributed homogeneously throughout the sample. The prepared mixture was then placed inside the cylindrical plastic container. The carbon rods were then inserted at a specified depth for each electrode. For the anode electrode, it was inserted at the lower part of the soil sample to ensure that anaerobic conditions were met. For the cathode electrode, it was inserted at the surface of the soil sample to ensure that oxygen was available for the cathodic reactions. The electrodes were kept at a specified distance of about 6 cm.

Copper wires were then connected to the electrodes securely to prevent any electrical short circuits. The voltage reading was obtained using a NI FlexLogger software digital multimeter. The reading was taken every minute for a period of 45 hours for one experiment and for a period of 24 hours for 7 experiments for a period of 30 days. Some experimental conditions were repeated twice to ensure that the data obtained was statistically reliable. The data was recorded in a tabulated form before being analyzed to determine the average values. The experiment was conducted for a batch reactor for a period of 30 days. After the experiment, the data was recorded before the soil sample was disposed of properly according to safety guidelines.

### **3.3 System Components**

This section presents an overview of the system components of the soil-based biofuel cell (BFC) designed for voltage production. The system was designed to function under controlled laboratory conditions and to accommodate variations of environmental and chemical factors for eight experiments. The system comprises various mechanical, biological, electrochemical, and electrical components to ensure stable and reproducible bioelectricity production.

The biofuel cell system comprises six cylindrical plastic containers used to function as separate electrochemical reactors. Each reactor contains a microbial medium of soil, an electrolyte facilitator of water, electron collectors of graphite, additives of glucose and sodium chloride, and a temperature-controlled incubation component. Table 3.1 presents

the main experimental instruments and measurement tools used in the experiment: balance, plastic container, carbon plate, pH strips, data logger, and multi-position magnetic stirrer.

### **3.4 Test Samples**


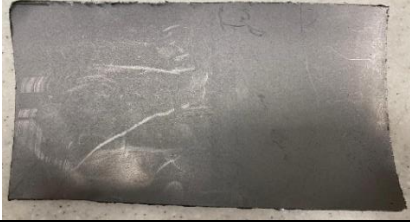


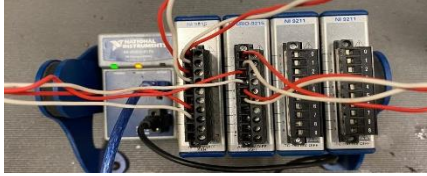

This section discusses the preparation, classification, and experimental design of the test samples used to evaluate voltage generation in the soil-based biofuel cell (BFC) system. The experimental setup was developed using cylindrical plastic containers with a capacity of 500 mL, which functioned as individual reactors under controlled laboratory conditions. Each reactor was configured by systematically varying key parameters, including soil mass, water content, operating temperature, glucose concentration, sodium chloride (NaCl) concentration, and pH level. These parameters were selected due to their significant influence on microbial activity, electrochemical reactions, and overall system performance.

In total, multiple experimental configurations were established to investigate the individual and combined effects of physicochemical and environmental factors on bioelectricity production. The preparation of each test sample involved homogenizing 100 g of soil with a defined volume of water, followed by the controlled addition of substrates (glucose) and electrolytes (NaCl), along with pH adjustment where required. This approach ensured consistency across experiments while allowing for targeted variation of specific parameters.

The core experimental instruments utilized in this study are summarized in Table 3.1. These include the Mettler Toledo AB204-S/FACT analytical balance for precise mass measurements, carbon electrode plates serving as the anode and cathode for electron transfer, cylindrical plastic containers acting as reactor vessels, pH test strips for monitoring acidity/alkalinity, a multi-channel data acquisition (DAQ) system for continuous voltage recording, and a multi-position magnetic stirrer to ensure uniform mixing and mass transfer within the system. The specifications of these instruments highlight their suitability for accurate monitoring and control of experimental conditions.

Table 3.1 summarizes the key instruments used to ensure accurate and consistent experimental measurements. The analytical balance provided precise mass measurements, while carbon electrodes enabled effective electron transfer within the system. The data acquisition system ensured continuous voltage monitoring, and the magnetic stirrer maintained uniform mixing conditions to support stable microbial activity.




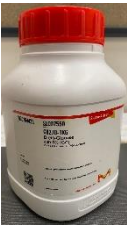


**Table 3.1** Core experimental instruments and their specifications

| Equipment Model                     | Image   | Specifications  |
|-------------------------------------|---|---|
| Mettler Toledo, AB204- S/FACT Scale |    | <p>Range: Up to 220 g</p> <p>Temperature Sensitivity (10 to 30 °C): 2.5 ppm/ °C</p> <p>Time (setting): 4s.</p>  |
| Carbon Electrode Plate              |    | <p>Material: carbon sheet</p> <p>Shape: flat plate</p> <p>Thickness: ~3–5 mm</p> <p>Surface: Smooth conductive surface</p> <p>Application: MFC anode/cathode electrode</p>  |
| Cylindrical Plastic Container       |    | <p>Material: Clear glass body</p> <p>Capacity: 520 mL</p> <p>Height: 12 cm</p> <p>Diameter: 8 cm</p>  |
| pH Test Strips                      |  | <p>pH Range: 0–14</p> <p>Quantity: 125 strips</p> <p>Type: Color comparison strips</p> <p>Application: Liquid pH measurement</p>  |
| Data Logger                         |  | <p>Type: Multi-channel DAQ system</p> <p>Channels: Multiple analog inputs</p> <p>Input Range: ±10 V (typical)</p> <p>Application: Voltage/data monitoring and recording</p>   |
| Multi-Position Magnetic Stirrer     |  | <p>Positions: 6 stirring stations</p> <p>Speed Range: 0–2000 rpm)</p> <p>Plate Material: Stainless steel top</p> <p>Control: Individual speed control knobs</p> <p>Display: Digital RPM display</p> <p>Power Supply: 220–240 V AC</p> |

Eight experimental configurations were developed using six 500 mL cylindrical plastic containers as reactors. Each test sample was prepared by varying soil mass, water volume, temperature, glucose and NaCl concentrations, and pH. The objective was to

evaluate the effects of physicochemical and environmental factors on bioelectricity generation. The test samples and their descriptions are listed in Table 3.2.

**Table 3.2** Chemicals used in the experimental work

| Material       | Sample  | Photo  | Description  |
|----------------|---|--|--|
| Soil           |  |   | I used 100g for each cylindr   |
| glucose Powder |  |   | Reagent grade anhydrous glucose powder   |
| NaCl           |  |  | Represents the sea salt addition to creating simulated seawater with purity > 99%. |

### 3.5 Experimental Uncertainty and Error Analysis

Analysis of errors is an integral component of any experiment, which guarantees the reliability and accuracy of the results obtained. In this particular study, the uncertainties relating to the measurement devices were properly evaluated, since any form of inaccuracy in the components of an experiment is likely to affect the data obtained. Table 3.3 shows the systematic errors relating to each of the devices used in the experiment.

It is worth noting that experimental errors can be divided into two major categories. These categories include systematic errors and random errors. Systematic errors are usually inherent in the measuring device. These errors are mainly due to limitations of the device or the manufacturer. In this work, the systematic errors of the components, such as the data logger, multi-position magnetic stirrer, and analytical balance, were obtained from the standard specifications of the devices.

On the other hand, random errors are usually caused by fluctuations during the experiment. These errors include environmental factors, sensitivity of the device, and

human factors. These errors were obtained by repeating the experiment three times for every parameter.

**Table 3.3** A list of measuring devices and their accuracies

| Component                           | Parameter     | Component Accuracy/Error                 |
|-------------------------------------|---------------|--|
| Data Logger                         | Potential     | ± 1%                                     |
|                                     | Temperature   | ± 0.1 °C                                 |
| Multi-Position Magnetic Stirrer     | Input voltage | ± 10%                                    |
| Mettler Toledo, AB204- S/FACT Scale | Input mass    | ±0.1 mg (typical)<br>±0.2 mg (linearity) |

The total experimental uncertainty was obtained by applying Equation (4.1).

$$U = \sqrt{(R^2 + S^2)} \quad (3.1)$$

where (U) is the total uncertainty, (R) is the random error, and (S) is the systematic error (%).

The random error was obtained by applying Equation (4.2).

$$R = (SD / AVG) \times 10 \quad (3.2)$$

where (SD) is the standard deviation, and (AVG) is the average of the experiment.

The uncertainty analysis results are shown in Table 3.4. Three repeated readings of each component were recorded to ensure the variability and reliability of the experiment.

The results obtained for the measurement of the data logger voltage gave a systematic error of 0.0498% and an uncertainty of 0.0703%. This indicates that there was a stable and constant reading of the voltage. The results for temperature measurement gave a systematic error of 0.0266% and an uncertainty of 0.0376%. This indicates high precision and minimal fluctuations. The results for the multi-position magnetic stirrer gave a systematic error of 0.02494% and an uncertainty of 0.03526%. This indicates reliable results with minimal variations. The analytical balance (Mettler Toledo AB204-S/FACT) gave results with a very low systematic error of 0.0099% and an uncertainty of 0.0141%. This indicates high precision and sensitivity of the analytical balance.

**Table 3.4** Errors and uncertainties of the system components

| Component                          | Parameter   | Reference Measurement |       |       | Systematic Error (%) | Uncertainty (%) |
|------------------------------------|-------------|-----------------------|-------|-------|----------------------|-----------------|
|                                    |             | 1                     | 2     | 3     |                      |                 |
| Data Logger                        | Voltages    | 20.2                  | 20.1  | 20    | 0.0498               | 0.0703          |
|                                    | Temperature | 37.5                  | 37.7  | 37.6  | 0.0266               | 0.0376          |
| Multi-Position Magnetic Stirrer    | Voltages    | 40.1                  | 40.2  | 40    | 0.02494              | 0.03526         |
| Mettler Toledo, AB204-S/FACT Scale | Gram        | 100                   | 100.2 | 100.1 | 0.0099               | 0.0141          |

The results obtained for the different components of the experiment show very low uncertainties. This indicates that the experiment setup is reliable and accurate.

The performance coefficient (PC) of the system can be calculated as follows:

$$\text{Performance Coefficient (PC)} = \frac{\frac{W_{\text{out,actual}}}{\dot{Q}_{\text{in,actual}}}}{\frac{W_{\text{out,reference}}}{\dot{Q}_{\text{in,reference}}}} \quad (3.3)$$

The performance coefficient is a dimensionless metric used to evaluate how effectively a system operates relative to a defined reference condition. It is calculated as the ratio of the actual system efficiency to the reference efficiency, where efficiency is expressed as the ratio of useful output rate to required input rate.

Specifically, the numerator represents the real operating performance of the system, while the denominator corresponds to a baseline or ideal/reference case. A PC value greater than 1 indicates that the system performs better than the reference condition, whereas a value less than 1 suggests lower performance. This formulation allows for a normalized comparison of system efficiency under varying operating conditions and is particularly useful in thermodynamic and energy system analyses.

## 4. RESULTS AND DISCUSSION

This study presents the experimental results and discussion of the voltage generation obtained from the soil-based microbial fuel cell system. The objective of this chapter is to analyze the effect of various operational parameters on bioelectricity generation. The experiment was carried out using a soil mass of 100 g and various operational variables such as temperature, glucose concentration, sodium chloride (NaCl) concentration, volume of water, and pH conditions. These variables are important since they have a major impact on the bioelectricity generation of the microbial fuel cell. The results of this experiment are divided into several sections to analyze the effect of each of the variables individually and together.

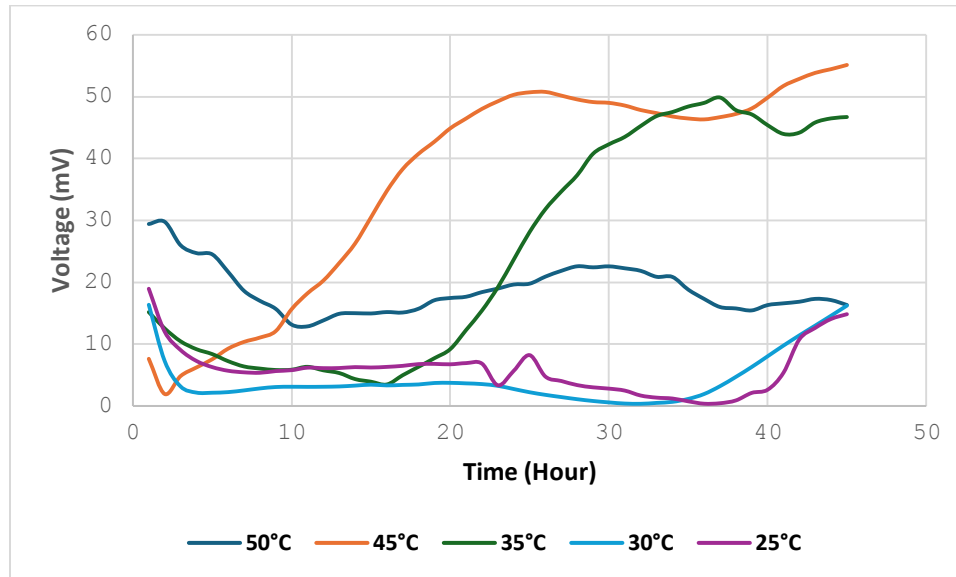
First, the effect of temperature on voltage generation was analyzed at two different conditions of operation, namely 45 hours and 24 hours. Then, the effect of substrate concentration was analyzed by varying the glucose concentration. Glucose is added as an electron donor to the MFC. The impact of electrolyte concentration was also analyzed by varying the concentration of NaCl. The combined effect of pH and temperature was analyzed to find the best environmental conditions for bioelectricity generation. Finally, the effect of soil moisture was analyzed by varying the volume of water. The results of this experiment are presented as a comprehensive analysis of the voltage generation of the soil-based MFC. The findings of this experiment are presented as a basis of the conclusions and recommendations of the study, as discussed in the following chapter.

### 4.1 Effect of Temperature

As depicted from the voltage profile, the temperature dependence in the soil-based MFC can be observed clearly. The best performance was observed at 45°C, where the voltage increased from 10 mV at start-up to nearly 55 mV at 45 hours (Figure 4.1). Moreover, the 35°C temperature also demonstrated significant enhancement, where the voltage increased from 15 mV to 50 mV before leveling off at 47 mV. Conversely, a peak in the voltage was observed at 50 °C, followed by a gradual decrease in the voltage from 30 mV to 16 mV, which might be due to thermal stress on the MFC. In addition, the lower temperatures, i.e., 30 °C and 25 °C, remained constant at levels lower than 16 mV, as depicted in Figure 4.1.

According to literature, temperature increase enhances the metabolic kinetics of microbes, which in turn results in a decrease in activation loss and increases the rate of

EET, whereas high temperature can be detrimental to the MFC performance due to the destruction of the biofilm structure and the inhibition of electrogenic bacteria [73].



**Figure 4.1** Voltage production results in soil-based MFC for 45 hours.

Temperature is one of the key factors that affect the performance of a microbial fuel cell. This is mainly attributed to the effects of temperature on the biochemical reactions that take place within the system. In this regard, an increase in the temperature enhances the enzymatic activity and the substrate oxidation reaction, thus increasing the rate at which electrons are released at the anode according to the Arrhenius equation [70]. At the same time, electrolyte viscosity decreases, thus reducing the internal resistance and increasing the voltage output.

Therefore, the optimal performance observed at 45°C (final voltage 55 mV) suggests that the soil environment was optimal for the electrogenic microbial activity. This is an indication that the enzymatic activity was high enough to increase the electron transfer at the anode. Similar observations were noted by other researchers where the use of moderate thermophilic conditions resulted in the improvement of the power density compared to the mesophilic conditions [72].

On the other hand, the 35°C condition was observed to develop a considerable amount of voltage (50 mV peak), which is consistent with the literature indicating this condition to be optimal for the growth and activity of *Geobacter* and other electrogenic bacteria [73]. The fact that the voltage continued to increase over the entire period suggests

the progressive maturation of the biofilm and the improvement in the efficiency of the electron transfer from the anode over the entire period. The fact that the voltage remained constant over the last part of the experiment suggests that the system had reached a steady state.

On the other hand, the 50°C condition started by producing a considerable amount of voltage (30 mV), only to decrease over the entire period to 16 mV. Although the use of high temperatures enhances enzymatic activity, high temperatures can also affect the stability of the microbial membrane and the proteins involved in the electron transfer. This may result in the shift from electrogenic activity to other metabolic pathways. At the same time, the metabolic energy required by the microbes may increase at high temperatures, thus reducing the availability of the electrons for the anode [71].

The findings show that the optimal temperature range for this soil-based MFC is between 35 and 45 °C, as shown in Table 4.1. Within this range, the kinetics and electron transfer rates are high and efficient. On the other hand, low temperatures result in low metabolic efficiency. These findings are consistent with bioelectrochemical theories indicating the relationship between activation loss, ohmic loss, and biological stability as factors affecting MFC voltage output [75].

**Table 4.1** Voltage production performance of soil-based MFC at different temperatures for 45 hours.

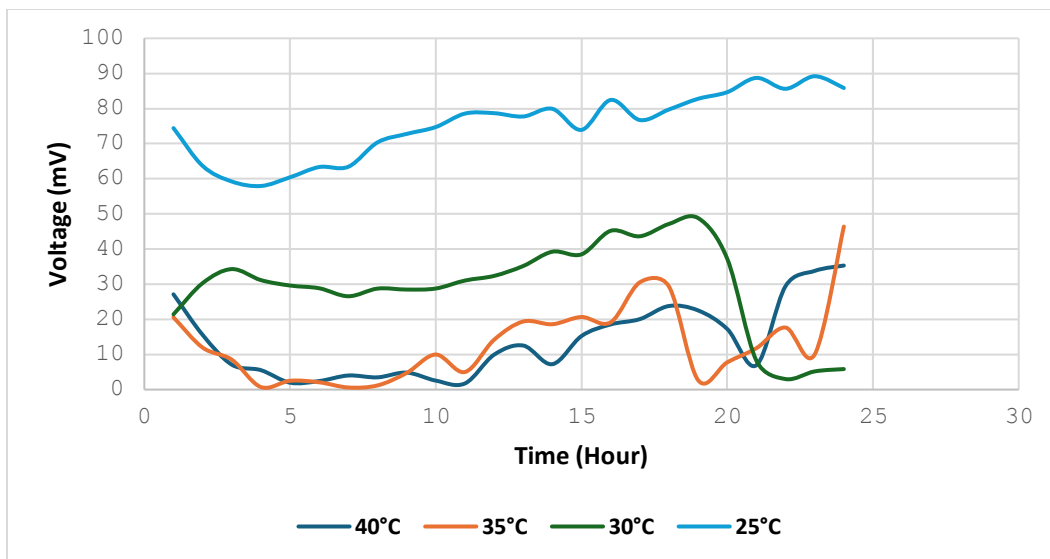
| Temperature | Initial (mV) | Peak (mV) | Peak Time (h) | Minimum (mV) | Minimum Time (h) | Final (mV) | Trend                             |
|-------------|--------------|-----------|---------------|--------------|------------------|------------|-----------------------------------|
| 50 °C       | 30           | 30        | 1             | 12           | 10               | 16         | Early higher than gradual decline |
| 45 °C       | 10           | 55        | 45            | 3            | 2                | 55         | Strong continuous increase        |
| 35 °C       | 15           | 50        | 38            | 5            | 15               | 47         | Slow rise than plateaus           |
| 30 °C       | 8            | 16        | 45            | 2            | 3                | 16         | Low output with late recovery     |
| 25 °C       | 18           | 18        | 1             | 1            | 36               | 15         | Early drop than slight recovery   |

The observed voltage fluctuations are primarily attributed to the dynamic interplay between microbial growth phases, substrate consumption rates, and electrochemical processes. During early operation, instability arises from microbial acclimation and lag-phase behavior. As biofilm develops, voltage increases; however, periodic drops occur due to substrate depletion, internal resistance changes, and limitations in extracellular electron transfer (EET). At elevated temperatures, fluctuations become more pronounced due to thermal stress, while at lower temperatures, delayed microbial activity leads to irregular and unstable voltage profiles.

These findings show the importance of the role played by temperature in the regulation of microbial activity and the generation of voltage. In further exploring the short-term effects of temperature on the production of voltage, a 24-hour experiment was done under similar conditions, as shown in the next section:

In the influence of temperature on the generation of voltage by the soil-based microbial fuel cell (MFC), the system containing 100 g soil and 100 mL water produced the optimal voltage output at 25°C (Figure 4.2). The system started by producing 75 mV of voltage output within the first hour before slightly declining to 58-60 mV between hours 3-5. The system gradually increased the output to a maximum of 88-90 mV between hours 22-23 before settling at 85 mV. This shows the adaptation of the microbes and the progressive biofilm formation on the anode surface. Mesophilic electrogenic microbes such as *Geobacter* and *Shewanella* have optimal activity for the transfer of electrons outside their cell membrane (extracellular electron transfer (EET)) between 20-30°C. The decline in the early stages may be attributed to the acclimation of the microbes.

Figure 4.2 describes that the voltage increased gradually from around 22 mV at 30°C up to around 48-50 mV at hour 19, then declined sharply to less than 10 mV after hour 21. This sharp decline may have been caused by substrate depletion or competition for substrates among microorganisms. Literature also supports that temperatures above optimum mesophilic ranges may cause increased internal resistance and proton transfer limitations.



**Figure 4.2** Voltage production results of soil-based MFC for 24 hours.

The performance of the system at 35°C was unstable; the voltage increased irregularly between almost 0 mV and around 30-32 mV before a sharp peak to almost 45 mV at hour 24. According to , high temperatures may cause changes in the microbial population structure, where non-electrogenic microorganisms may dominate the electrodes, competing for substrates without transferring electrons.

At 40°C, the voltage declined sharply from around 25 mV to less than 5 mV during the first 10 days before gradually increasing to around 35 mV on day 24. This may have been caused by the adaptation of microorganisms to high temperatures, enriching thermotolerant microorganisms (Table 4.2). According to Ahn et al. [77], it was observed that while moderate increases in temperatures may cause increased reaction rates, high temperatures may cause membrane destruction and lower the efficiency of the electron transport chains.

**Table 4.2** Voltage Performance of Soil-Based MFC at Different Temperatures for 24 hours.

| Temperature (°C) | Initial Voltage (mV) | Peak Voltage (mV) | Final Voltage (mV) |
|------------------|----------------------|-------------------|--------------------|
| 25               | 75                   | 90                | 85                 |
| 30               | 22                   | 50                | 10                 |
| 35               | 20                   | 45                | 45                 |
| 40               | 25                   | 35                | 35                 |

On the other hand, the 25°C condition had the maximum peak voltage (90 mV), followed by 30°C (50 mV), 35°C (45 mV final spike), and 40°C (35 mV), as shown in Table 4.2. This further validates the fact that the optimal performance for soil-based MFC systems lies within the mesophilic range. At the same time, the rise in voltage is a result of the maturation of biofilms and the efficient oxidation of substrates, while the fall is a result of substrate limitation, competition, and the effects of thermal stress.

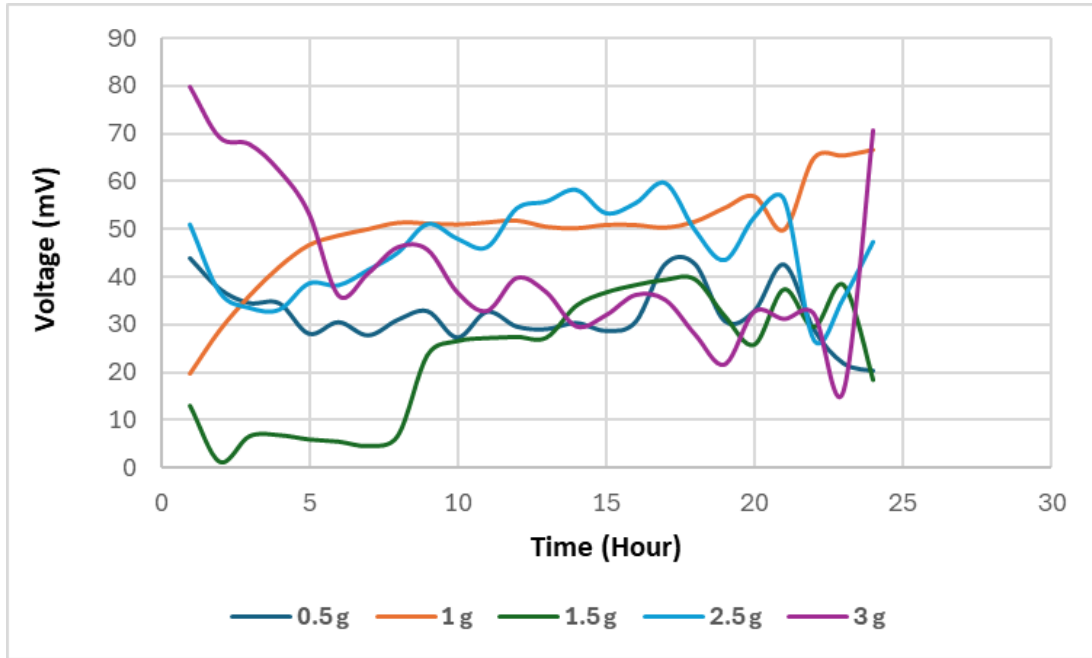
The voltage profiles presented in Figure 4.2 exhibit significant and abrupt fluctuations across all temperature conditions, highlighting the dynamic and non-steady-state nature of the soil-based MFC system. At 25 °C, although high voltage outputs were achieved, noticeable oscillations were observed due to intermittent substrate utilization and electron transfer limitations. In contrast, the system at 30 °C demonstrated severe instability, characterized by strong fluctuations followed by a sharp voltage collapse, indicating possible substrate depletion or biofilm disruption. The 35 °C condition showed the most erratic behavior, with repeated peak–drop cycles reflecting unstable microbial activity and inconsistent electrochemical performance. Meanwhile, at 40 °C, fluctuations were comparatively moderate, with a gradual recovery in voltage suggesting improved microbial acclimation despite initial thermal stress. Overall, these fluctuations confirm that voltage generation in soil-based MFCs is governed by transient microbial processes, substrate dynamics, and temperature-dependent electrochemical limitations.

#### **4.2 Effect of Glucose Concentration**

This section presents the results on the effect of glucose dose on the generation of voltage in a soil-based MFC operated at 25 °C with 100 g soil and 100 mL water, where glucose was added in different concentrations: 0.5 g, 1.0 g, 1.5 g, 2.5 g, and 3.0 g, and the voltage in mV was measured over a 24-hour period.

In all conditions, the variations in the MFC voltage over time can be attributed to (I) acclimation and establishment of biofilm, (II) periods of enhanced EET as electroactive biofilm matures, and (III) instabilities due to substrate depletion, fermentation product, pH gradient, and/or growth of competing non-electrogenic microbes. These mechanisms are in agreement with the MFC literature on the use of readily fermentable substrates, such as glucose, which can increase the rate of biodegradation, thereby reducing Coulombic efficiency due to the diversion of electrons for biomass production and soluble

fermentation products (e.g., VFAs) rather than for current production. In glucose-fed MFCs, electron loss in the form of intermediates and competing sinks, such as methanogens in MFCs with mixed cultures, is well documented as a factor for decreased current production, particularly in glucose-fed MFCs with excessive glucose supply [82].



**Figure 4.3** Voltage production results of soil-based MFC operated at 25 °C and soil to water ratio of 1:1 with different glucose concentrations.

The 1.0 g glucose condition (orange) had the strongest evidence for a "beneficial dose" response: starting from 21 mV at the beginning of the experiment, increasing to 45-50 mV by hours 5-8, and then settling into a long plateau at 50-55 mV before finally peaking at 66 mV by hours 23-24 as shown in Figure 4.3. This profile implies that the 1.0 g condition supplied an ample electron donor for continued electrogenic metabolism without supporting excessive competing fermentation pathways. A plateau response for the MFC voltage is often a good sign that a stable anode biofilm and constant internal resistance have been achieved for the quasi-steady-state condition. A moderate feeding rate for the mixed cultures has been shown to maximize the electron harvesting efficiency without accumulating excessive byproduct, supporting the notion that the 1.0 g condition was approaching an optimal dosage.

The 0.5 g glucose condition (dark blue) indicated a lower final performance with a gradual decrease in performance over time. Voltage levels were initially 43 mV (first hour)

before leveling off in the 28-35 mV range for a large portion of the experiment, finally falling off to a minimum and final value of 20 mV by hour 23-24. This decrease in performance over time can be attributed to electron donor limitation and exhaustion of the added substrate, as readily biodegradable carbon sources are limiting in these systems, slowing the rate of electrogenic respiration, and causing a decrease in the overall voltage as the anode potential increases due to a shift in the biofilm towards maintenance respiration.

The 1.5 g glucose (green) condition indicated a delayed start-up with a peak in performance in the middle of the experiment. Voltage levels were initially 12 mV (first hour) before falling off to 2 mV (second hour) before rising significantly from hours 8-10, reaching 25-30 mV, with a peak of 40 mV at hour 17, finally falling off to 22 mV by hour 25. The lower levels in the early hours can be attributed to a longer acclimation period or oxygen/electron acceptor consumption before the anode takes over as the electron sink. Once the anode biofilm was established, the voltage increased, as established biofilms are known to increase EET capacity [83]. The decrease in performance can be attributed to a decrease in the glucose concentration and a diversion of electrons towards fermentation products, which can be expected in glucose-fed systems where syntrophic reaction mechanisms can be significant in the anode carbon flow.

At higher dosages, the system showed stronger evidence of excess substrate side effects. When using 2.5 g glucose (light blue), the voltage started relatively high (49 mV), dropped to a range of 33-38 mV during hours 2-5, then increased to a higher level around 52-58 mV during hours 11-18, peaking at a high of around 59 mV around hour 17. After this peak, increased fluctuations were observed, with a sharp dip to 28 mV around hour 22 before leveling off again to 46 mV around hour 25. Such fluctuations are consistent with dynamic changes between electrogenic respiration and other processes (e.g., fermentation and methanogenesis), which periodically impede the rate of electron transfer to the anode, with pH splitting and mass transfer limitations that become more serious at higher biodegradation rates [81]. Glucose MFCs are particularly susceptible to poor Coulombic efficiency because glucose is readily fermented to intermediates (e.g., propionate), which may accumulate and represent a loss of electrons to the current-generating process.

The strongest signature of the initial overfeeding and subsequent instability was seen in the 3.0 g glucose condition (purple). The voltage was initially very high, at 79 mV at the first hour, but dropped precipitously to 37 mV by the sixth hour, remained variable at levels around 30 to 40 mV between hours 8 and 20, dropped to a minimum at about 19 mV at hour 23, and then rose sharply to 63 mV at hour 25. The rapid decline in the early hours is consistent with the effects of excessive substrate, where the high glucose concentration would be expected to increase the rate of fermentation, increase the rate of biomass production (thereby increasing the internal resistance of the biofilm), and produce acidic by-products that would tend to decrease the pH near the anode, thereby decreasing the overall rate of electron transfer as current. The late rise may be the result of the consumption of intermediates (volatile fatty acids) by the adapted biofilm once the readily consumable glucose was depleted. A similar consumption of glucose-derived intermediates has been observed in adapted MFC biofilms on the anode.

**Table 4.3** Voltage production performance of soil-based MFC at 25 °C with different glucose concentrations.

| Glucose (g) | Initial (mV) | Peak (mV) | Peak Time | Minimum (mV) | Minimum Time | Final (mV) |
|-------------|--------------|-----------|-----------|--------------|--------------|------------|
| 0.5         | 43.2         | 43.2      | 1         | 20.5         | 24           | 20.5       |
| 1.0         | 21.1         | 65.8      | 24        | 21.1         | 1            | 65.8       |
| 1.5         | 12.3         | 40.0      | 17        | 2.3          | 2            | 21.8       |
| 2.5         | 49.4         | 59.5      | 17        | 28.3         | 22           | 46.1       |
| 3.0         | 78.6         | 78.6      | 1         | 18.8         | 23           | 62.5       |

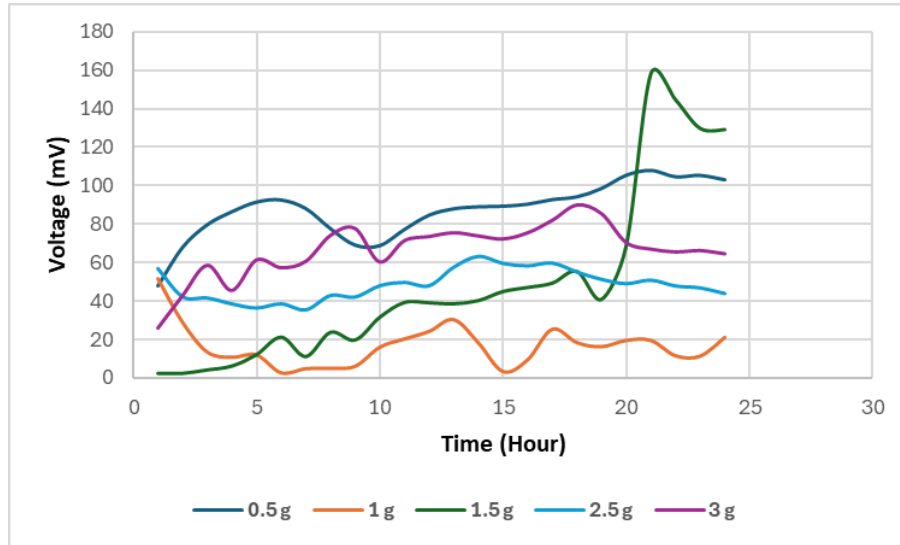
The voltage profiles corresponding to different glucose concentrations exhibit pronounced and irregular fluctuations, highlighting the strong influence of substrate loading on system stability. At lower concentrations (0.5 g), fluctuations were moderate, reflecting limited but stable microbial activity. The 1 g condition demonstrated the most stable behavior, with minimal oscillations and consistently high voltage output, indicating optimal substrate utilization. In contrast, higher concentrations (1.5 g and 2.5 g) showed increased fluctuation intensity, characterized by delayed activation, oscillatory behavior, and periodic voltage drops due to metabolic imbalance and substrate oversupply. The most extreme fluctuations were observed at 3 g, where sharp voltage spikes and drops indicated severe instability, likely caused by microbial inhibition and accumulation of metabolic by-products. These findings confirm that while substrate addition enhances

bioelectrochemical activity, excessive concentrations disrupt system stability and lead to highly transient voltage behavior.

Among the tested quantities, the maximum sustained and final voltage was recorded by 1.0 g glucose, which exhibited relatively stable mid-run performance. The second best was 2.5 g glucose, which recorded similar mid-run voltage but was less stable. The third best was 3.0 g glucose, which recorded the maximum voltage at the beginning and exhibited inhibitory-like declines and recoveries. The lowest dose of glucose (0.5 g) was seen to perform poorly over the long term due to electron donor limitations, as shown in Table 4.3. This observation further supports the general principle that an “optimal substrate window” exists in MFCs, such that too little carbon substrate leads to impaired electrogenic respiration, and too much substrate leads to “competing sinks” and “pH and mass transfer limitations. This demonstrates that the concentration of glucose has a strong effect on voltage generation, depending on the availability of electron donors and the metabolic activity of microorganisms in the soil-based MFC. In order to assess the interrelationship between substrate concentration and temperature, the effect of glucose dosage on voltage generation was also investigated at a higher temperature (40°C), as discussed in the following section:

At a temperature of 40°C, the effect of glucose dosage is likely to result in a trade-off between the availability of electron donors and the electrochemical limitations, which become increasingly severe with temperature. Glucose is not usually oxidized directly by electroactive bacteria in the anodic biofilm of a mixed culture MFC, but is instead fermented to an intermediate, which is then used by electroactive bacteria for current generation. When an excess of glucose is present, a greater proportion of electrons is wasted on biomass, soluble metabolic by-products (such as propionate), and methanogenesis, which reduces the Coulombic efficiency and voltage output. Operation of MFCs at and above 40°C will also affect the electroactive bacteria, which are usually mesophilic, and will instead allow faster fermenters and methanogens, which will decrease the voltage output at the anode, even though the overall rate of biodegradation is increased [84]. As a consequence, any increases in voltage output during start-up within the dataset for a temperature of 40°C are most likely due to acclimation and biofilm formation (improved extracellular electron transfer), whereas any decreases and oscillations in

voltage output are likely due to bursts of excess substrate fermentation, proton gradients, and diversion of electrons from the anode, particularly with higher concentrations of glucose. When the figure is digitized, the discussion will also report the initial, maximum, minimum, and ultimate voltage output (mV) for each glucose dosage (0.5–3.0 g), as shown in Figure 4.4.



**Figure 4.4** Voltage production results of soil-based MFC operated at 40 °C and soil to water ratio of 1:1 with different glucose additions.

The results from the 40 °C tests are also significant in that they represent a contrasting set of conditions from the 25 °C results, in which increased temperature simultaneously accelerates microbial kinetics and exacerbates biological and transport rate limitations that can act as a barrier to electricity generation. Temperature can affect MFC performance in a variety of ways, with multiple mechanisms interacting simultaneously, for example, in microbial kinetics, membrane/solution conductivity, diffusion, and the relative rates of electrogenic respiration and other competing anaerobic metabolic pathways. In general, a temperature increase from a cold temperature to a mesophilic temperature can enhance MFC performance in terms of increased current and power due to decreased start-up times and increased microbial kinetics, though MFCs operated at 40 °C were found to have a negative impact on EAB on the anode and a concomitant increase in methane production, which can decrease current density and voltage stability. Accordingly, the trends in the 40 °C results should be understood in terms of (I) substrate

conversion mechanisms with glucose as the fed substrate, and (II) temperature effects on the electroactive biofilm.

Glucose, as a highly fermentable substrate, is also commonly associated with low CE in MFCs with mixed cultures, as fermentation occurs before electroactive respiration can capture the electrons as current. Freguia et al. [82] also showed that glucose oxidation in MFC anodes occurs in a multi-step syntrophic process, where glucose first undergoes fermentation, primarily to acetate and H<sub>2</sub>, and then these intermediates are oxidized by electricity-producing bacteria. This process also does not linearly relate glucose dosage with MFC voltage, as with a low dosage, the electrogenic process can become electron donor limited, causing a decrease in MFC voltage as the glucose is used, whereas with a high dosage, the MFC can become unstable due to conditions caused by excessive fermentation, which can result in a decrease in current production due to the production of intermediates, such as propionate, which can also cause the production of methane and biomass, in addition to causing a decrease in CE in MFCs, as also shown by Kim et al. [81].

These substrate-driven effects are magnified at 40°C, as the elevated temperatures may favor the growth of the fast-growing fermenters and methanogens and disfavor the competitiveness of the typical mesophilic EAB, e.g., *Geobacter*-dominated communities are often enriched at lower temperatures under acetate-fed systems. In their study, they reported that temperatures above 40°C can inhibit EAB activity on the anode and discussed the relationship between current production and methane production; the balance between the two may be particularly relevant at elevated temperatures. This information is shown in Figure 4.4. This means that the 40°C voltage curves may show more fluctuations, sudden drops, and subsequent recoveries. This may be the result of the sudden depression of the pH in the anode microenvironment by the rapid activity of the fermenters, followed by a subsequent increase in the anode potential as the intermediates are consumed and the system returns to a new equilibrium. In addition, the thicker biofilms and increased biomass under high substrate loading can cause unstable anode potentials over time.

For 40 °C, the following comparative analysis can be reached for each glucose condition (0.5 g, 1.0 g, 1.5 g, 2.5 g, 3.0 g): (a) initial voltage (start-up), (b) maximum voltage and day of occurrence, (c) minimum voltage and day of occurrence, and (d) final

voltage at the end of the experiment. In addition, the stability of the voltages will be qualitatively assessed by determining whether the curves show a plateau (which would indicate a stable electrogenic response) or whether the curves show oscillations (which would indicate the presence of competition between the different processes). This format for the results is consistent with the recommended format for the reporting of MFC results [95].

**Table 4.4** Voltage production performance of soil-based MFC at 40 °C with different glucose concentrations

| Glucose (g) | Initial Voltage (mV) | Peak Voltage (mV) | Time of Peak | Minimum Voltage (mV) | Time of Minimum | Final Voltage (mV) | Overall Trend                        |
|-------------|----------------------|-------------------|--------------|----------------------|-----------------|--------------------|--------------------------------------|
| 0.5 g       | 55                   | 108               | 20           | 50                   | 1               | 102                | Gradual increase than stable plateau |
| 1.0 g       | 52                   | 30                | 13           | 3                    | 15              | 20                 | Early decline and fluctuations       |
| 1.5 g       | 2                    | 160               | 21           | 2                    | 1               | 128                | Slow growth than sharp spike         |
| 2.5 g       | 58                   | 63                | 14           | 35                   | 6               | 45                 | Moderate fluctuations                |
| 3.0 g       | 28                   | 90                | 18           | 28                   | 1               | 65                 | Gradual rise then mild decline       |

As a whole, the expected outcome for the 40°C tests is that an intermediate glucose dosage will yield the best balance of electron donor supply and minimized diversion; a very low dosage will be poor due to limitations; and a very high dosage will be unstable with a decreased voltage due to electron loss from fermentation and methanogenesis as indicated in Table 4.4. All these are based on the well-accepted fact that MFCs with glucose-fed anodes operate as anaerobic bioreactors where syntrophic conversion and

competitive sinks are key determinants in electricity generation, and high temperatures above or near 40°C can impede EABs and favor electron flow in non-electricity-generating pathways.

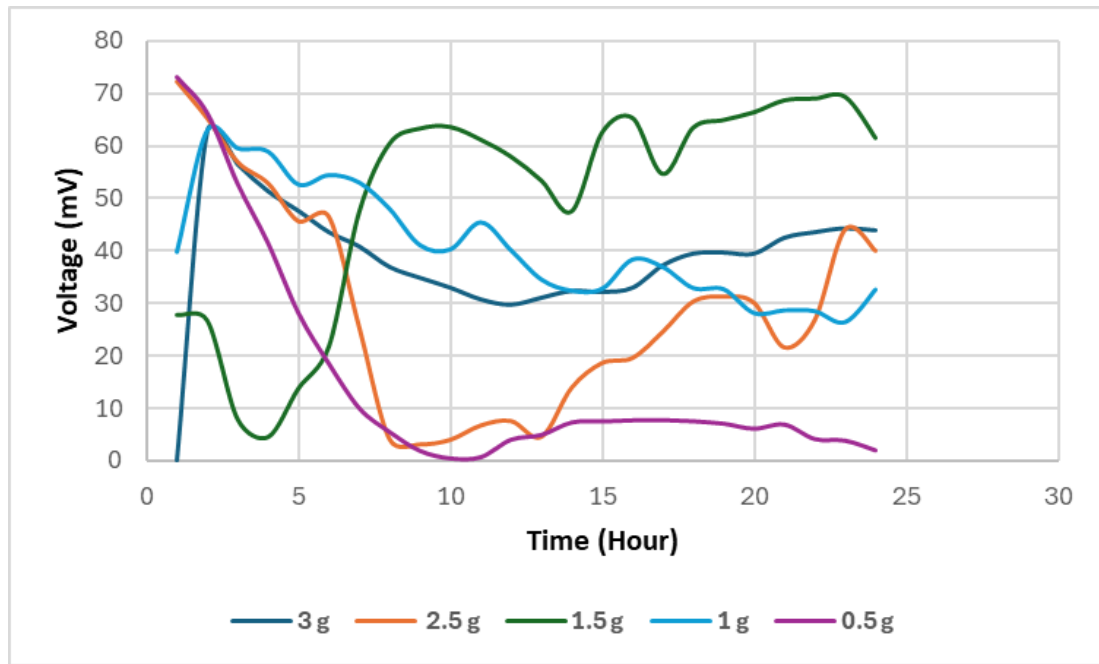
The voltage profiles presented in Figure 4.4 exhibit pronounced and abrupt fluctuations across all glucose concentrations, indicating highly dynamic system behavior. At 0.5 g, fluctuations were relatively controlled, with a steady increase in voltage reflecting stable microbial activity. In contrast, the 1 g condition showed repeated collapse–recovery cycles, indicating unstable substrate utilization and poor electrochemical performance. The most significant fluctuation was observed at 1.5 g, where a sudden voltage surge to approximately 160 mV occurred following a prolonged low-activity phase, suggesting delayed microbial adaptation and rapid oxidation of accumulated substrates. Higher concentrations (2.5 g and 3 g) exhibited continuous oscillations and instability, with voltage outputs fluctuating widely due to metabolic imbalance and possible inhibitory effects. These results confirm that substrate concentration plays a critical role in governing both the magnitude and stability of voltage generation in soil-based MFC systems.

#### **4.3 Effect of Sodium Chloride (NaCl) Concentration**

Variation in the dosage of sodium chloride (NaCl) in a soil-based microbial fuel cell (MFC) operated at 25°C is expected to yield a non-linear relationship in the voltage response due to the concomitant increase in ionic strength and conductivity, which can decrease ohmic loss and internal resistance, and inhibition of electroactive biofilm due to high levels of osmotic/ionic stress (Figure 4.5). Previous MFC research supports the hypothesis that ionic strength, increased by the addition of NaCl, can increase power production due to decreased solution resistance, which in turn can increase the voltage produced by the MFC, as indicated by higher and more stable voltage plateaus [71]. In a similar study, Lefebvre et al. observed improved performance due to increased ionic strength from added NaCl, which decreased the internal resistance, while higher levels of added salt were detrimental, suggesting an optimum salinity range for MFC performance [86].

In detail, increased conductivity can improve the rate of proton transfer, which can increase the potential generated in the MFC, thereby increasing the voltage, while high levels of added salt can decrease the ability of exoelectrogens in the biofilm, which can decrease the rate of extracellular electron transfer (EET) and thereby decrease the voltage

generated in the MFC. Therefore, within the 25 °C dataset (0.5-3.0 g NaCl), the increase in voltage can be best explained by a decrease in internal resistance due to increased ionic strength, while the decrease in voltage and oscillation can be best explained by salinity inhibition and changes in the microbial community structure. The results will be expressed in terms of the actual numerical values in millivolts for the different levels of added NaCl in the MFC, which will be digitized from the plotted curves.



**Figure 4.5** Voltage production results of soil-based MFC operated at 25 °C and soil to water ratio of 1:1 with different NaCl concentrations.

The NaCl series at 25 °C constitutes a well-defined test case for the combined influence of ionic strength and salt tolerance on electricity generation in a soil-based MFC. In MFCs, the output voltage is a measure of the difference between cathode and anode potentials adjusted for internal losses, as depicted in Figure 4.5. One of the key internal losses is ohmic resistance in the electrolyte and separator material, which is also a function of ionic strength. Increasing NaCl concentration should therefore improve the conductivity of the electrolyte, reducing ohmic loss and thereby increasing the output voltage under load. This effect is well established in the MFC literature: Liu et al. demonstrated that increased ionic strength in the electrolyte, achieved by addition of NaCl, increased MFC output voltage, consistent with reduced ohmic loss in the electrolyte [71]. If the curves

show that moderate amounts of NaCl (0.5-1.5 g) have a higher plateau or a smoother curve than very low salt concentration, then this is consistent with a conductivity effect.

Nevertheless, NaCl is not entirely a positive factor as the electroactive community can be inhibited by increased salinity. In fact, the electrogenic biofilms have limited salinity tolerance. Consequently, increased salinity may cause a change in the microbial community composition and the metabolic pathways, as well as the efficiency of the EET process. Miyahara et al. observed that the optimal salinity concentration is a result of the balance between the improvement of the conductivity and the salt tolerance of exoelectrogenic bacteria. Moreover, the optimal salinity is not without limits. In fact, the optimal salinity can even enable the growth of *Geobacter* bacteria in the anode biofilms of the MFC. Lefebvre et al. observed that the addition of NaCl can improve the performance of the MFC but only to a certain limit. In fact, increased salinity can be detrimental to the performance of the MFC. In the results section, if the performance of the system is affected by the increased salinity dosage (e.g., 2.5–3.0 g), where the voltage decreases and becomes unstable compared to the moderate dosage, it can be inferred that the negative effects of increased salinity are greater than the positive effects of increased conductivity.

In soil-based MFC systems, there are also mechanisms that may cause increases and decreases in the voltage output over time. The voltage may increase during the start-up period as the biofilm develops on the anode, allowing the EET mechanisms to be established. The voltage may also decrease as the substrates are depleted in the soil, as inhibitory compounds are produced, and as the moisture level fluctuates, as indicated in Figure 4.6. The presence of NaCl may also affect some of the mechanisms that increase and decrease the voltage output, as indicated in Table 4.5, where the presence of NaCl may inhibit the microbial respiration, enzyme production, and osmoregulation, among other factors, as the conductivity of the saline solution increases. It has also been indicated that the increase in the salinity of the saline MFC may cause the microbial community to shift, as the metabolic pathways are altered, with the highest output being achieved at moderate conductivity levels, rather than at low or high conductivity levels, as indicated in Figure 5.7[88].

**Table 4.5** Voltage production performance of soil-based MFC at 25 °C with different NaCl concentrations

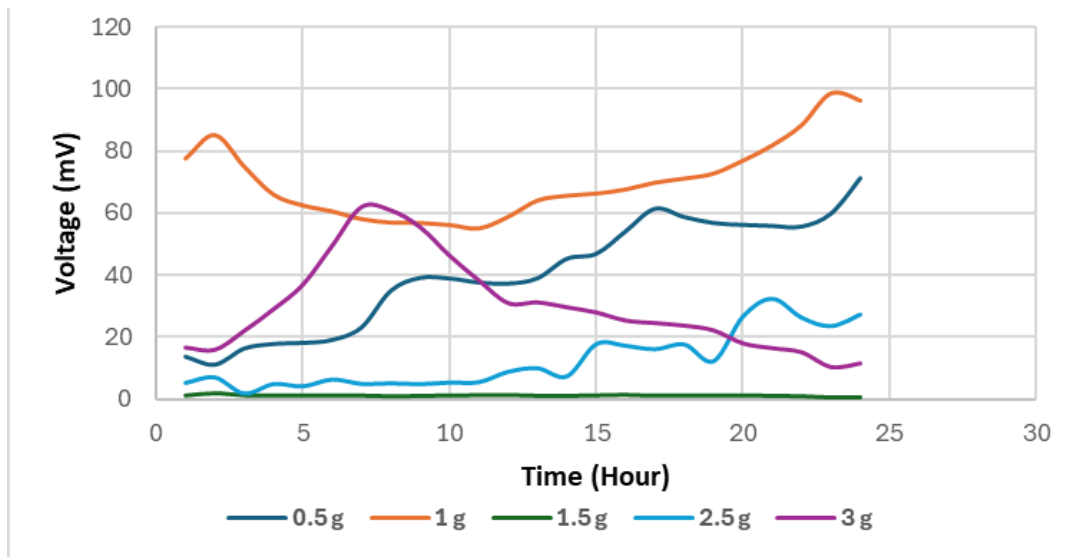
| NaCl (g) | Initial Voltage (mV) | Peak Voltage (mV) | Time of Peak | Minimum Voltage (mV) | Time of Minimum | Final Voltage (mV) | Overall Trend                             |
|----------|----------------------|-------------------|--------------|----------------------|-----------------|--------------------|---|
| 3.0 g    | 0                    | 44                | 23           | 0                    | 1               | 44                 | Sharp early rise, stable moderate plateau |
| 2.5 g    | 72                   | 72                | 1            | 3                    | 9               | 40                 | Early decline, gradual recovery           |
| 1.5 g    | 28                   | 70                | 23           | 5                    | 4               | 62                 | Dip, strong increase, and high stability  |
| 1.0 g    | 40                   | 65                | 2            | 26                   | 23              | 32                 | Moderate fluctuations                     |
| 0.5 g    | 72                   | 72                | 1            | 2                    | 10              | 2                  | Strong decline and low output             |

The voltage profiles in Figure 4.5 exhibit pronounced fluctuations across all glucose concentrations, reflecting the dynamic and non-steady-state behavior of the system. At low concentration (0.5 g), a rapid voltage decline followed by sustained low output indicated substrate depletion and system instability. The 1 g condition showed moderate and controlled fluctuations, suggesting balanced substrate utilization and stable microbial activity. The 1.5 g concentration demonstrated the most stable and highest performance, with sustained voltage output and minor oscillations indicative of efficient biofilm activity. In contrast, higher concentrations (2.5 g and 3 g) exhibited severe early-stage voltage drops followed by partial recovery, reflecting substrate inhibition and metabolic imbalance. These findings confirm that optimal substrate concentration is critical for maintaining both high voltage output and system stability in soil-based MFCs.

The results showed that the concentration of sodium chloride plays a significant role in voltage production, affecting both ionic conductivity and microbial salt tolerance in

the soil-based microbial fuel cell. To further assess the influence of both increased temperature and salinity levels on voltage production, NaCl concentration was also tested at a temperature of 40 °C, as discussed in the next section:

The results of voltage production at a temperature of 40 °C showed a significant influence of NaCl concentration on voltage production in the soil-based microbial fuel cell. The voltage output was nonlinearly proportional to NaCl concentration. Among all tested concentrations, 1.0 g of NaCl showed the highest voltage output. The voltage was initiated at about 80 mV, increased up to almost 100 mV, and remained at about 95 mV, as shown in Figure 5.6. This indicates that the addition of NaCl at a concentration of 1.0 g improved both ionic conductivity and reduced the overall internal resistance of the soil-based microbial fuel cell. Ionic conductivity is known to increase voltage output in microbial fuel cells. It is reported that increasing ionic strength enhances voltage output because it can reduce ohmic losses, thus increasing voltage output [77].



**Figure 4.6** Voltage production results of soil-based MFC operated at 40 °C and soil to water ratio of 1:1 with different NaCl concentrations.

On the other hand, the lowest salinity condition (0.5 g NaCl) started from a low voltage of around 5 mV and gradually increased to around 70 mV by the 24th hour. This may be attributed to the high internal resistance at the beginning due to low ionic conductivity. As stated by Liu et al. [71], the ionic strength of the electrolyte has a significant impact on the internal resistance; increasing the ionic strength decreases the internal resistance and hence the voltage stability.

Under the 1.5 g NaCl condition, the voltage values were extremely low throughout the entire experiment (2-5 mV, ending at 1 mV), indicating a high level of microbial inhibition. High salinity levels impose osmotic stress on electrogenic bacteria, affecting membrane integrity and the efficiency of the extracellular electron transfer (EET), as shown in Figure 4.6. Previous research has indicated that high concentrations of NaCl have a negative impact on the diversity and activity of microbes present on the anode of the MFC [86]. Although the conductivity may be high at high salinity levels, the inhibitory effects on the microbes are more pronounced.

The intermediate 2.5 g NaCl condition showed a moderate performance where the output increased from 7 mV to 30 mV by the 21st day. This shows a partial improvement in conductivity while the microbes were under stress. In the 3.0 g NaCl condition, the performance showed an early peak at around 65 mV by the 6th day before declining to 12 mV by the 24th hour. This is a typical behavior where high salinity enhances the electrolyte conductivity before inhibiting the electrogenic microbes, as indicated by the literature [88].

As shown by the results above, the existence of an optimal salinity window is established at 40°C. A moderate concentration of NaCl (1.0 g) strikes a balance between ionic conductivity and microbial tolerance, producing the maximum sustained voltage as shown by the results below in Table 4.6.

**Table 4.6** Voltage production performance of soil-based MFC at 40 °C with different NaCl concentrations

| NaCl (g) | Initial (mV) | Peak (mV) | Peak Time | Minimum (mV) | Minimum Time | Final (mV) |
|----------|--------------|-----------|-----------|--------------|--------------|------------|
| 0.5 g    | 5            | 60        | 17        | 2            | 2            | 70         |
| 1.0 g    | 80           | 100       | 1         | 60           | 10           | 95         |
| 1.5 g    | 2            | 5         | 3         | 0            | 1            | 1          |
| 2.5 g    | 7            | 30        | 21        | 3            | 3            | 30         |
| 3.0 g    | 15           | 65        | 6         | 10           | 23           | 12         |

Lower concentrations suffer from ohmic losses, while higher concentrations suffer from osmotic stress. This is consistent with established literature for typical MFC performance where moderate ionic strength enhances performance before declining at high salinity levels [74].

The voltage profiles in Figure 4.6 exhibit structured yet noticeable fluctuations, reflecting the influence of glucose concentration on system dynamics. At 0.5 g, a gradual

increase in voltage with mild oscillations indicated stable but substrate-limited performance. The 1 g condition demonstrated the most stable behavior, with minimal fluctuations and a steady increase in voltage, confirming optimal substrate utilization. In contrast, the 1.5 g condition showed negligible voltage output throughout the experiment, suggesting severe microbial inhibition. Higher concentrations (2.5 g and 3 g) exhibited delayed activation and irregular fluctuations, with the 3 g condition showing an initial voltage peak followed by a continuous decline due to metabolic imbalance and inhibitory effects. These results confirm that substrate concentration critically governs both the magnitude and stability of voltage generation in soil-based MFC systems.

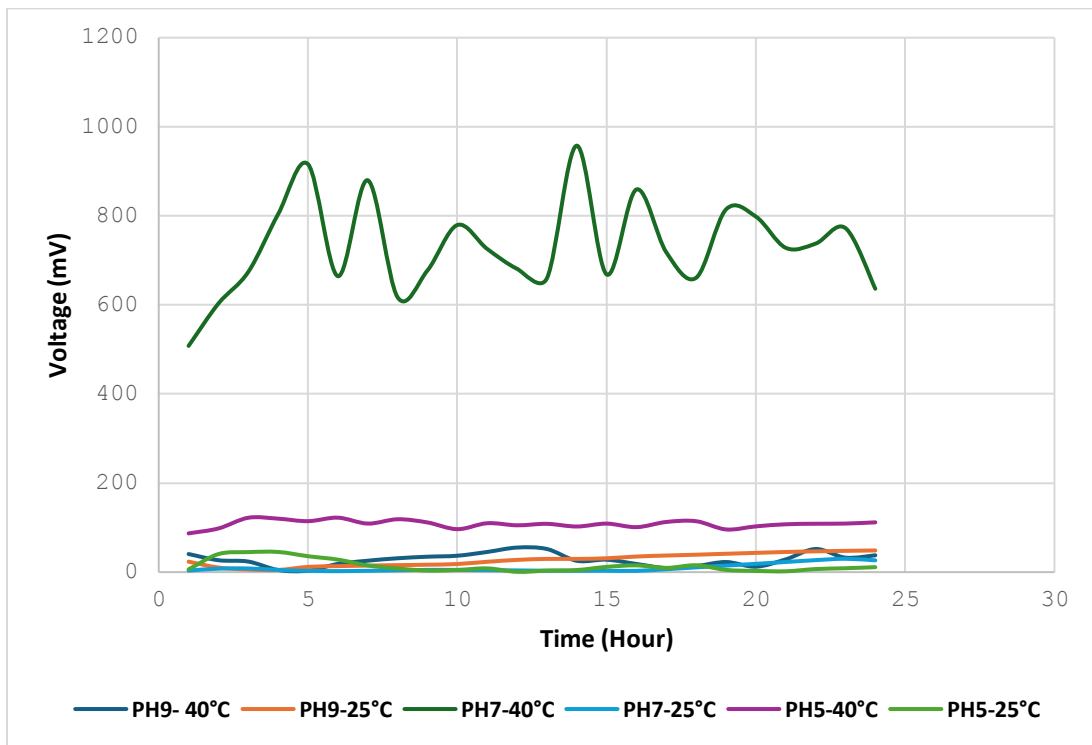
#### **4.4 Combined Effect of pH and Temperature**

The pH-temperature comparison shows that MFC voltage is strongly controlled with respect to both pH and temperature. The highest voltages were recorded at a pH of 7 and a temperature of 40 °C (dark green), oscillating between 500 mV during start-up to a peak around 950 mV (peak at hour 14) before dropping off around 630 mV (Figure 4.7).

Neutral pH conditions are generally conducive to efficient extracellular electron transfer by electroactive biofilms, and also minimize any issues of acid/alkali stress, whereas the increase in temperature can increase the rate of reaction, allowing the voltage to increase and the solution resistance to decrease, thus increasing the voltage under load; however, temperature may also destabilize the microbial biofilm and increase issues of transport, resulting in the peaks and troughs observed in the curve [95]. At the pH of 7, and the temperature of 25 °C (light blue), the voltages were significantly lower, ranging between 10 mV and 55 mV, which is likely due to the slower rate of metabolism at the reduced temperature range [73].

The pH of 9 conditions resulted in reduced voltage levels compared to the neutral pH conditions, where the voltage increased at both temperatures, although the rate of increase was slower at the reduced temperature of 25 °C, where the voltage increased to reach a maximum of 40 mV at 40 °C (dark blue) and reached a maximum of 50 mV at 25 °C (orange) after 21 hours. Alkaline pH conditions may increase cathode overpotentials and also shift the microbial biofilm, whereas both acidic and alkaline pH conditions, i.e., conditions that are not at neutral pH, may increase internal losses due to pH splitting of the biofilm and membrane systems [83]. The pH of 5 conditions, i.e., the acidic conditions,

resulted in intermediate levels of voltage, where the voltage was relatively high at the increased temperature of 40 °C (purple), whereas at the reduced temperature of 25 °C (light green), the voltage remained relatively low to moderate and increased later on. While the inhibiting effect of acidic bulk pH on MFC performance is well known to impede anodic metabolism and cause increased proton concentration inside the anode biofilms, some degree of adaptation and kinetics enhancement with increased temperature may help alleviate some of the inhibiting effect, thus explaining the increased pH 5 output at 40 °C compared to 25 °C in the data set [89]. The optimum pH for MFCs is around the neutral pH with enhanced performance at increased temperatures, while any deviation to pH 5 or 9 leads to a reduction in both voltage and/or stability due to a combination of both microbial stress and pH gradient-induced losses.



**Figure 4.7** Voltage production of a soil-based MFC operated at pH 5, 7, and 9 under 25 °C, soil to water ratio of 1:1, and temperature of 40 °C.

One of the most significant MFC operating parameters is pH, which affects both the microbial activity at the anode, the cathodic kinetics, ionic conductivity, and the magnitude of internal losses. In a conventional MFC design, protons are produced at the anode during substrate oxidation reactions and have to be transported through the

electrolyte phase to the cathode to facilitate the continuation of the current output. If proton transport is insufficient to meet the rate of current output, then a pH gradient is created with a consequent acidification of the anode biofilm and alkalization of the cathode biofilm, thereby creating additional activation and ohmic losses (termed pH splitting). Such a pH gradient reduces the cell voltage even if the open circuit voltage is high; in addition, the pH gradient also inhibits the activity of bacteria residing in the biofilms, especially in the deeper parts of thick anode biofilms [83].

From my data set, the most prominent feature is the high and fluctuating voltage at pH 7 and 40 °C (dark green), with the starting voltage at approximately 500 mV and a consistent peak at 800-950 mV with a maximum at approximately 950 mV at hour 14. Neutral pH is beneficial for the growth and activity of EAMs because it keeps enzymatic activity and membrane potentials at optimal levels while minimizing the cost for maintaining pH homeostasis. From the electrochemical point of view, MFCs with a lower electrolyte resistivity due to increased operation temperatures (like 40 °C) may have increased voltages due to increased mass transfer rates under constant external resistance. Improvements in MFC performance with increased temperatures have been reported in MFC research and review articles; however, high temperatures may cause a destabilization of the MFC communities with a more fluctuating output [72]. The peaks observed in the pH 7 curve at 40 °C may be attributed to the feedback between increased current generation and increased local pH gradients that cause increased voltage loss; therefore, with increased current generation, local pH gradients may become more prominent, causing a reduction in the output voltage. This feedback between current generation and local pH gradients is known to cause non-steady-state responses in MFCs with biofilms [75].

When temperature decreased to 25 °C at pH 7 (light blue), voltage decreased dramatically to a much smaller range (10-55 mV, last value at 35 mV). This is expected since microbial kinetics are slower at lower temperatures, i.e., substrate oxidation occurs at a slower rate. At lower temperatures, more voltage is lost. Furthermore, it might take longer for a mature electroactive biofilm to develop at lower temperatures, which can extend the start-up time and lead to a lower voltage plateau [73]. Overall, it appears from the pH 7 comparison that temperature in this system greatly contributed to increased voltage output.

At pH 9, both temperature profiles resulted in relatively low voltage outputs, although it is clear that both profiles would eventually increase voltage output. The pH 9 at 25 °C curve increased from 10 mV up to 50 mV at peak hour 21, whereas the pH 9 at 40 °C curve increased from 20 mV up to 40 mV at peak hour 22. At pH 9, it is expected that microbial communities might change, increasing the cost of maintaining cytoplasmic pH homeostasis. From an electrochemical perspective, it is also expected that an alkaline cathode environment can influence oxygen reduction reactions, increasing voltage loss. Several studies have indicated that MFC voltage is optimized at pH levels near or at neutral pH, or even at mildly alkaline pH levels (pH 7-8 or even higher, depending on inoculum source and substrate), but voltage output is decreased at more extreme pH levels [89]. The fact that pH 9 does not increase voltage output in this system suggests that voltage loss due to alkaline pH is significant.

As can be observed in Fig. 4.7, the temperature effect was again observed at acidic pH 5, where at 40 °C (purple) the voltages were high and stable, with a final voltage of 105 mV, while at 25 °C (light green) the voltages were still very low, with a final voltage of 20 mV. As mentioned in Table 4.7, acidic conditions are known to decrease current and power due in part to the inhibitory effect of low bulk pH on the biofilm, where increased proton concentrations are expected in the anode biofilm; modeling and experimental results suggest that large proton gradients can result in significant low-pH inhibition in biofilms [83].

The voltage profiles presented in Figure 4.7 exhibit significant variations in both magnitude and fluctuation intensity across different pH and temperature conditions. The most pronounced fluctuations were observed at pH 7 and 40 °C, where voltage outputs reached up to approximately 950 mV, characterized by repeated peak-drop cycles indicative of highly active microbial metabolism and efficient extracellular electron transfer. In contrast, conditions at pH 5 and pH 9 showed substantially lower voltage outputs with reduced fluctuation intensity, reflecting microbial inhibition and increased internal resistance under non-neutral pH conditions. Furthermore, at 25 °C, all pH conditions exhibited significantly lower voltage outputs and minimal fluctuations, highlighting the limiting effect of low temperature on microbial kinetics. These results

demonstrate that both pH and temperature play a critical role in governing not only the magnitude but also the dynamic behavior of voltage generation in soil-based MFC systems.

As can be observed in the results, the combined pH-temperature conditions support the formulation of three different thesis statements, which are as follows: First, neutral pH (pH 7) conditions are best for high voltage in the soil MFC, especially at high temperature conditions. Second, as can be observed in the results, acidic (pH 5) and alkaline (pH 9) conditions decrease the voltage and/or stability of the MFC compared with neutral pH, which can be attributed to pH stress and increased inner losses due to pH gradient, as can be observed in Table 4.7.

**Table 4.7** Voltage production performance of soil-based MFC at 25 °C and 40 °C with different pH values.

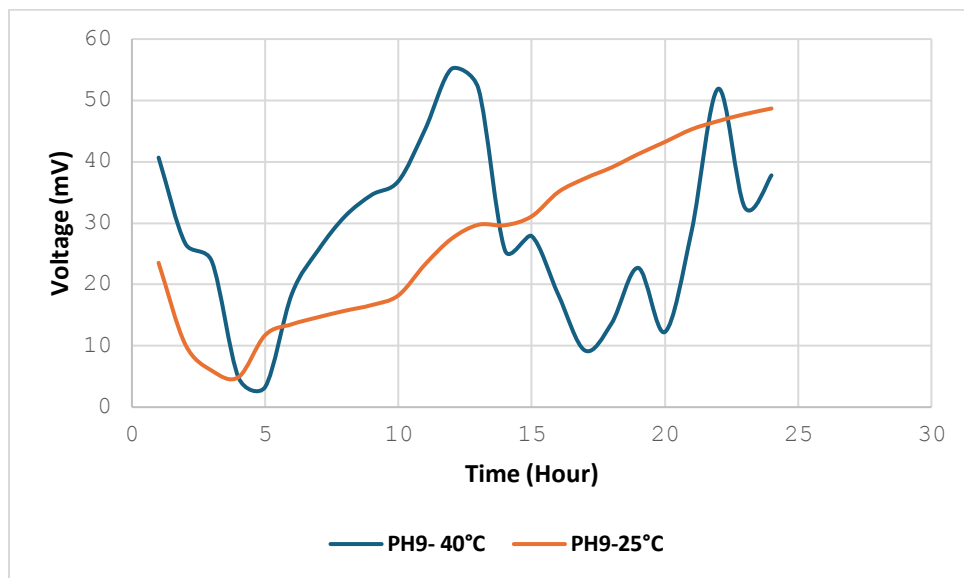
| Condition    | Initial (mV) | Peak (mV) | Peak Time | Minimum (mV) | Minimum day | Final (mV) | Trend   |
|--------------|--------------|-----------|-----------|--------------|-------------|------------|---|
| pH 9 at 40°C | 20           | 40        | 22        | 5            | 2           | 30         | Low–moderate with late increase                           |
| pH 9 at 25°C | 10           | 50        | 21        | 5            | 4           | 25         | Low start; gradual rise then mild decline                 |
| pH 7 at 40°C | 500          | 950       | 14        | 500          | 1           | 630        | Very high; oscillatory peaks (biofilm/transport dynamics) |
| pH 7 at 25°C | 20           | 55        | 20        | 10           | 6           | 35         | Moderate; small oscillations                              |
| pH 5 at 40°C | 90           | 115       | 4         | 85           | 11          | 105        | High early; stable band ~90–110                           |
| pH 5 at 25°C | 15           | 35        | 22        | 5            | 2           | 20         | Low–moderate; late improvement                            |

This is in line with established MFC literature that emphasizes pH control and temperature as critical operating parameters for biofilm activity, internal resistance, and cell voltage operation [73].

The results have shown that the combined effect of pH and temperature significantly impacts the operation of voltage generation in soil-based microbial fuel cells. To further demonstrate and understand the impact of temperature under constant pH conditions, a comparative study between 25 and 40 °C under pH 9 conditions is presented in the following section. The comparative study between 40 °C and pH 9, and 25 °C and pH 9, indicates the impact of temperature on MFC voltage performance under constant pH conditions (Figure 4.8).

At 40 °C, the voltage varied from an initial 20 mV to a peak of 40 mV. However, for 25 °C, the voltage varied from an initial 10 mV to a peak of 50 mV, as shown in Figure 4.8. An increase in temperature generally increases microbial metabolic kinetics and reduces internal resistance for improved electron transfer rate. However, too high a temperature may result in increased instabilities in biofilm.

At this pH level, temperature influenced proton movement rate, enzymatic activity, and ionic conductivity in the soil medium. An increase in temperature reduces solution resistance and increases rate of reactions in both electrodes. However, there is a possibility of thermal stress for microbes.



**Figure 4.8** Voltage production comparison between 40 °C and 25 °C under pH 9.

At this pH level, 40 °C exhibited a reduced peak voltage compared to 25 °C. This indicates that thermal enhancement was not significant. The microbial limitations are shown in Table 4.8. The variation in voltage stability indicates that temperature affects biofilm development and internal resistance. This study is in line with established MFC research that indicates temperature significantly impacts MFC performance.

The comparison of results indicates that temperature is a critical factor in MFC operation. Temperature performance is subject to a balance between kinetic and microbial limits.

This suggests that the temperature effect on the generation of voltage performance depends on the pH conditions in the soil-based microbial fuel cell system. In order to

further evaluate the temperature effect on the performance of the voltage in a more suitable pH environment, a similar comparison between 40 °C and 25 °C at a pH of 7 will be discussed in the next section. The comparison between 40 °C, pH 7, and 25 °C, pH 7, shows the temperature effect on MFC voltage performance in a similar pH environment (Figure 4.9).

**Table 4.8** Voltage production performance of soil-based MFC at 25 °C and 40 °C with pH 9

| Condition   | Initial (mV) | Peak (mV) | Peak Time | Minimum (mV) | Final (mV) |
|-------------|--------------|-----------|-----------|--------------|------------|
| 40 °C, pH 9 | 20           | 40        | 22        | 5            | 30         |
| 25 °C, pH 9 | 10           | 50        | 21        | 5            | 25         |

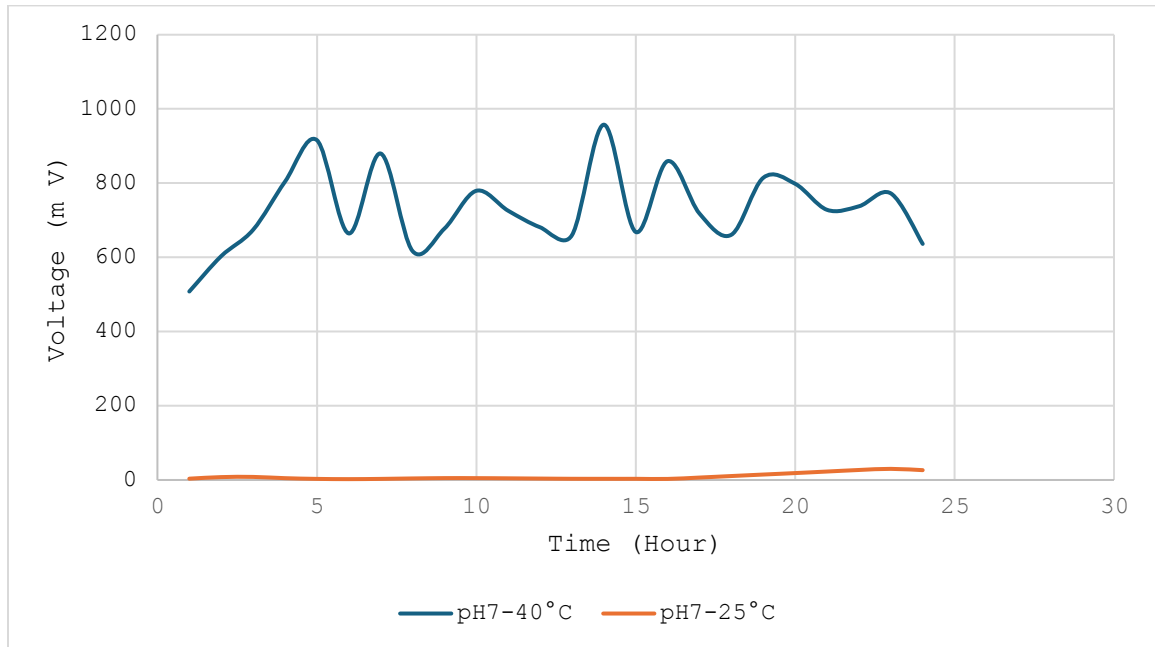
The voltage profiles at pH 9 demonstrate markedly different fluctuation behaviors under varying temperature conditions. At 40 °C, the system exhibited pronounced and abrupt fluctuations, characterized by repeated peak–drop cycles, indicating unstable microbial activity and electrochemical performance due to combined thermal and alkaline stress. In contrast, at 25 °C, the voltage profile showed a gradual and steady increase with only minor oscillations, reflecting stable microbial acclimation and consistent extracellular electron transfer. These findings highlight that while elevated temperature can enhance reaction kinetics, under alkaline conditions it may lead to increased instability, whereas lower temperature promotes more stable but moderate voltage generation.

At 40 °C, the range of the voltage was from 500 mV initially, reaching a peak of 950 mV, as opposed to 25 °C, where the range of the voltage was from 20 mV, reaching a peak of 55 mV, as depicted in Figure 4.9. An increase in temperature generally increases the kinetics of microbial metabolic rates and reduces the inner resistance, thereby improving the rate of electron transfer [82]. However, high temperature also increases the instabilities in biofilms.

At the same pH, temperature affects the mobility of protons, enzymatic activity, and ionic conductivity in the soil matrix. An increase in temperature also reduces the solution resistance, which can increase the rate of reaction in the electrodes.

At this pH, the 40 °C temperature demonstrated a higher peak voltage compared to 25 °C, which suggests that temperature enhancement was greater than the limitations of the microbes. The differences in the stability of the voltage suggest that temperature affects

the dynamics of biofilm growth, as depicted in Table 4.9, which presents the inner resistance.



**Figure 4.9** Voltage production comparison between 40 °C and 25 °C under pH 7.

As depicted in the above results, temperature significantly influences MFC performance, as demonstrated in previous MFC research, where temperature was identified as a key factor in MFC performance, as depicted in Figure 4.9 [95]. Therefore, the results of the above experiment suggest that temperature plays a key role in MFC performance, as demonstrated in the differences in the results at different temperatures, which proves that temperature is a key factor in MFC performance, as depicted in the above results.

The voltage profiles at pH 7 reveal a significant contrast in both magnitude and fluctuation behavior between 40 °C and 25 °C. At 40 °C, the system exhibited high voltage outputs ranging from approximately 500 to 950 mV, characterized by repeated cyclic fluctuations indicative of active microbial metabolism and efficient extracellular electron transfer. These oscillations correspond to dynamic substrate utilization and biofilm activity. In contrast, at 25 °C, the voltage output remained significantly lower, with minimal fluctuations, reflecting reduced microbial kinetics and limited electrochemical performance. These results confirm that neutral pH combined with elevated temperature provides optimal conditions for enhanced and dynamic voltage generation in soil-based MFC systems.

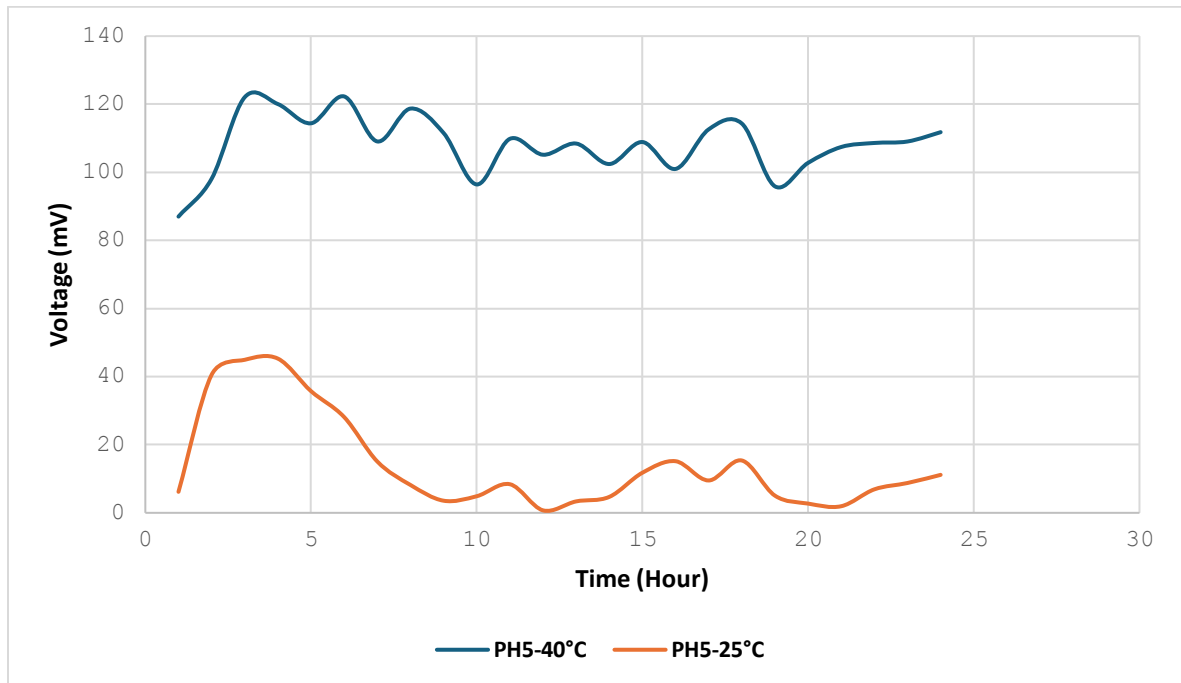
**Table 4.9** Voltage production performance of soil-based MFC at 25 °C and 40 °C with pH 7.

| Condition   | Initial (mV) | Peak (mV) | Peak Time | Minimum (mV) | Final (mV) |
|-------------|--------------|-----------|-----------|--------------|------------|
| 40 °C, pH 7 | 500          | 950       | 14        | 500          | 630        |
| 25 °C, pH 7 | 20           | 55        | 20        | 10           | 35         |

This shows that temperature has a significant effect on voltage generation, especially when the pH is kept neutral. This is an important factor that needs to be considered for the soil-based MFC. In order to compare the effect of temperature on voltage generation under acidic conditions, a similar comparison between 40°C and 25°C, pH 5, is given in the following section:

The comparison between 40°C, pH 5, and 25°C, pH 5, shows the effect of temperature on voltage generation for the MFC.

When the temperature is kept at 40°C, the voltage generation ranges from 90 mV initially to a maximum of 115 mV, whereas when the temperature is kept at 25°C, the voltage generation ranges from 15 mV initially to a maximum of 35 mV, as explained in Figure 4.10. It is generally known that an increase in temperature has a positive effect on the rate of metabolism for microorganisms, which increases the rate of electron transfer.



**Figure 4.10** Voltage production comparison between 40 °C and 25 °C under pH 5.

However, an increase in temperature also has a negative effect on biofilm instability. At the same pH, temperature affects the mobility of protons, enzymatic activities, and ionic conductivity. Increasing temperature decreases solution resistance and may accelerate reaction rates at both electrodes [72]. However, microorganisms may suffer from thermal stress depending on pH and substrate conditions.

The voltage profiles at pH 5 demonstrate that temperature significantly influences both the magnitude and stability of system performance under acidic conditions. At 40 °C, the system exhibited moderate voltage outputs with controlled oscillations, indicating partially sustained microbial activity despite pH-induced limitations. In contrast, at 25 °C, the voltage output was significantly lower and characterized by irregular fluctuations, reflecting suppressed microbial metabolism due to the combined effects of low temperature and acidic conditions. These findings suggest that while elevated temperature can enhance system performance under acidic conditions, it does not fully overcome the inhibitory effects of low pH.

Under this pH, the result at 40 °C showed higher peak voltage compared to the result at 25 °C. This proves that temperature enhancement is more dominant compared to microbial limitations. The differences in voltage stability prove that temperature affects biofilm growth dynamics. The internal resistance is listed in Table 4.10. These results confirm previously established MFC research findings, which prove that temperature is a major factor affecting MFC performance [96].

The comparison proves that temperature is a major operational factor affecting MFC performance, and the result depends on a balance between enhanced kinetics and microbial limitations.

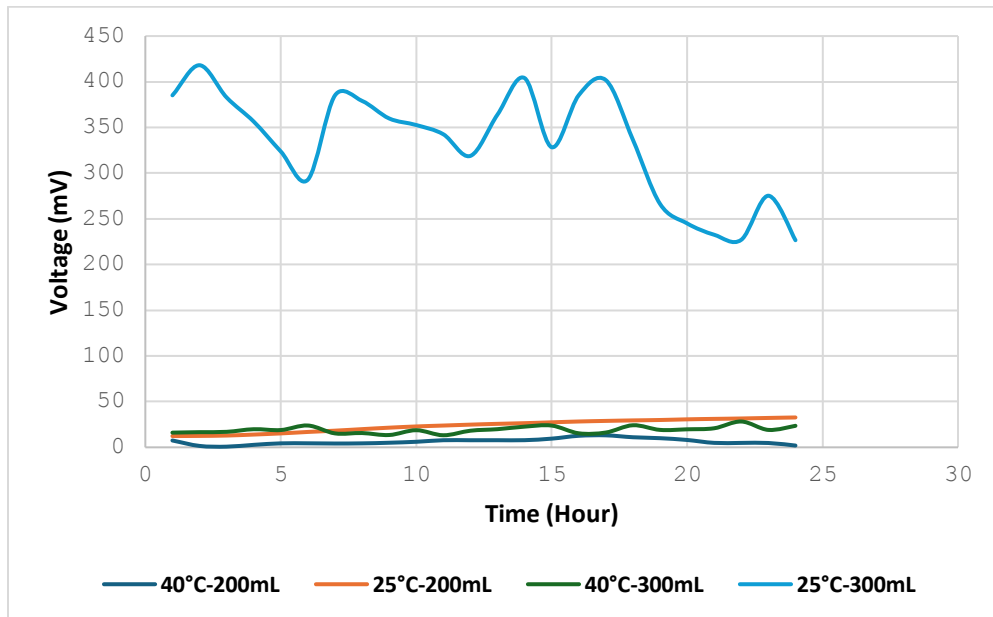
**Table 4.10** Voltage production performance of soil-based MFC at 25 °C and 40 °C with pH 5.

| Condition   | Initial (mV) | Peak (mV) | Peak Time | Minimum (mV) | Final (mV) |
|-------------|--------------|-----------|-----------|--------------|------------|
| 40 °C, pH 5 | 90           | 115       | 4         | 85           | 105        |
| 25 °C, pH 5 | 15           | 35        | 22        | 5            | 20         |

#### 4.5 Effect of Different Water Volume

Soil moisture, connectivity, and conductivity are affected by the varying volume of water in the soil microbial fuel cells, which are key parameters that affect the internal resistance

of the SMFC. From the dataset (see Figure 5.11), the highest voltage was recorded at 25 °C, where the volume of water was 300 mL (yellow), starting at 427 mV at the first hour, fluctuating between 300 mV and 420 mV, and then decreasing to about 232 mV at the 24th hour (minimum of 230 mV at about the 21st hour). All research on SMFCs indicates that increasing the moisture level of the soil to the optimum level increases the voltage/power generated by the SMFC because of the increased conductivity of the electrolyte, which reduces the ohmic losses, although excessive moisture levels may destabilize the SMFC by increasing the oxygen level in the anode area, which may affect the microbial fuel cell performance [90]. At 40 °C, where the volume of the water was also 300 mL (grey), the voltage was significantly reduced, fluctuating between 19 mV initially, increasing to about 30 mV at the 21st hour, and finally stabilizing at 26 mV, where the temperature may affect the microbial community, which may increase or decrease the voltage generated by the SMFC depending on whether the microbial activity is electrogenic and the internal losses are minimized.



**Figure 4.11** Voltage production of a soil-based MFC (100 g soil) operated with different water volumes at 25 °C and 40 °C.

For the 200 mL tests, 25 °C–200 mL (orange) increased gradually from 13 mV to a late peak of ~8 mV (hour 24), whereas 40 °C–200 mL (light blue) was very low (3–14 mV), peaking at 14 mV at hour 16. This implies that (i) higher moisture (300 mL) greatly reduces internal resistance and increases voltage at 25 °C but also causes a gradual decrease

over time, possibly due to transport limitations or redox imbalances, and (ii) for 40 °C, the combination of temperature and lower water content may have caused increased stress on the microbe population and/or increased internal losses, limiting voltage even though reaction rate may be increased [93].

Water volume in a soil MFC is a readily accessible parameter for adjusting the soil moisture content. Moisture affects the thickness and continuity of the aqueous phase in the soil pores, which, in turn, affects ionic conductivity and thus ohmic loss. Low water content limits the transport of protons and ions, thereby increasing the internal resistance and voltage under load. As water content increases, the water in the pores facilitates ionic conductivity, thereby decreasing the internal resistance and increasing the voltage.

This phenomenon is commonly observed in SMFC research designed for soil monitoring and energy harvesting, where the performance increases with moisture content up to a certain optimum range [91]. However, high water content can also impose another set of limiting factors, for example, by facilitating oxygen transfer pathways, especially in SMFCs with exposed electrodes, by increasing the redox potential of the anode, which in turn decreases the potential difference between the anode and cathode, and by changing the microbial consortia; hence, the performance-moisture relationship tends to be non-linear [90].

As observed in the results, the 25°C-300 mL (yellow) treatment significantly outperformed the other conditions with an initial voltage of 427 mV, with multiple peaks above 380-420 mV, then declining after approximately 18-20 hours, reaching 232 mV by day 24 as illustrated in Figure 4.11. This could be due to a high conductivity state with rapid biofilm formation on the anode, causing high voltage, then a decrease in performance due to possible transport limitations and redox gradients, which are commonly observed in SMFCs, especially in the later stages of operation. A possible explanation for the observed decrease in voltage in the later stages of SMFC operation is due to the development of transport limitations and redox gradients within the biofilm, where the increase in biofilm thickness and current production leads to the accumulation of protons within the biofilm, causing a pH gradient in the biofilm and pore water, which in turn increases the internal resistance, causing a decrease in the cell voltage.

In addition, substrate depletion or the production of inhibitory metabolites can also affect the rate of microbial electron transfer. The changing nature of the SMFC output over time has also been commonly observed in the literature on soil MFCs, where the microbial activity and conditions result in a changing power output rather than a constant plateau [92].

A comparison of the 25°C-300 mL and 25°C-200 mL (orange) curves shows the effect of moisture/conductivity. The 25 °C-200 mL curve increased gradually from 13 mV, with a peak in the later stages of 38 mV, without a high voltage phase similar to the 300 mL test. This indicates that, with 200 mL, the soil matrix was still a relatively resistive phase, limiting the voltage even as the biofilm was becoming established. In moisture-dependent MFCs, it has been demonstrated that a low or moderate level of moisture can maintain a high level of resistance, which prevents the cell from utilizing the microbial activity as a useful voltage [85]. Therefore, the large difference in performance between the 25°C-200 mL and 25°C-300 mL curves supports the conclusion that 300 mL of water provided a much more conductive, connected phase.

From the temperature comparisons, it is evident that the voltage was not affected by the increase to 40 °C, even though the electrolyte resistance decreases with temperature and the reaction rate increases. At 40 °C-300 mL (grey), the voltage remained stable at medium levels (19-30 mV), while at 40 °C-200 mL (light blue), the voltage remained at extremely low levels (3-14 mV). There are two possible reasons for this phenomenon. Firstly, the microbial community may have changed with the increase in temperature, as the increase in temperature may favor the growth of different microbial populations, which may have reduced the voltage by diverting the electrons elsewhere, as some microbes, such as methanogens, are known to be capable of this. Reviews and studies on the operation of MFC systems discuss the effect of temperature on the overall performance, as it increases the internal resistance, but also significantly affects the biological activity, which may increase or decrease the performance of the overall MFC system, depending on the conditions [72].

Secondly, the increase in temperature may have resulted in the loss of moisture, as the voltage was extremely low at 40 °C, even though the amount of water was reduced to 200 mL, indicating that the increase to 40 °C may have resulted in the loss of moisture, as

the conductivity at the interface of the soil and the electrodes may have been reduced due to the increase in temperature, resulting in the increase of the rate of evaporation of the moisture present in the soil.

One of the interesting aspects of the results is that the 40°C-300 mL curve (grey) is higher than both of the 200 mL curves for part of the run (peak 30 mV), suggesting that there was still some benefit to the presence of moisture. This supports the general conclusion that improvements in ionic strength and/or transport conditions (through conductivity improvements) can mitigate performance losses to a degree, as was seen in various research projects where increased ionic strength and/or transport conditions increased output by reducing impedance and improving output current [74].

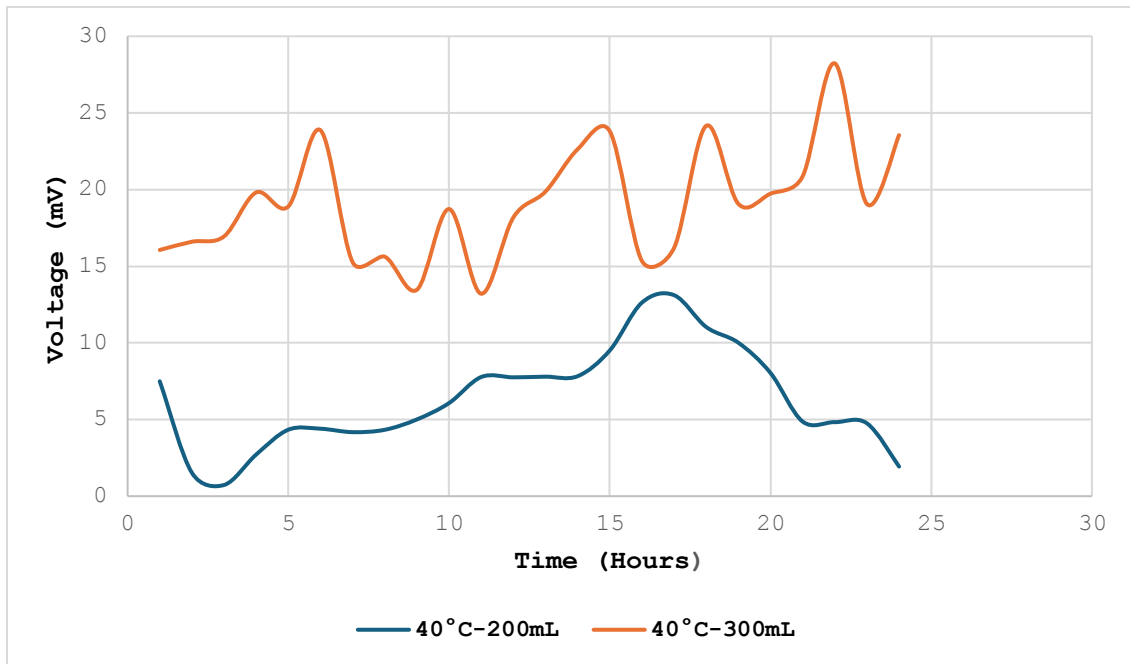
The voltage profiles in Figure 4.11 demonstrate the combined influence of soil moisture content and temperature on system performance and fluctuation behavior. The condition at 25 °C with 300 mL exhibited the highest voltage output, characterized by large and repeated oscillations, indicating enhanced ionic conductivity and active electrochemical processes. In contrast, conditions with lower moisture content (200 mL) showed significantly reduced voltage output and minimal fluctuations due to limited ion transport. At 40 °C, despite increased temperature, voltage outputs remained relatively low, suggesting microbial inhibition and possible moisture loss effects. These results confirm that higher moisture content enhances electrochemical activity, while excessive temperature may negatively impact system stability and performance.

Overall, the results of this experiment suggest that water volume and thus moisture was the biggest factor in determining voltage output for this soil MFC. The 300 mL of water at 25°C produced the maximum output but also demonstrated long-term decline, possibly due to the development of internal losses and/or redox and/or transport changes. This is discussed in greater detail in Table 4.11. The lower output voltages of the 200 mL and/or 40°C conditions were possibly due to internal resistance and/or temperature-related biological changes. This is in keeping with the results of SMFC research papers, which point to (I) high moisture dependency with an optimum window of operation, (II) time-varying output due to microbial activity, and (III) temperature dependency where temperature reduces resistance while simultaneously affecting microbial activity and stability [93].

**Table 4.11** Voltage production performance of soil-based MFC at 25°C and 40°C with different water volumes.

| Condition                        | Initial (mV) | Peak (mV) | Peak Time | Minimum (mV) | Minimum hour | Final (mV) | Trend  |
|----------------------------------|--------------|-----------|-----------|--------------|--------------|------------|--|
| 40 °C, 200 mL water (light blue) | 2.8          | 14.1      | 16        | 1.9          | 2            | 1.9        | Very low; small hump mid-run then declines         |
| 25 °C, 200 mL water (orange)     | 13.1         | 37.5      | 24        | 13.1         | 1            | 37.5       | Gradual increase to late peak                      |
| 40 °C, 300 mL water (grey)       | 18.8         | 30.0      | 21        | 15.0         | 8            | 26.2       | Moderate; mild rise then stable                    |
| 25 °C, 300 mL water (yellow)     | 426.7        | 426.7     | 1         | 230.0        | 21           | 231.7      | Very high; oscillatory then declines after hour 18 |

This shows that soil moisture is a major factor that affects voltage generation, considering its effect on ionic conductivity, internal resistance, and microbe activity within the soil-based microbial fuel cell. In order to compare the effect of water volume within the same temperature conditions, a comparison between 300 mL water and 200 mL water, both at 40°C, is discussed in the following section:



**Figure 4.12** Voltage production comparison between water volumes of 200 mL and 300 mL at 40 °C.

This comparison between 40°C and 300 mL, and 40°C and 200 mL, shows the combined effect of temperature and soil moisture on voltage output in a microbial fuel cell (MFC). When the temperature is 40°C and water volume is 300 mL, the voltage output

ranges from 18.8 mV to a maximum of 30.0 mV, compared with a range from 2.8 mV to a maximum of 14.1 mV when the temperature is 40°C and water volume is 200 mL, as shown in Figure 4.12. Soil moisture affects ionic conductivity and internal resistance, whereas temperature affects the metabolic rate of microbes and electrochemical reaction rate.

Increased levels of moisture also generally help in reducing ohmic resistance, especially due to improved proton transport mechanisms. Insufficient levels of moisture, on the other hand, result in increased levels of internal resistance, which can affect the voltage generated.

The voltage profiles at 40 °C demonstrate that moisture content significantly influences both the magnitude and fluctuation behavior of the system. At 300 mL, the system exhibited higher voltage output with pronounced oscillations, indicating enhanced ionic conductivity and active electrochemical processes. In contrast, at 200 mL, voltage output was significantly lower and characterized by minimal fluctuations, reflecting limited ion transport and reduced microbial activity. These results confirm that increased moisture enhances electrochemical performance but also introduces greater variability in voltage output due to dynamic substrate utilization and electron transfer processes.

As indicated in Table 4.12, differences in peak voltage and stability were also observed, implying that for optimal performance, a balance between ionic transport and microbial activity must be achieved. When increased levels of voltage were observed, improved conductivity, as well as efficient biofilm development, must have played a significant role in the process.

**Table 4.12** Voltage production performance of soil-based MFC at 40°C at different volumes

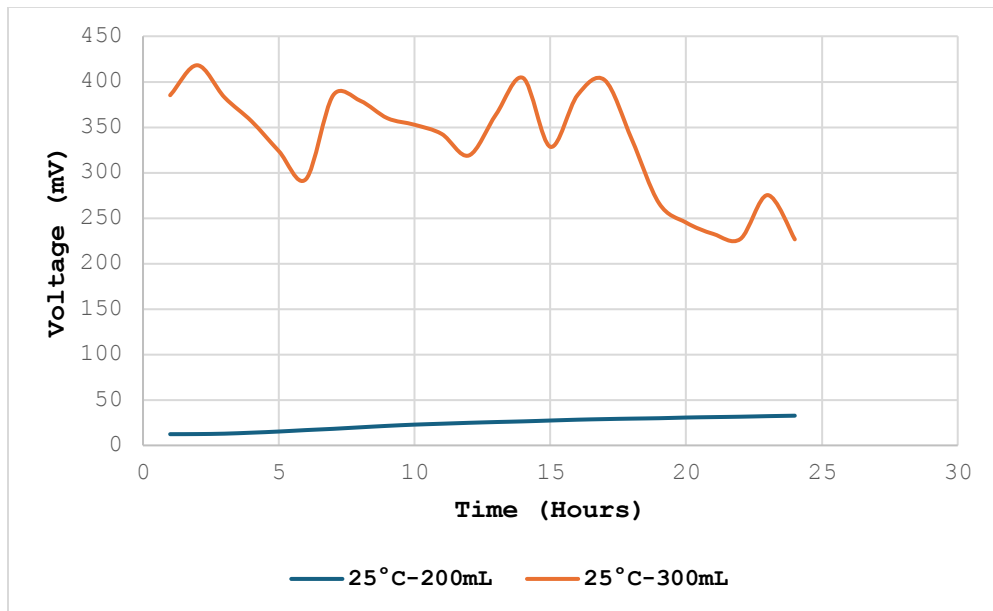
| Condition      | Initial (mV) | Peak (mV) | Peak Time | Minimum (mV) | Final (mV) |
|----------------|--------------|-----------|-----------|--------------|------------|
| 40 °C – 300 mL | 18.8         | 30.0      | 21        | 15.0         | 26.2       |
| 40 °C – 200 mL | 2.8          | 14.1      | 16        | 1.9          | 1.9        |

This study also supports the existing MFC theory on the role of ionic strength, moisture content, and temperature in regulating internal resistance as well as the efficiency of extracellular electron transfer [98]. The results also support the fact that for optimal

performance, a balance between water volume and temperature must be achieved in order to enhance sustainable levels of voltage generation in the MFC process in soils.

This shows that soil moisture is a very important factor that affects voltage generation when the temperature is increased. This is due to the effect of soil moisture on ionic conductivity and internal resistance in the soil-based microbial fuel cell. In order to compare the effect of water volume when the temperature is low, a comparison between 25°C – 200 mL and 25°C – 300 mL is shown in the following figure:

This comparison between 25°C – 300 mL and 25°C – 200 mL is used to analyze the effect of temperature and soil moisture on voltage output in the microbial fuel cell. When the temperature is 25°C – 300 mL, the voltage output ranges from 426.7 mV to a maximum of 426.7 mV, whereas when the temperature is 25°C – 200 mL, the voltage output ranges from 13.1 mV to a maximum of 37.5 mV, as shown in Figure 4.13. Soil moisture has a direct effect on ionic conductivity and internal resistance, whereas temperature affects the metabolic rate of the microbe and electrochemical reaction rate [99].



**Figure 4.13** Voltage production comparison between water volumes of 200 mL and 300 mL at 25 °C

On the other hand, higher moisture tends to decrease ohmic resistance by improving proton transport. Conversely, insufficient moisture tends to increase internal resistance and lower voltage output. Temperature can accelerate reaction rates and lower electrolyte

resistance. Nevertheless, higher temperature may interfere with microbial communities or induce alternative pathways.

The voltage profiles at 25 °C demonstrate that moisture content plays a critical role in determining both voltage magnitude and fluctuation behavior. At 300 mL, the system exhibited high voltage output characterized by large and repeated oscillations, indicating enhanced ionic conductivity and active electrochemical processes. In contrast, at 200 mL, voltage output was significantly lower and showed a gradual increase with minimal fluctuations, reflecting limited ion transport and reduced microbial activity. These results confirm that higher moisture levels enhance system performance but also introduce greater variability due to dynamic substrate utilization and electron transfer processes.

The differences in peak voltage and stability imply that the best performance is achieved by finding a balance between ionic transport and microbial activities. The higher voltage was achieved through improved conductivity and efficient biofilm formation, as shown in Table 4.13. Conversely, lower voltage was achieved through limitations of moisture and temperature-related microbial stress.

The results obtained are consistent with the theoretical framework of MFCs regarding the role of ionic strength, moisture content, and temperature in controlling internal resistance and improving the efficiency of extracellular electron transfer [70]. The results obtained confirm the importance of optimizing both moisture and temperature to achieve the best performance of voltage generation.

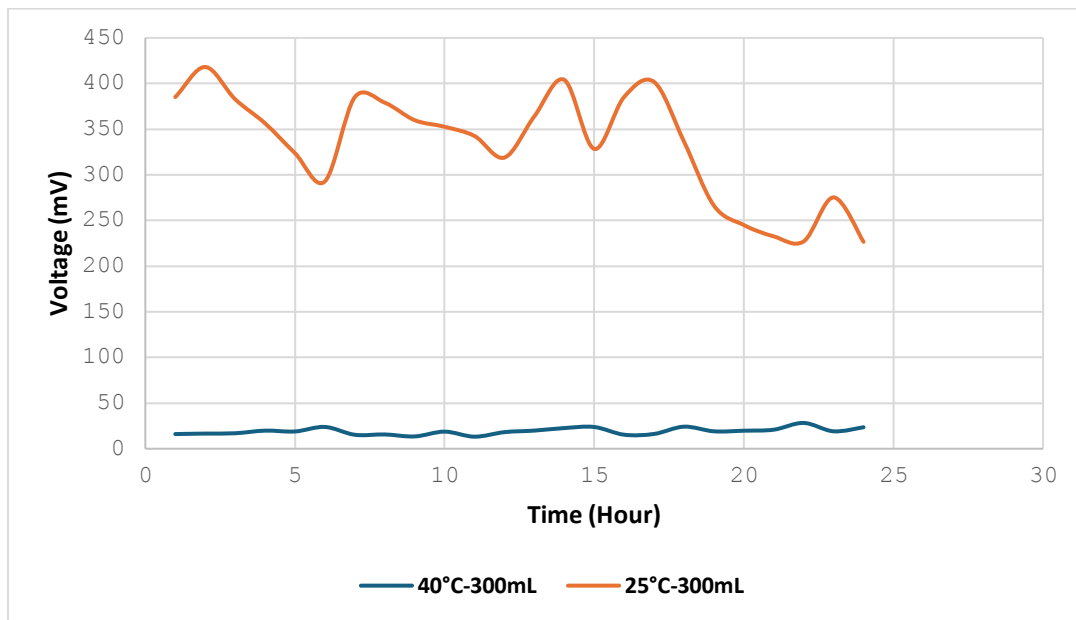
**Table 4.13** Voltage production performance of soil-based MFC at 25°C at different water volumes

| Condition      | Initial (mV) | Peak (mV) | Peak Time | Minimum (mV) | Final (mV) |
|----------------|--------------|-----------|-----------|--------------|------------|
| 25 °C – 300 mL | 426.7        | 426.7     | 1         | 230.0        | 231.7      |
| 25 °C – 200 mL | 13.1         | 37.5      | 24        | 13.1         | 37.5       |

This result shows that soil moisture plays a crucial role in the generation of voltage at the same temperature by controlling the ionic conductivity and internal resistance of the soil-based microbial fuel cell. To further assess the combined effect of temperature at the same soil moisture, a comparative result of 40 °C and 25 °C at the same soil moisture of 300 mL water is shown below:

This comparison of 40 °C and 300 mL, and 25 °C and 300 mL illustrates the combined effect of temperature and soil moisture on the voltage output of the microbial fuel cell. The range of voltage at 40 °C and 300 mL was from approximately 18.8 mV to a maximum of 30.0 mV, while the range of voltage at 25 °C and 300 mL was from approximately 426.7 mV to a maximum of 426.7 mV, as shown in Figure 4.14. Soil moisture directly affects the ionic conductivity and internal resistance of the soil-based microbial fuel cell, while temperature affects the microbial metabolic rate and electrochemical reaction kinetics [79].

Higher moisture content typically results in an increased ohmic resistance due to improved proton transfer. However, insufficient moisture results in increased internal resistance and consequently limits the output voltage. Temperature may improve the rate of biochemical reactions and reduce electrolyte resistance. However, high temperatures may disrupt microbial activity and result in alternative metabolic pathways [82].



**Figure 4.14** Voltage production comparison between 25 and 40 °C with water volume of 300 mL

The results for peak voltage and stability indicate that for maximum efficiency, both ionic and microbial activity need to be optimized. This is presented in Table 4.14. Improved voltage was seen in one of the experiments. Improved conductivity and efficient biofilm development were probably the main contributing factors. Lower voltages were seen in

both experiments. This was probably due to insufficient moisture content and temperature-related microbial stress.

The voltage profiles at 300 mL demonstrate that temperature strongly influences both the magnitude and fluctuation behavior of the system. At 25 °C, the system exhibited high voltage output characterized by large and cyclic oscillations, indicating enhanced ionic conductivity and active electrochemical processes. In contrast, at 40 °C, voltage output was significantly lower and showed only minor fluctuations, reflecting suppressed microbial activity and reduced electrochemical performance. These results suggest that while high moisture content enhances system conductivity, elevated temperature may negatively impact microbial stability and overall system efficiency.

The results agree with current theory on MFCs and how ionic strength, moisture content, and temperature interact to control internal resistance and efficiency of EET. It is therefore evident that optimization of both water volume and temperature is of utmost importance for maximum sustainable voltage output in soil-based MFCs.

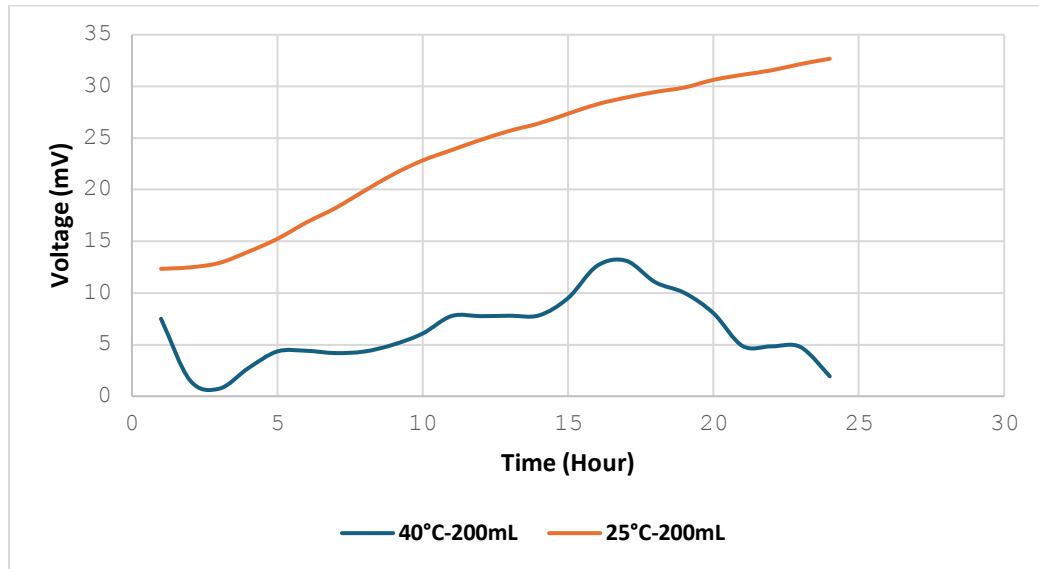
**Table 4.14** Voltage production performance of soil-based MFC at 25°C and 40°C using water volume of 300 mL

| Condition      | Initial (mV) | Peak (mV) | Peak Time | Minimum (mV) | Final (mV) |
|----------------|--------------|-----------|-----------|--------------|------------|
| 40 °C – 300 mL | 18.8         | 30.0      | 21        | 15.0         | 26.2       |
| 25 °C – 300 mL | 426.7        | 426.7     | 1         | 230.0        | 231.7      |

This is an indicator that temperature has a substantial impact on voltage generation even when the conditions of moisture are kept constant, as shown in the results obtained from the soil-based microbial fuel cell. A comparison of the results obtained when the temperature is 40°C and 200 mL, and when the temperature is 25°C and 200 mL, is shown in the following section, which aims to provide a better understanding of the effect of temperature on the voltage output of the microbial fuel cell (MFC) when the conditions of soil moisture are low:

This comparison of the voltage output when the temperature is 40°C and 200 mL, and when the temperature is 25°C and 200 mL, indicates that temperature has a substantial impact on the voltage output of the microbial fuel cell (MFC), as shown by the range of voltage, which is between 2.8 mV and a maximum of 14.1 mV when the temperature is

40°C and 200 mL, and between 13.1 mV and a maximum of 37.5 mV when the temperature is 25°C and 200 mL, as shown in Figure 4.15.



**Figure 4.15** Voltage production comparison between 25 and 40 °C using water volume of 200 mL

Soil moisture content directly influences ionic conductivity and internal resistance, while temperature regulates the metabolic activity of microbes and the electrochemical reaction rate. Increased moisture content would likely decrease ohmic resistance by facilitating the proton transport path. Conversely, low moisture content would increase the internal resistance and subsequently limit the output voltage. Temperature can increase the biochemical reaction rate and decrease the electrolyte resistance; however, high temperatures may impair the microbial community and/or increase the competing pathways.

The voltage profiles at 200 mL demonstrate that temperature significantly affects fluctuation behavior under limited moisture conditions. At 40 °C, the system exhibited noticeable fluctuations with a peak followed by a gradual decline, indicating unstable microbial activity and limited electrochemical performance due to combined thermal stress and reduced ionic conductivity. In contrast, at 25 °C, the voltage increased steadily with minimal fluctuations, reflecting stable microbial metabolism and consistent electron transfer. These results suggest that under low moisture conditions, lower temperature favors stability, whereas higher temperature leads to increased variability and reduced performance.

Differences observed for peak voltage and stability indicate optimal performance when ionic and microbial activity are balanced, as indicated by the optimal performance zones shown in Table 4.15. When increased voltage was observed, increased conductivity and efficient biofilm formation likely played a key role. When decreased voltage was observed, the performance may have been limited by moisture content and/or temperature stress on the microbial community.

**Table 4.15** Voltage production performance of soil-based MFC at 25°C and 40°C using water volume of 200 mL

| Condition      | Initial (mV) | Peak (mV) | Peak Time | Minimum (mV) | Final (mV) |
|----------------|--------------|-----------|-----------|--------------|------------|
| 40 °C – 200 mL | 2.8          | 14.1      | 16        | 1.9          | 1.9        |
| 25 °C – 200 mL | 13.1         | 37.5      | 24        | 13.1         | 37.5       |

These results are consistent with the theory for MFC performance, where ionic strength, moisture content, and temperature work collectively to regulate the internal resistance and efficiency of EET [98].

These findings verify the importance of optimizing water volume and temperature for maximum sustainable voltage output from the soil-based MFC.

#### 4.6 Comparison of Glucose and NaCl Concentration

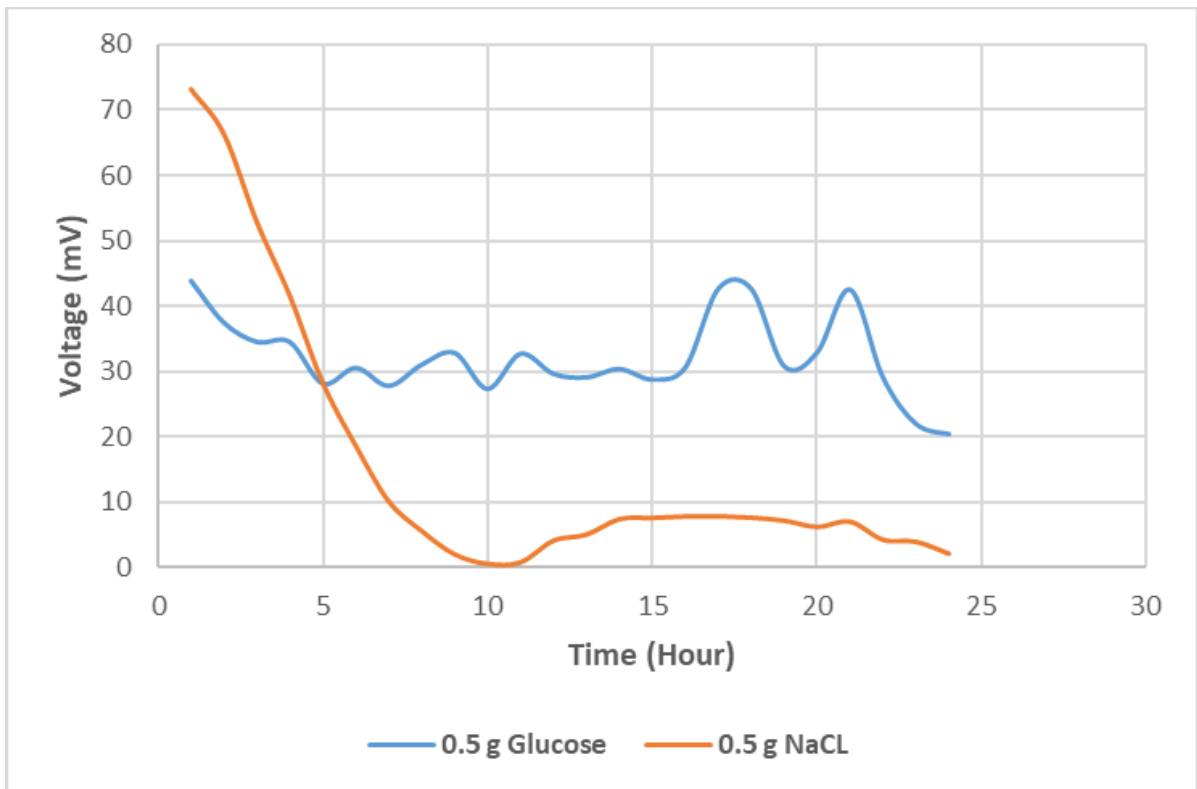
The effect of substrate addition (0.5 g glucose) and electrolyte enhancement (0.5 g NaCl) on voltage generation was investigated in a soil-based microbial fuel cell at 25°C using 100 g of soil and 100 mL of water for 24 hours, as shown in Figure 4.16.

In the glucose-added system (blue curve), the voltage initially started at around 43-45 mV for the first hour and gradually decreased to 28-30 mV by the 5th hour. This may be attributed to the microbial acclimation and biofilm formation on the anode's surface. As cited by Logan et al. [70], the startup procedure for an MFC often involves a temporary change in the voltage output as the electroactive microbes adapt to the electrode and start the process of electron transfer. After this adaptation period (5-15 hours), the voltage remained constant at around 29-32 mV.

At around 16-21 hours, the voltage increased significantly to a maximum of 44-46 mV at the 17th hour and 42-43 mV at the 20th hour, as shown in Figure 4.16. This may be attributed to the improvement in biofilm maturation and the direct transfer of electrons

through conductive pili and cytochromes, as cited by Lovley [94]. The availability of glucose as a readily biodegradable substrate enhances the microbial respiration and electron transfer to the anode. At around 21 hours, the voltage started to decline to 20 mV by the 24th hour.

In contrast, the performance of the NaCl-amended system (orange curve) showed different behavior. The voltage was significantly higher at the beginning, between 72 and 75 mV, during the first hour of operation, probably because of the improved conductivity and reduced internal resistance of the electrolyte solution. It is known that the concentration of the electrolyte reduces ohmic losses in the MFC, thereby increasing the voltage temporarily [76]. The voltage, however, dropped significantly between the second and sixth hours, reaching as low as 10 mV, almost approaching zero at the tenth hour of operation. Excessive salinity has been known to affect microbial performance by causing osmotic stress, thereby inhibiting microbial metabolism and affecting enzymatic activities, as mentioned in the literature [99].



**Figure 4.16** Voltage production in 24 hours for soil-based MFC using 0.5 g glucose and 0.5 g NaCl concentrations at 25°C

After the twelfth hour, the performance of the NaCl-amended MFC showed some improvement, as the voltage remained between 6 and 8 mV between the fourteenth and twentieth hours, after which the voltage dropped to almost 2 to 3 mV at the twenty-fourth hour of operation.

It can be noted that although the NaCl-amended MFC showed improved performance in terms of the voltage peaks, the performance was not sustainable, as the glucose-amended MFC showed better performance during the entire period of operation, except during the first hour.

It can be noted that the NaCl-amended MFC showed improved performance during the first hour, as the voltage was significantly higher at the beginning, at about 75 mV, whereas the glucose-amended MFC showed only about 45 mV of voltage during the first hour of operation. The glucose-amended MFC showed relatively stable voltage between 28 and 32 mV during most of the period, whereas the NaCl-amended MFC showed significantly higher peaks of up to 45 mV, as mentioned in Table 4.16.

It can be noted that the performance of the glucose-amended MFC was significantly better, as the voltage was relatively stable, whereas the performance of the NaCl-amended MFC was not sustainable, thereby indicating that the availability of the substrate plays a more important role in the production of bioelectricity than the enhancement of the electrolyte solution, as the voltage generated in the MFC depends on the microbial metabolism, as mentioned in the established theory of the MFC, where the voltage depends on the microbial metabolism, electron transfer, and internal resistance balance in the MFC [82].

The voltage profiles comparing 0.5 g glucose and 0.5 g NaCl demonstrate distinct fluctuation behaviors associated with different system mechanisms. The glucose condition exhibited moderate and sustained oscillations, reflecting active microbial metabolism and continuous extracellular electron transfer. In contrast, the NaCl condition showed a high initial voltage followed by a rapid decline to near-zero levels, indicating that while ionic conductivity was initially enhanced, the absence of an organic substrate and possible osmotic stress led to system instability and suppressed performance. These results confirm that sustained voltage generation in soil-based MFCs is primarily driven by microbial activity rather than electrolyte concentration alone.

The results obtained from these experiments reveal that an increase in organic substrate concentration results in a stable and sustainable source of voltage compared to an increase in electrolyte concentration. To further understand how an increase in substrate and electrolyte concentration affects bioelectricity production in an MFC, a comparison of 1 g glucose and 1 g NaCl is presented in the next section:

**Table 4.16** Voltage production performance comparison at 25°C using 0.5 g glucose and 0.5 g NaCl.

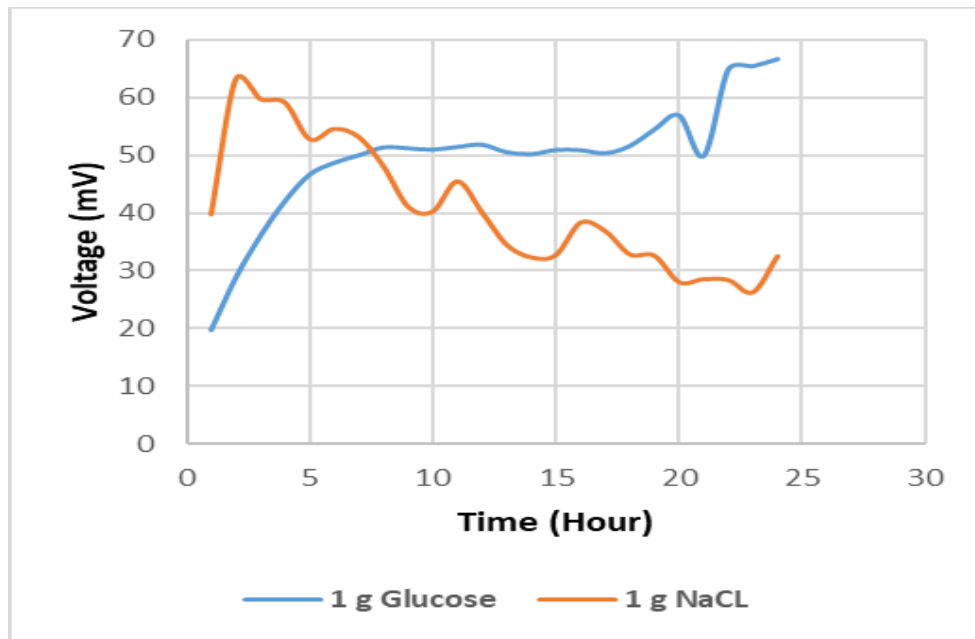
| Time (Hour) | 0.5 g Glucose (mV) | 0.5 g NaCl (mV) |
|-------------|--------------------|-----------------|
| 1           | 44                 | 74              |
| 5           | 30                 | 28              |
| 10          | 29                 | ~0              |
| 17          | 45                 | 7               |
| 24          | 20                 | 3               |

This experiment was conducted to observe and analyze the effect of an increased concentration of substrate (1.0 g glucose) and electrolyte (1.0 g NaCl) on voltage production in a soil-based microbial fuel cell operated under a temperature of 25°C with 100g of soil and 100mL of water for a period of 24 hours. Voltage produced in an MFC is a result of microbial activity and electron transfer and internal resistance. As shown in the results of this experiment (blue curve), when 1.0 g glucose was used in the experiment, the voltage produced increased gradually from 20 mV to almost 45-48 mV by hour 5, as shown in Figure 4.17.

This is due to the startup phase of an MFC, where microbes start to colonize on the anode surface and start to transfer electrons. Between hours 6 and 18, the voltage produced was constant and stable between 49-52 mV. This indicates that microbes were able to oxidize glucose in a steady state. By hour 21, there was a sudden drop in voltage to almost 49-50 mV. However, there was a sudden increase in voltage to almost 65-67 mV by hour 24. This sudden increase in voltage by microbes in the later stages of the experiment may be due to an increase in direct electron transfer via outer membrane cytochromes and conductive pili. This increased concentration of glucose was enough to produce and enhance bioelectricity. On the other hand, the performance of the 1.0 g NaCl system (represented by the orange curve) depicted a different trend. The voltage was initially at

approximately 40 mV and then sharply rose to approximately 63/65 mV at hours 2 and 3, respectively.

This could be due to improved ionic conductivity and reduced ohmic resistance of the electrolyte, as a higher salt concentration improves ion mobility [85]. However, the voltage gradually decreased over time, dropping to approximately 40 mV at hour 10 and continuing to decrease to approximately 27/32 mV at hours 18 and 22, respectively. The decrease could be due to osmotic stress and microbial inhibition caused by a high salt concentration. Excessive salt concentration can impair membrane structure, enzyme activity, and proton exchange efficiency, thus affecting electron generation [77]. Although minor fluctuations were noted, the performance of the MFC was not improved. Comparing the two MFC systems, it was noted that the MFC using NaCl as a substrate amendment initially recorded a higher peak voltage of approximately 65 mV at the early hours compared to the glucose-fed MFC. However, the glucose-fed MFC recorded a higher voltage at the end of the experiment and was noted to have recorded the highest voltage of approximately 67 mV at hour 24, as shown in Table 4.17.



**Figure 4.17** Voltage production in 24 hours for soil-based MFC with 1 g glucose and 1 g NaCl at 25°C.

This could be due to improved ionic conductivity and reduced ohmic resistance of the electrolyte, as a higher salt concentration improves ion mobility [85]. However, the

voltage gradually decreased over time, dropping to approximately 40 mV at hour 10 and continuing to decrease to approximately 27/32 mV at hours 18 and 22, respectively. The decrease could be due to osmotic stress and microbial inhibition caused by a high salt concentration. Excessive salt concentration can impair membrane structure, enzyme activity, and proton exchange efficiency, thus affecting electron generation [77]. Although minor fluctuations were noted, the performance of the MFC was not improved. Comparing the two MFC systems, it was noted that the MFC using NaCl as a substrate amendment initially recorded a higher peak voltage of approximately 65 mV at the early hours compared to the glucose-fed MFC. However, the glucose-fed MFC recorded a higher voltage at the end of the experiment and was noted to have recorded the highest voltage of approximately 67 mV at hour 24, as shown in Table 4.17.

The voltage profiles comparing 1 g glucose and 1 g NaCl exhibit distinct fluctuation patterns associated with their respective roles in the system. The glucose condition showed a gradual increase in voltage with minimal fluctuations, indicating stable microbial activity and efficient extracellular electron transfer. In contrast, the NaCl condition exhibited an initial high voltage followed by a progressive decline with irregular oscillations, reflecting the lack of sustained microbial metabolism and possible osmotic stress effects. These findings confirm that while electrolyte addition enhances initial conductivity, long-term voltage generation is primarily governed by substrate-driven microbial processes.

**Table 4.17** Voltage production comparison at 25°C using 1 g glucose and 1 g NaCl

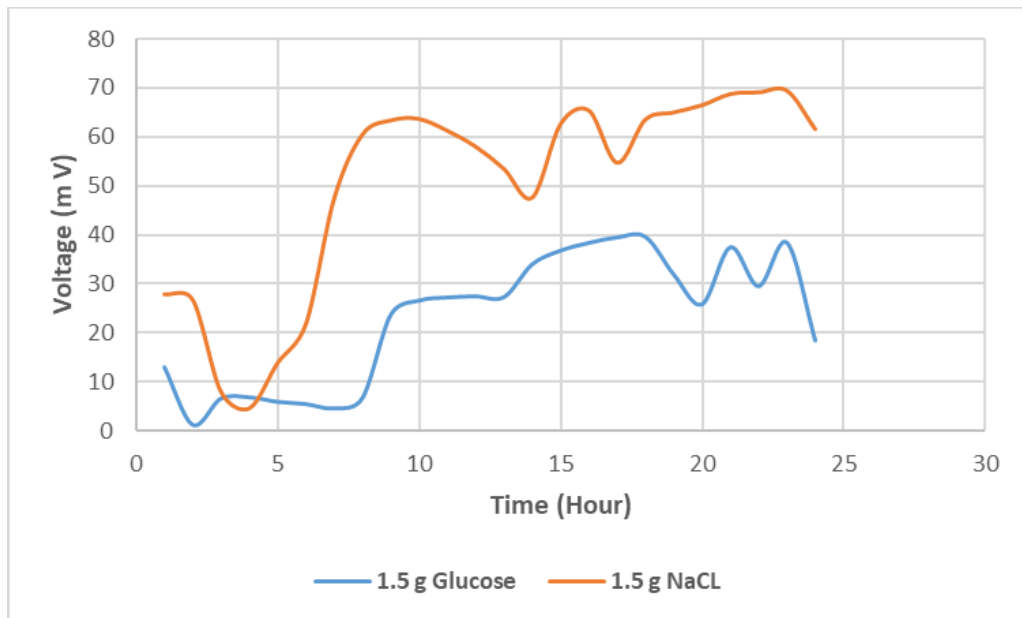
| Time (Hour) | 1.0 g Glucose (mV) | 1.0 g NaCl (mV) |
|-------------|--------------------|-----------------|
| 1           | 20                 | 40              |
| 3           | 35                 | 65              |
| 10          | 50                 | 40              |
| 20          | 56                 | 28              |
| 24          | 67                 | 32              |

This indicates that substrate concentration is the major factor affecting the sustainability of bioelectricity generation, while electrolyte concentration affects the short-term performance of the MFC. The findings of this study are consistent with MFC theory, which states that the voltage generated by an MFC depends on the availability of electron donors, the efficiency of microbial respiration, and the internal resistance of the MFC [95]. The findings of this study show that increasing glucose concentration improves

bioelectrochemical performance, while increasing NaCl concentration improves the short-term performance of the MFC.

As can be seen from these results, a significant increase in the concentrations of the substrate and the electrolyte can have a substantial impact on the generation of the voltage in the microbial fuel cell, as measured in the soil-based microbial fuel cell experiment. In order to further analyze the impact of increased levels of these additives on the generation of bioelectricity in the MFC, a comparison of 1.5 g glucose and 1.5 g NaCl will be made in the next section:

This experiment was designed to evaluate the impact of increased levels of an organic substrate (1.5 g glucose) and an electrolyte (1.5 g NaCl) on the generation of a voltage in a soil-based microbial fuel cell operated at 25 degrees Celsius with 100 g of soil and 100 mL of water for 24 hours. The results of the experiment are presented in the form of a voltage profile, which illustrates the complex inter-relationship between microbial metabolic activity, biofilm development, substrate availability, and ionic conductivity in the MFC.



**Figure 4.18** Voltage production in 24 hours for soil-based MFC with 1.5 g glucose and 1.5 g NaCl at 25°C.

As can be seen in Figure 4.18, in the 1.5 g glucose-based MFC, the voltage starts off at 12-13 mV at hour 1, then drops significantly to 1-2 mV by hour 2. This could be due to an adaptation phase, where microbes adapt to the increased concentration of glucose in the

MFC. According to [89], during the start-up of a MFC, a transient drop in voltage can be observed as microbes adapt to the conditions in the MFC, which can be due to a change in the microbial community composition and an increase in the internal resistance of the MFC. Between hours 3 and 7, the voltage in the 1.5 g glucose-based MFC remained at a low level, around 4-6 mV, indicating that a biofilm was still in the process of development.

This increase was substantial between hours 8 and 10, where the voltage increased from 6 mV to a range of 25–27 mV. This increase indicates that a functional electroactive biofilm was formed, and extracellular electron transfer was enhanced as glucose oxidation increased. Between hours 10 and 14, the voltage remained stable between 26–28 mV, indicating a steady state. Then, between hours 14 and 18, the voltage increased again, reaching a maximum of 39–40 mV at hour 18. This increase is attributed to the maturation of the biofilm, which enhanced direct electron transfer through conductive pili and outer membrane cytochromes [79]. After this, the voltage of the glucose system decreased until it reached 25–27 mV again at hour 20, followed by oscillations reaching peaks of 36–38 mV between hours 21 and 23, and then decreased again until it reached 18–20 mV by hour 24. The voltage of the 1.5 g NaCl system (orange curve) was higher compared to that of the glucose system. The voltage started between 27–28 mV, decreased until it reached 6–8 mV by hour 3, and then increased sharply after hour 5. Between hours 6 and 9, the voltage increased dramatically from 20 mV to a range of 62–65 mV, indicating a strong reduction of ohmic losses due to increased ionic strength. A higher electrolyte concentration enhances charge transfer and minimizes internal resistance. This could lead to a dramatic increase in voltage output. In the early and middle stages of operation, this effect could be critical [97].

In the period between hours 9 and 12, the voltage of the NaCl system was high at approximately 63-65 mV. The voltage then decreased to 48-50 mV at approximately hour 14. The voltage then improved to 65-67 mV at approximately hour 16. The voltage then improved gradually from hour 18, reaching a peak of 68-70 mV at hours 21 and 23. The voltage then decreased to 62 mV at hour 24. Although high salinity enhances conductivity, it could also impede microbial metabolism as a result of osmotic stress [74]. The persistence of high voltage suggests a certain adaptation of the microorganisms. The benefits of high ionic strength on conductivity outweighed its inhibitory effects. Comparing the two MFC systems, the MFC operating on the NaCl-amended substrate

recorded a higher voltage compared to the glucose-amended MFC. The MFC operating on the NaCl-amended substrate recorded a peak voltage of 70 mV compared to a peak of 40 mV recorded by the glucose-amended MFC, as shown in Table 4.18. The MFC operating on the glucose-amended substrate recorded higher voltage as the substrate was depleted. The MFC operating on the NaCl-amended substrate recorded higher voltage but dipped at certain stages of operation. The results show that MFC performance is a delicate balance of substrate concentration and electrolyte conditions.

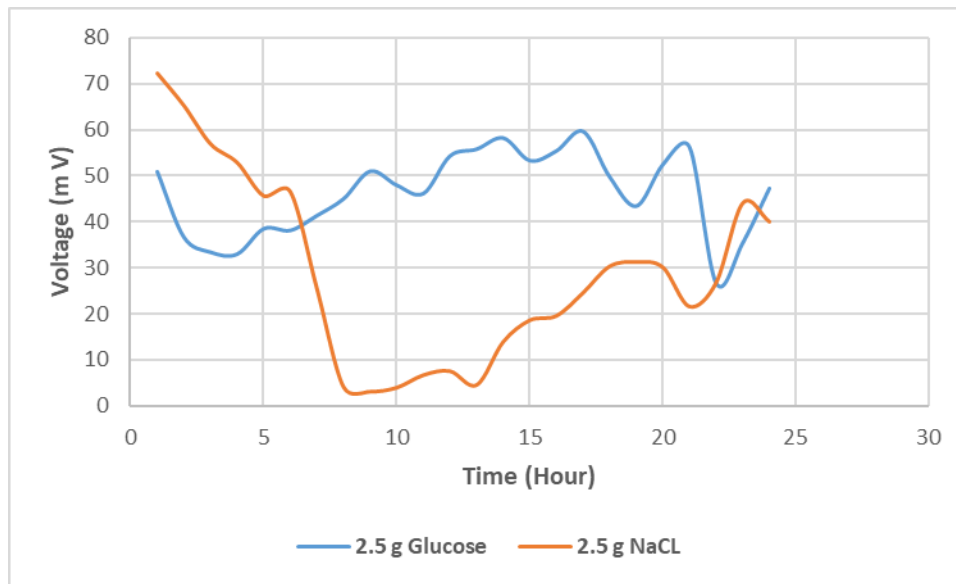
The voltage profiles comparing 1.5 g glucose and 1.5 g NaCl demonstrate distinct fluctuation behaviors associated with their respective roles in the system. The glucose condition exhibited delayed activation with moderate fluctuations, indicating initial substrate inhibition followed by gradual microbial adaptation. In contrast, the NaCl condition showed significantly higher voltage output with structured oscillations, reflecting enhanced ionic conductivity and efficient electrochemical processes. These results suggest that at higher concentrations, electrolyte addition can dominate system performance by improving charge transport, whereas excessive substrate loading may initially suppress microbial activity before partial recovery.

**Table 4.18** Voltage production comparison at 25°C using 1.5 g glucose and 1.5 g NaCl

| Time (Hour) | 1.5 g Glucose (mV) | 1.5 g NaCl (mV) |
|-------------|--------------------|-----------------|
| 1           | 13                 | 28              |
| 3           | 5                  | 7               |
| 9           | 26                 | 64              |
| 14          | 28                 | 49              |
| 18          | 40                 | 66              |
| 23          | 37                 | 70              |

These results show that the increase in substrate and electrolyte concentrations can significantly affect the generation of voltage through the metabolic activity of microbes, biofilm formation, and ionic conductivity in the soil-based microbial fuel cell. In order to further understand the impact of the increased additive concentrations on the production of bioelectricity, a comparison between 2.5 g glucose and 2.5 g NaCl is shown in the following section (Figure 4.19). This experiment was designed to evaluate the impact of high substrate concentration (2.5 g glucose) and high electrolyte concentration (2.5 g NaCl) on the production of voltage in a soil-based microbial fuel cell (MFC) under 25°C using 100 g soil and 100 mL water for 24 hours.

For the 2.5 g glucose system (blue curve), the voltage started at an initial value of about 50 mV for the first hour, after which the voltage decreased to a range of 32-35 mV for hours 2-4, as shown in Figure 4.19. This initial voltage drop could be due to the adaptation of the microbe and the temporary imbalance between the substrate concentration and the capability of the microbe for oxidation. Excessive substrate loading, as reported by [98], was found to increase internal resistance due to a quick buildup of protons near the anode. Between hours 5 and 10, the voltage for the glucose system increased gradually from 38 mV to 50-52 mV, which could mean that the microbe has adapted well to the increased concentration of glucose and has established a good pathway for electron transfer.



**Figure 4.19** Voltage production in 24 hours for soil-based MFC with 2.5 g glucose and 2.5 g NaCl at 25°C.

Between hours 11 and 17, the voltage for the glucose system remained at a high range of 55-60 mV, with a maximum of 59-60 mV at hour 17. This could mean that the glucose was being oxidized very well, and the extracellular electron transfer was also very good, as explained in the biofilm formation by [94]. However, the voltage decreased sharply to a range of 43-45 mV at hour 19, and even lower, ranging from 26 to 28 mV, at around hour 22. This could be due to substrate depletion, metabolic by-products, and pH effects on proton transport. The voltage increased again to 47 mV at around hour 24.

On the contrary, the 2.5 g NaCl system (orange curve) started with a substantially higher reading (72 mV at hour 1) due to lower ohmic resistance resulting from a higher concentration of ions. Nevertheless, a sharp decline in reading was observed between hours 3 and 8, where the reading dropped substantially to between 2 mV and 4 mV. Such a sharp decline in reading may imply that there was a high degree of osmotic stress inhibiting microbial activity [76].

The voltage profiles for 2.5 g glucose and 2.5 g NaCl exhibit pronounced fluctuations, reflecting different system dynamics. The glucose condition showed sustained voltage output with significant oscillations, indicating active but unstable microbial metabolism and continuous substrate utilization. In contrast, the NaCl condition exhibited a high initial voltage followed by a rapid collapse and delayed recovery, reflecting the absence of sustained microbial activity and the effects of osmotic stress. These results demonstrate that while both conditions produce fluctuating behavior, only the substrate-driven system maintains long-term electrochemical performance.

After hour 9, the NaCl system started to show a gradual recovery pattern. The reading increased from 4 mV to around 20 mV at hour 15, then rose further to between 30 mV and 32 mV at around hour 18-20 (Table 4.19). The reading dropped temporarily to around 22 mV at around hour 21 before rising again to between 40 mV and 45 mV at hour 24. It is noteworthy that while the NaCl system recorded the highest reading at the beginning (72 mV), its reading was also the most unstable throughout the experiment period, with a sharp decline in reading during the mid-experiment period, as indicated in Table 4.19

**Table 4.19** Voltage production comparison at 25°C using 0.5 g glucose and 0.5 g NaCl

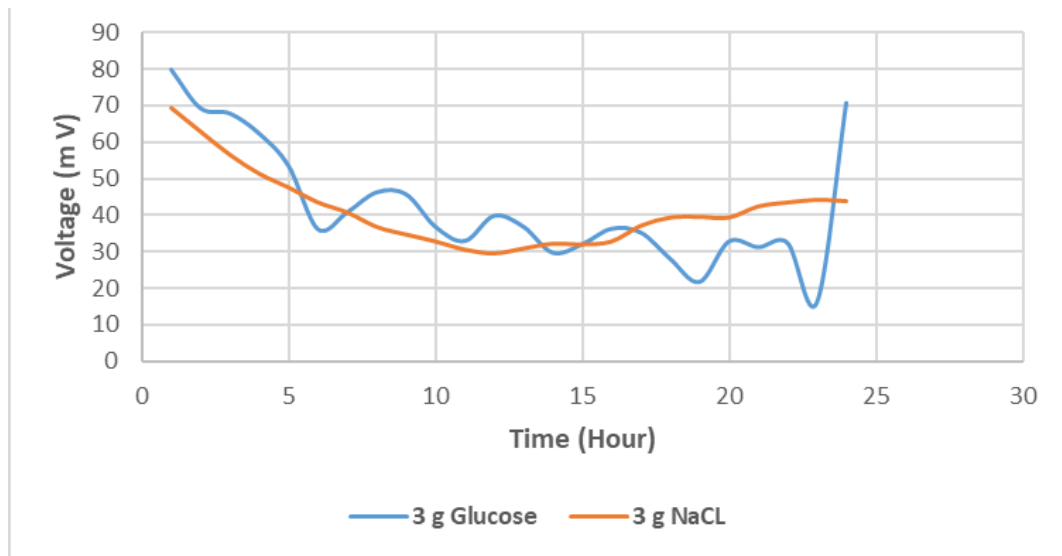
| Time (Hour) | 2.5 g Glucose (mV) | 2.5 g NaCl (mV) |
|-------------|--------------------|-----------------|
| 1           | 50                 | 72              |
| 4           | 33                 | 45              |
| 8           | 50                 | 3               |
| 15          | 57                 | 20              |
| 20          | 45                 | 31              |
| 24          | 47                 | 42              |

The glucose system also indicated a higher and more stable voltage production, reaching a peak of 60 mV, with higher average voltage production over a longer period of time. These results support the theory that the availability of a substrate controls the

production of sustainable electrons, while the concentration of an electrolyte primarily controls the inner resistance, which could also introduce inhibitory effects in high concentrations [82].

The results also support the established theory of a microbial fuel cell, which states that for a microbial fuel cell to operate at optimal levels, the concentration of a substrate must be sufficient for sustaining microbial life, while also avoiding high ionic stress, which could impede bioelectrochemical activity.

As indicated in the results, the experiment proves that high concentrations of substrates and electrolytes significantly affect the production of voltage in a microbial fuel cell by changing the metabolic rate of microbes, the rate of substrate consumption, and ionic conductivity in the soil-based microbial fuel cell. In order to further test the hypothesis that high levels of added substances in a microbial fuel cell could affect the production of bioelectricity, a comparative analysis of 3 g glucose and 3 g NaCl will be conducted in the next section (Figure 4.20).



**Figure 4.20** Voltage production in 24 hours for soil-based MFC with 3 g glucose and 3 g NaCl at 25°C.

This experiment was conducted in order to analyze the effect of high levels of a substrate (3.0 g glucose) and high levels of an electrolyte (3.0 g NaCl) on the production of voltage in a soil-based microbial fuel cell (MFC) operated at 25 degrees Celsius, with 100 g of soil and 100 mL water for 24 hours. The results show the combined effect of the availability of a substrate, high ionic concentration, microbes adapting to the high levels of

glucose, and inner resistance on the production of bioelectricity in the MFC. In the case of the 3.0 g glucose MFC, the results indicated a declining rate from 68-69 mV in hour 1, reaching 55 mV in hour 5, which could be due to high levels of glucose in the MFC, causing microbes to produce extra electrons, which could increase inner resistance in the MFC [95]. Between hour 6 and 10, the MFC produced a fluctuating voltage between 35-47 mV, which could be due to microbes adapting to the high levels of glucose in the MFC.

Between hours 11 and 17, the voltage varied between 30 and 40 mV. This could be an indication of a quasi-steady metabolic state. The most notable drop was recorded between hours 19 and 20, where the voltage was as low as nearly 22 mV. This could be a result of a pH gradient, substrate overload, or the accumulation of metabolic intermediates inhibiting electron transfer. [77] indicated that high organic loading could result in a decrease in the efficiency of the electroactive material if proton transport was a limiting factor.

A sudden drop was recorded at hour 23, where the voltage was as low as nearly 15 mV. This was followed by a sudden rise to as high as approximately 70 mV at hour 24 (maximum recorded voltage of 79.86 mV as indicated by the legend of the graph) as shown in Figure 4.20.

On the other hand, the 3.0 g NaCl (orange curve) started at approximately 62 and 63 mV, gradually decreasing to approximately 45 mV by hour 6. The sudden decrease could be as a result of stress caused by the high salinity. High salinity could result in a decrease in ohmic resistance but could also result in osmotic pressure on the cells of the microorganisms. [70] indicated that high ionic strength could result in osmotic pressure on the cells of microorganisms.

During hours 7-12, the voltage continued to fall further to a minimum range of 30-32 mV. Nevertheless, during hours 13-18, the voltage started to rise gradually to a range of 38-40 mV before continuing to rise to a range of approximately 43-44 mV during hours 23-24 (final reading of 69.21 mV indicated in the legend likely representing cumulative or peak reading). This indicates that there was some degree of adaptation to the saline environment.

The voltage profiles for 3 g glucose and 3 g NaCl demonstrate pronounced differences in fluctuation behavior at excessive loading conditions. The glucose system

exhibited highly irregular and large fluctuations, including a sharp decline followed by intermittent oscillations and a final voltage spike, indicating metabolic instability and substrate inhibition. In contrast, the NaCl system showed a smoother and gradually declining trend with minor fluctuations, reflecting conductivity-driven behavior without sustained microbial activity. These findings confirm that excessive substrate loading negatively impacts system stability, while electrolyte addition alone cannot support long-term voltage generation.

From Table 4.20 above, it is evident that the 3.0g Glucose system was more volatile with sharp peaks during the experiment. The NaCl system also indicated a gradual pattern with lower mid-experiment readings before improving towards the end of the experiment. Even though NaCl helped to reduce internal resistance, the high salinity also acted as a limiting factor for microbial electron generation. On the other hand, high Glucose ensured a high supply of electrons for the circuit but led to a metabolic imbalance during some parts of the experiment.

The experiment has proven that for MFCs to operate optimally, there is a need to balance the substrate loading rate with the electrolyte concentration. Too much organic material may cause some degree of instability during the experiment, while too much salt may impede the metabolism process despite reducing the internal resistance. This is in line with the theory that has been developed for MFCs regarding balancing the availability of electrons with the control of internal resistance.

**Table 4.20** Voltage production comparison at 25°C using 3 g glucose and 3 g NaCl

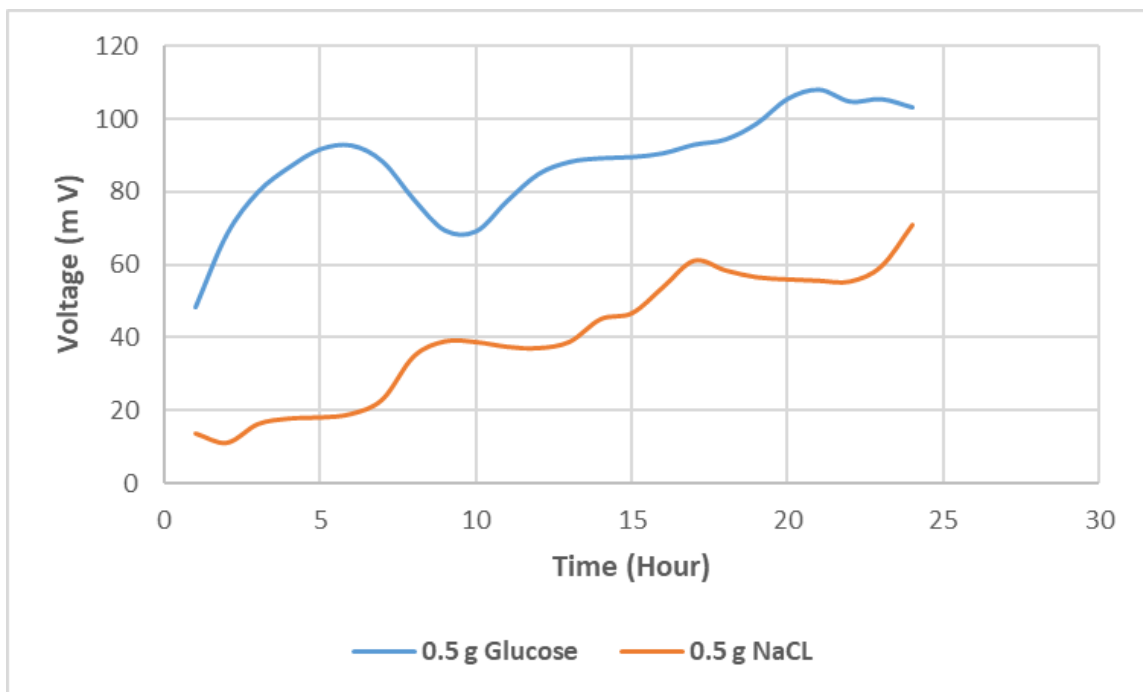
| Time (Hour) | 3.0 g Glucose (mV) | 3.0 g NaCl (mV) |
|-------------|--------------------|-----------------|
| 1           | 69                 | 63              |
| 5           | 55                 | 45              |
| 10          | 35                 | 32              |
| 17          | 36                 | 39              |
| 20          | 22                 | 40              |
| 24          | 70                 | 44              |

These results show that extremely high substrate and electrolyte concentrations can significantly impact the voltage behavior by affecting microbial metabolism, internal resistance, and system stability in the soil-based microbial fuel cell. To further examine the effect of temperature elevation on the voltage production with similar conditions of

additive, the following section compares the results of the 0.5 g glucose and 0.5 g NaCl at 40°C:

Experiment: The effect of temperature elevation on the voltage production with low substrate and electrolyte concentrations in the soil-based microbial fuel cell.

In this experiment, the effect of temperature elevation (40°C) with low substrate and electrolyte concentrations (0.5 g glucose and 0.5 g NaCl) on the voltage production in the soil-based microbial fuel cell with 100 g soil, 100 mL water, and 24 hours of operation was investigated. The role of temperature in the MFC systems is critical because it plays a vital role in microbial metabolism, enzymatic kinetics, proton mobility, and internal resistance.



**Figure 4.21** Voltage production in 24 hours for soil-based MFC with 0.5 g glucose and 0.5 g NaCl at 40°C.

In the 0.5 g glucose system (blue curve), the voltage production was initially at 48-50 mV at hour 1, increasing rapidly to reach 80-85 mV at hours 3-4, as illustrated in Figure 4.21. The rapid increase in the voltage production at the beginning of the experiment may be due to the improvement of microbial metabolism at 40°C because, at this temperature, the enzymatic oxidation of glucose is greatly improved. According to [85], the increase in temperature has the potential to significantly increase the current density because of the improvement of bioelectrochemical activity.

Between hours 5 and 7, the voltage readings were around 90-92 mV. This was followed by a short-lived drop to 68-70 mV around hour 10. This may be attributed to the effects of proton buildup, pH fluctuations, and possibly temporary substrate diffusion limitations within the soil matrix. After hour 10, the readings began to rise steadily again. This time the readings were around 88-90 mV around hour 14 and a peak of 105-108 mV around hour 20-21. This was the peak reading for this condition, indicating optimal microbial activity and low internal resistance under the elevated temperatures. A slight drop to 102-104 mV was observed around hour 24.

On the other hand, the 0.5 g NaCl system (orange curve) had a lower voltage reading compared to the 1.0 g NaCl system. The readings started at 12-14 mV around hour 1 and gradually increased to 18 mV around hour 5. Between hours 7 and 9, the readings increased more sharply to 38-40 mV. This may be attributed to the effects of increased ionic conductivity at elevated temperatures. At elevated temperatures, the mobility of the ions is increased, reducing the ohmic resistance within the electrolyte [96].

From hours 10-15, it was observed that voltage was stable at a range of 36-45 mV. Another increase was observed at around hour 17-18, reaching a value of around 58-60 mV. The voltage was maintained at around 55-57 mV until hour 22, and another increase was observed at around hour 24, reaching a value of around 70 mV. This increase indicates gradual adaptation of microbes to saline conditions and better proton transfer at 40°C.

**Table 4.21** Voltage production comparison at 40°C using 0.5 g glucose and 0.5 g NaCl

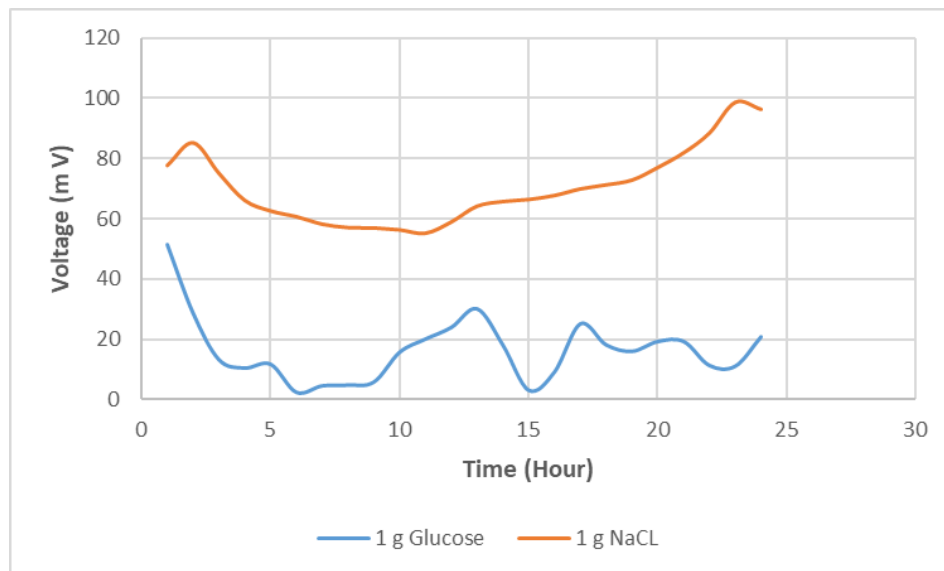
| Time (Hour) | 0.5 g Glucose (mV) | 0.5 g NaCl (mV) |
|-------------|--------------------|-----------------|
| 1           | 50                 | 13              |
| 5           | 90                 | 18              |
| 10          | 69                 | 38              |
| 15          | 89                 | 45              |
| 20          | 107                | 56              |
| 24          | 103                | 70              |

The voltage profiles comparing 0.5 g glucose and 0.5 g NaCl exhibit distinct fluctuation behaviors. The glucose condition showed a rapid increase in voltage followed by controlled oscillations and sustained high output, indicating stable microbial activity and efficient extracellular electron transfer. In contrast, the NaCl condition exhibited a gradual increase in voltage with minor fluctuations, reflecting improved ionic conductivity but limited biological contribution. These findings confirm that glucose addition enhances

both the magnitude and stability of voltage generation, while NaCl primarily influences conductivity without sustaining long-term electrochemical activity.

In comparison, it is observed from both systems that the glucose-fed MFC showed a much higher voltage compared to the NaCl-fed system. The voltage was increased to a maximum of around 108 mV, whereas it was increased to a maximum of around 70 mV in the NaCl system, as indicated in Table 4.21. This indicates that substrate concentration plays a major role in increasing voltage, whereas electrolyte concentration plays a major role in increasing internal resistance [99].

The results showed significant improvement in voltage output at 40°C compared to other temperatures, as supported by other literature sources, which stated that mesophilic and moderately thermophilic temperatures can improve microbial fuel cell performance [89]. Nevertheless, it is also possible that temperatures above the optimum for microbial growth can lead to enzyme denaturation.



**Figure 4.22** Voltage production in 24 hours for soil-based MFC with 1 g glucose and 1 g NaCl at 40°C.

The above experiments showed that temperature elevation can significantly influence the balance of substrate-generated electrons and electrolyte-generated conductivity in microbial fuel cells. To further assess the performance of increased additive concentration under elevated temperature, a comparison of 1 g glucose and 1 g NaCl at 40°C is provided in the next section (Figure 4.22).

The experiment was designed to assess the effect of increased substrate concentration (1.0 g glucose) and electrolyte concentration (1.0 g NaCl) on voltage generation in a microbial fuel cell using 100 g of soil, 100 mL of water, and operated at 40°C for 24 hours. Temperature is known to influence microbial growth kinetics, enzyme activity, electron transfer, and ionic mobility, which can improve microbial fuel cell performance within an optimum temperature range [79]. The voltage-time profiles showed significant differences for glucose- and NaCl-amended systems under elevated temperature.

Under the 1g glucose condition (blue curve), the voltage started high at around 50 mV at the beginning of the experiment (at the 1st hour), but this dropped sharply to 10-12 mV by the 4th hour. This may be attributed to the high levels of acids produced by the fermentation and oxidation of glucose at high temperatures, which can increase the internal resistance and decrease the anode potential [70]. The voltage remained low (between 2 mV and 6 mV) from the 5th to the 9th hours. This may indicate the inhibition of the electroactive biofilm or the activity of other metabolic pathways such as fermentation and methanogenesis.

However, from the 10th hour onwards, the glucose system started to improve gradually; the voltage increased to 15-20 mV by the 11th hour and a local maximum of 28-30 mV by the 13th hour. The voltage dropped sharply by the 15th hour to 3-5 mV; however, the system started to improve again by the 17th hour to 25 mV, as shown by the readings in Figure 4.22. This may be attributed to the various factors affecting the anode such as the composition of the microbial community, the diffusion of substrates through the soil matrix, and the redox reactions occurring at the anode. Ishii [98] states that for efficient electron transfer to occur from the anode to the cathode, the electroactive bacteria must be stably attached to the anode. Any interference with the pH may cause the voltage to drop temporarily. The voltage readings between the 18th and 24th hours were between 10 mV and 20 mV; the final voltage was around 20 mV.

On the contrary, for the 1.0 g NaCl condition (orange curve), higher voltages were consistently achieved. The system initially ranged between 78 and 85 mV for the first 1 to 2 hours before gradually decreasing to around 55 to 60 mV for hours 8 to 11. This high output during the initial period is due to reduced ohmic losses and increased ion mobility

in the electrolyte solution. An increase in salinity increases ionic conductivity. This results in reduced internal resistance and hence an increased output for the MFC system [76]. After hour 12, the NaCl system continued to increase its output. It increased from 60 mV to around 70 mV by hour 18 and continued to reach a maximum of around 98 to 100 mV by hour 23. It then decreased to around 95 to 97 mV by hour 24.

The voltage profiles comparing 1 g glucose and 1 g NaCl reveal contrasting fluctuation behaviors under this condition. The glucose system exhibited low and irregular voltage output with significant fluctuations, indicating unstable microbial activity and possible substrate inhibition. In contrast, the NaCl system showed higher voltage output with smooth and controlled fluctuations, reflecting enhanced ionic conductivity and stable electrochemical performance. These results demonstrate that under certain conditions, electrolyte-driven systems can outperform substrate-driven systems when microbial activity is limited or inhibited.

Table 4.22 indicates that for operation under 40°C temperature, the NaCl-amended MFC significantly outperformed the glucose-amended MFC in terms of both stability and peak voltage (100 mV compared to 30 mV peak for glucose-amended). This indicates that for operation under elevated temperatures, the NaCl-amended MFC would gain more from increased conductivity than from additional glucose.

**Table 4.22** Voltage production comparison at 40°C using 1 g glucose and 1 g NaCl

| Time (Hour) | 1.0 g Glucose (mV) | 1.0 g NaCl (mV) |
|-------------|--------------------|-----------------|
| 1           | 50                 | 78              |
| 4           | 11                 | 65              |
| 8           | 4                  | 58              |
| 13          | 30                 | 65              |
| 15          | 4                  | 66              |
| 23          | 12                 | 100             |

It is likely that, in the case of the 1.0 g glucose at 40°C, rapid fermentation and acidification, as well as internal resistance, may have inhibited electroactive respiration, whereas the NaCl may have improved the ionic conductivity without causing excessive metabolic imbalance.

It has been observed that the increase in the concentration of the substrate as well as the electrolyte, along with the effect of the high temperature, plays a significant role in

the overall microbial metabolism, ionic conductivity, and voltage production in the soil-based microbial fuel cells.

To assess the impact of the increase in the concentration of the substrate as well as the electrolyte at the elevated temperature of 40°C, the performance of the microbial fuel cell with the increase in the concentration of the substrate as well as the electrolyte, i.e., 1.5 g glucose and 1.5 g NaCl, is presented in the following section:

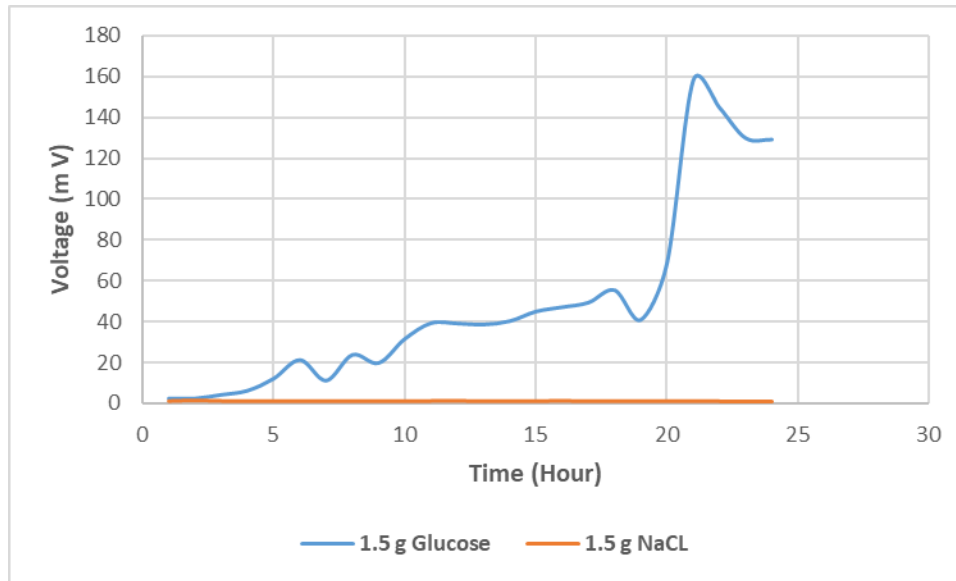
This experiment was conducted to assess the impact of the increase in the concentration of the substrate as well as the electrolyte on the voltage production in the soil-based microbial fuel cell at the elevated temperature of 40°C, using 100 g soil, 100 mL of water, and 1.5 g glucose and 1.5 g NaCl, and the experiment was conducted for 24 hours. It has been observed that the temperature plays a significant role in the overall microbial respiration rate, enzymatic activity, as well as membrane and proton mobility, resulting in the enhancement of the overall microbial fuel cell performance at the optimal mesophilic temperature range [82].

In the case of the microbial fuel cell with the increase in the concentration of glucose to 1.5 g (blue curve), the voltage was observed to be extremely low during the first 2-3 hours, i.e., the microbes were acclimatizing, and the voltage was between 2-3 mV, whereas the voltage increased gradually between hours 4 and 6, resulting in the production of 18-22 mV, as depicted in Figure 4.23.

The minor oscillations between 15 and 25 mV from hours 6 to 9 indicate continued biofilm development and stabilization of the extracellular electron transport pathways [97].

After hour 10, the voltage continued to rise steadily to a range of 38 to 40 mV near hour 11 and eventually to 45 mV by hour 15. There was a slight decrease to 40 mV near hour 18, which could be caused by localized proton accumulation or substrate diffusion limitations. The most dramatic feature was the dramatic rise in voltage after hour 20. The voltage rose sharply from 60 mV to a peak of approximately 155 to 160 mV at hour 21 (Table 4.23). This was the maximum voltage recorded. The high temperature could have enhanced the rate of reaction of the enzymes, proton conductivity, and minimized internal resistance, thus maximizing the electron flow [96]. The voltage then settled at approximately 130 mV by hour 24.

On the other hand, the 1.5 g NaCl solution (orange curve) recorded almost no voltage, remaining at approximately 0 to 2 mV over the entire period. The combined effects of high temperature and high salinity could have caused extreme osmotic stress, thus inhibiting microbial respiration and electron transfer. Excessive salinity could have disrupted membrane and enzymatic functions, thus inhibiting electron transfer and resulting in suppressed electrogenic activity despite enhanced theoretical conductivity [99].



**Figure 4.23** Voltage production in 24 hours for soil-based MFC with 1.5 g glucose and 1.5 g NaCl at 40°C.

Comparatively, the MFC fed with glucose was observed to have performed better than the NaCl MFC, with a maximum voltage of 160 mV compared to almost 0 mV for NaCl, as shown in Table 4.23. This implies that at a temperature of 40°C, substrate enhancement is dominant in MFCs, while high salt concentration may be beyond the limit that microorganisms can tolerate.

The voltage profiles for 1.5 g glucose and 1.5 g NaCl show distinct fluctuation behaviors. The glucose system exhibited an initial lag phase followed by gradual voltage increase and minor oscillations, culminating in a sharp spike to approximately 160 mV after 20 hours, indicating enhanced microbial activity and efficient extracellular electron transfer. In contrast, the NaCl system showed negligible voltage output throughout the experiment, with no significant fluctuations observed. These results confirm that substrate

availability is essential for sustained electrochemical activity, while electrolyte addition alone cannot generate measurable voltage.

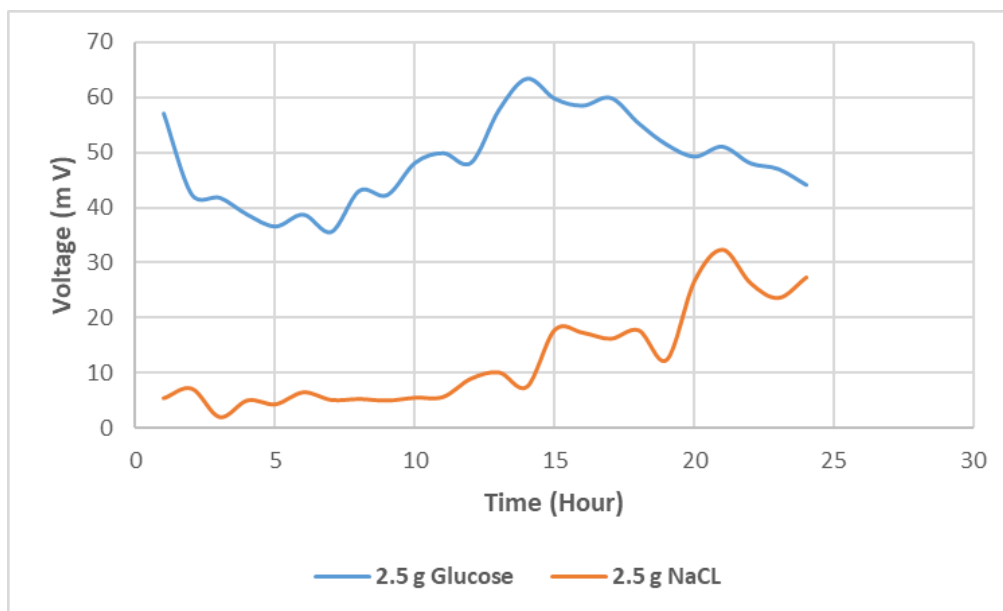
**Table 4.23** Voltage production comparison at 40°C using 1.5 g glucose and 1.5 g NaCl

| Time (Hour) | 1.5 g Glucose (mV) | 1.5 g NaCl (mV) |
|-------------|--------------------|-----------------|
| 1           | 2                  | 1               |
| 6           | 20                 | 1               |
| 11          | 40                 | 1               |
| 18          | 40                 | 1               |
| 21          | 160                | 1               |
| 24          | 130                | 1               |

The results have shown that the addition of a high concentration of substrates under high-temperature conditions enhances the activity of microorganisms in MFCs. Therefore, to further validate the effect of high concentrations of additives on the generation of electricity in MFCs at a temperature of 40°C, a comparison between the MFC fed with 2.5g glucose and NaCl is presented in the next section (Figure 4.24).

The objective of this experiment was to validate the effect of high substrate concentration (2.5g glucose) and high electrolyte concentration (2.5g NaCl) on the generation of electricity in a soil-based MFC at a constant temperature of 40°C using a constant amount of 100g soil and 100ml water for a period of 24 hours. Temperature enhances the rate of microbial metabolism; however, the performance of MFCs also depends on the concentration of substrates and electrolytes [77]. For the MFC fed with 2.5g glucose (blue curve), the voltage started at around 56-57mV at hour 1, then declined to around 37-40mV at hours 4-6, as explained in Figure 4.24. Then, the voltage started to rise again between hours 7 and 10 to around 48-50mV.

A considerable increase was observed between hours 12 and 15, where the voltage increased from 48 mV to around 62-63 mV (maximum peak). This may be an optimal metabolic activity at 40°C under sufficient substrate availability. After hour 15, the voltage gradually decreased to 49-51 mV by hour 20 and further decreased to 44-46 mV by hour 24. This may indicate a certain level of substrate depletion or increased diffusion resistance within the soil medium. Nevertheless, the glucose system remained at a moderate level of stability throughout the experiment.



**Figure 4.24** Voltage production in 24 hours for soil-based MFC with 2.5 g glucose and 2.5 g NaCl at 40°C.

The voltage profiles comparing 2.5 g glucose and 2.5 g NaCl show distinct fluctuation patterns under higher loading conditions. The glucose system exhibited moderate and controlled oscillations with relatively high voltage output, indicating sustained microbial activity and efficient electron transfer, although slight performance decline was observed at later stages. In contrast, the NaCl system showed low initial voltage followed by delayed and irregular increases, reflecting limited electrochemical activity driven primarily by ionic conductivity. These findings suggest that while glucose supports more stable and efficient performance, excessive loading begins to introduce minor instability, whereas NaCl results in weaker and less consistent voltage generation.

On the other hand, the 2.5 g NaCl system (orange curve) showed much lower voltage values during the early hours, starting from 5-6 mV and oscillating between 3-7 mV until around hour 10. This may indicate the inhibitory effect of high salinity on microbial activity under increased ionic conductivity. From hour 12 onwards, the voltage increased gradually. It increased to 17-18 mV around hour 15 and reached a maximum peak of around 31-32 mV around hour 21. The final reading at hour 24 was 26-27 mV, as shown in Table 4.24. This may indicate the adaptation response of the microbes to saline stress under increased proton transport at high temperatures [98].

**Table 4.24** Voltage production comparison at 40°C using 2.5 g glucose and 2.5 g NaCl

| Time (Hour) | 2.5 g Glucose (mV) | 2.5 g NaCl (mV) |
|-------------|--------------------|-----------------|
| 1           | 57                 | 6               |
| 6           | 38                 | 5               |
| 10          | 50                 | 6               |
| 15          | 63                 | 18              |
| 20          | 50                 | 28              |
| 24          | 45                 | 27              |

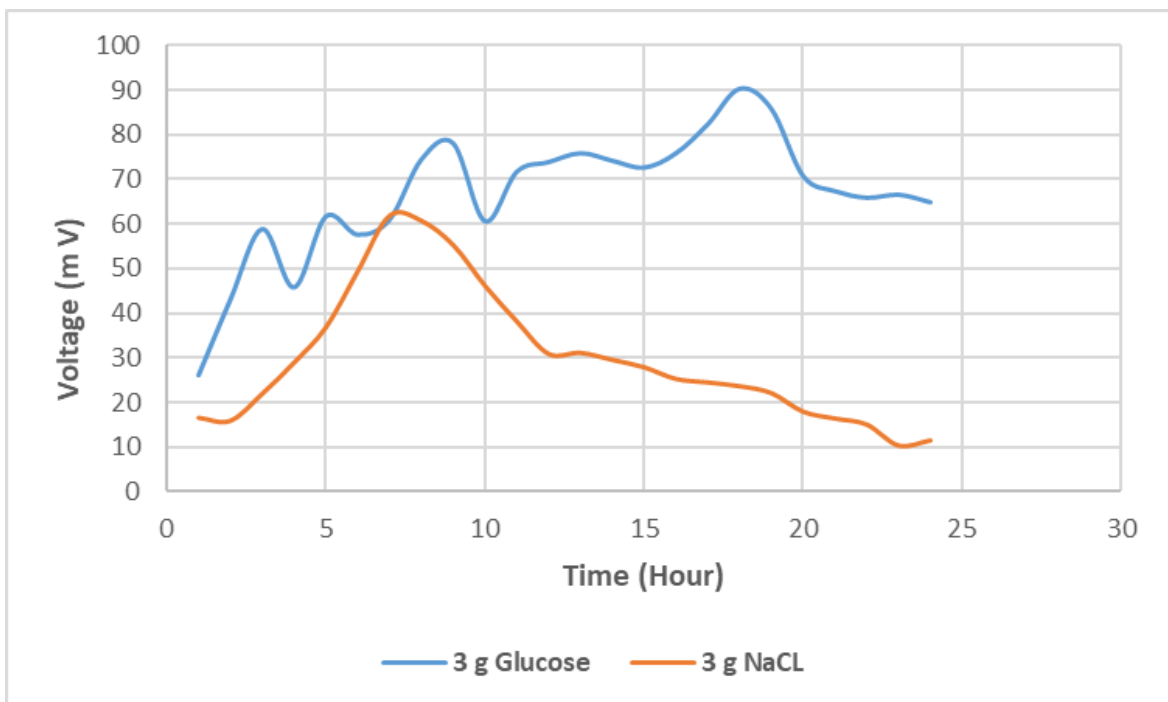
Comparatively, the glucose-fed system showed a higher voltage output compared to the NaCl-fed system over the entire duration of the experiment (63 mV vs. 32 mV). The glucose condition showed a higher metabolic-driven voltage enhancement, whereas the NaCl condition showed conductivity-driven improvement, albeit lower voltage output. This is consistent with established MFC principles, where substrate availability is considered a primary factor in electron generation, and the concentration of the electrolyte is considered a secondary factor that affects internal resistance, which may also produce adverse effects at higher concentrations [79]. It was also observed that an increase in temperature from 30°C to 40°C increased voltage output, although a higher salt concentration showed adverse effects on biochemical efficiency. It is, therefore, evident that for optimal MFC performance, substrate loading and electrolyte concentration need to be balanced for maximum electron flow and minimal metabolic stress.

This experiment showed that an increase in substrate and electrolyte concentrations, along with an increase in temperature, has a significant effect on voltage output in a soil-based MFC. A comparison between the 3g glucose and 3g NaCl concentrations is shown in the following section for a higher concentration of additives on voltage output in a soil-based MFC operated at 40°C for 24 hours:

This experiment was designed to assess the effect of increased substrate loading (3.0g glucose) and increased electrolyte concentration (3.0g NaCl) on voltage output in a soil-based MFC operated at 40°C for 24 hours with 100g soil and 100mL water. Increased temperature is known to increase the metabolic rate of microorganisms, which increases the rate of reaction, enzymatic activity, and proton transport, although increased substrate loading or increased salt concentration may interfere with the electrochemical reaction [89].

The voltage output for the 3.0g glucose system (blue curve) was observed to increase from 26–28 mV in the initial 1-hour period, followed by a sharp increase in voltage output, reaching 58–60 mV within the initial 3 hours, as shown in Figure 4.25. A drop in voltage output was also observed, reaching 45 mV within the 4th hour, followed by a sharp increase, reaching 60 mV within the 5th hour. A sharp increase was also observed between the 6th and 9th hours, reaching 78–80 mV within the 8th hour.

This is a result of increased glucose oxidation coupled with efficient EETs at a temperature of 40°C, where increased temperatures enhance microbial respiration [95]. The voltage then dropped slightly to approximately 60 mV around hour 10 before continuously increasing again to a peak of 75 mV around hour 13. The voltage then increased to approximately 88-90 mV around hour 18 before dropping again to a range of 65-70 mV around hour 24. This dropping off could have been a result of substrate depletion or increased internal resistance due to metabolite buildup.



**Figure 4.25** Voltage production in 24 hours for soil-based MFC with 3 g glucose and 3 g NaCl at 40°C.

The 3.0 g NaCl system (orange curve) started off with a voltage output of approximately 15-17 mV before increasing to a peak of approximately 60-62 mV around hour 7. This could have been a result of increased ionic conductivity at increased

temperatures, thus reducing ohmic loss in the cell [97]. After hour 7, the voltage then started dropping steadily before dropping to approximately 45 mV around hour 10. The voltage then dropped further to a range of 25-30 mV around hour 15 before dropping further to a range of approximately 10-12 mV between hours 18 and 24, as shown in Table 4.25.

This dropping off could have been a result of inhibitory action of high salinity coupled with increased temperatures that may have subjected the microbial cell to osmotic stress, thus interfering with membrane integrity and thereby reducing microbial electron generation [98].

In comparison, the glucose-fed MFC performed much better than the NaCl-fed MFC at 40°C, recording a peak voltage of 90 mV compared to 62 mV for NaCl. Although NaCl was observed to enhance conductivity, it was also observed that, in the long term, NaCl-fed MFCs showed decreased performance due to inhibitory effects [99].

**Table 4.25** Voltage production comparison at 40°C using 3 g glucose and 3 g NaCl

| Time (Hour) | 3.0 g Glucose (mV) | 3.0 g NaCl (mV) |
|-------------|--------------------|-----------------|
| 1           | 27                 | 16              |
| 5           | 60                 | 60              |
| 8           | 80                 | 62              |
| 13          | 75                 | 30              |
| 18          | 90                 | 25              |
| 24          | 67                 | 11              |

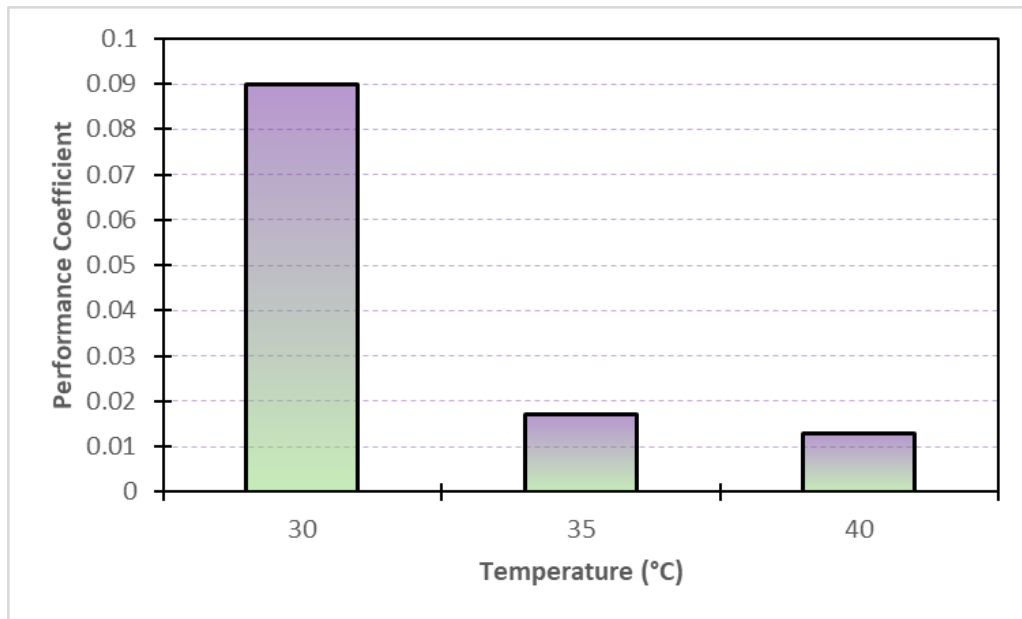
The voltage profiles comparing 3 g glucose and 3 g NaCl show pronounced fluctuation behavior under high loading conditions. The glucose system exhibited high voltage output with periodic oscillations, reaching a peak of approximately 90 mV before gradually declining, indicating active but increasingly unstable microbial performance. In contrast, the NaCl system showed a rapid increase to a peak value followed by continuous decline, reflecting transient conductivity enhancement without sustained electrochemical activity. These results demonstrate that excessive substrate loading leads to fluctuating and unstable performance, while electrolyte addition alone results in short-lived and unsustainable voltage generation.

In general, it was observed that operating at 40°C enhanced voltage generation for the glucose-fed MFC, thus proving that temperature can be increased for better

performance, provided substrate concentration is within limits. High salinity levels, however, seem to inhibit microbial activity at increased temperatures.

#### 4.7 Performance Evaluations

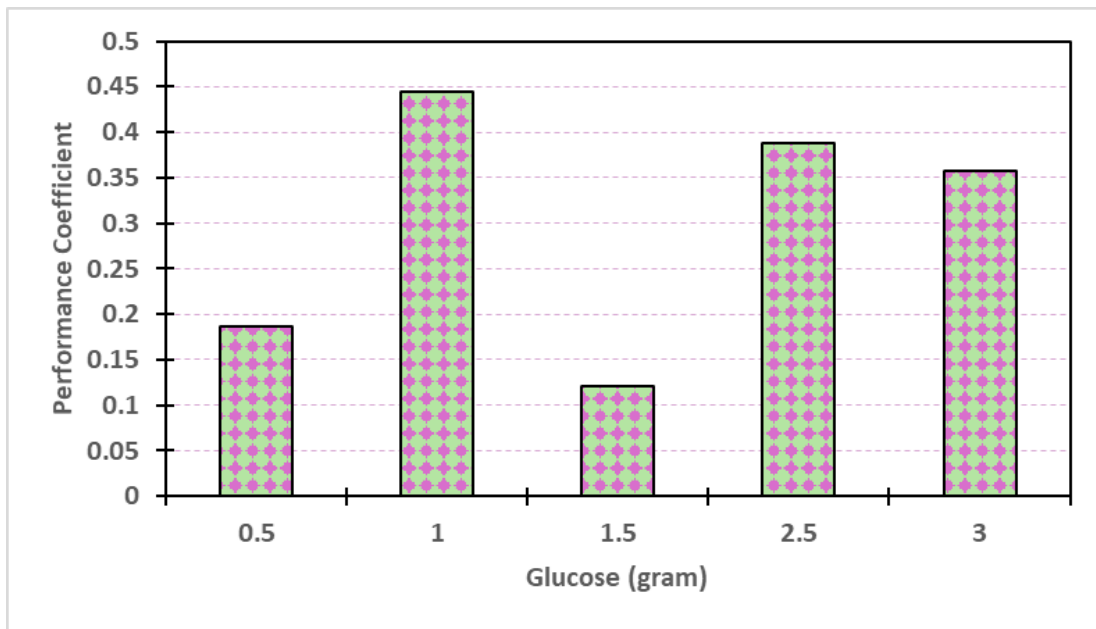
This section presents a comprehensive evaluation of the performance coefficient of the soil-based microbial fuel cell (MFC) under varying operational conditions, including temperature, substrate concentration, salinity, pH, and soil–water ratio. The analysis aims to identify optimal conditions that maximize electrochemical efficiency and microbial activity within the system. By examining the combined effects of these parameters, the study provides insight into the mechanisms governing extracellular electron transfer and internal resistance. The results contribute to a deeper understanding of how environmental and operational factors influence MFC performance. Ultimately, this section supports the development of more efficient and stable bioelectrochemical energy systems.



**Figure 4.26** Effect of temperature on the performance coefficient of soil-based microbial fuel cell

Figure 4.26 illustrates the effect of temperature on the performance coefficient of the soil-based microbial fuel cell (MFC), showing a strong inverse relationship beyond the optimal range. At 30 °C, the system achieved the highest performance coefficient of 0.0898, indicating optimal microbial activity, efficient extracellular electron transfer, and reduced internal resistance. However, increasing the temperature to 35 °C resulted in a

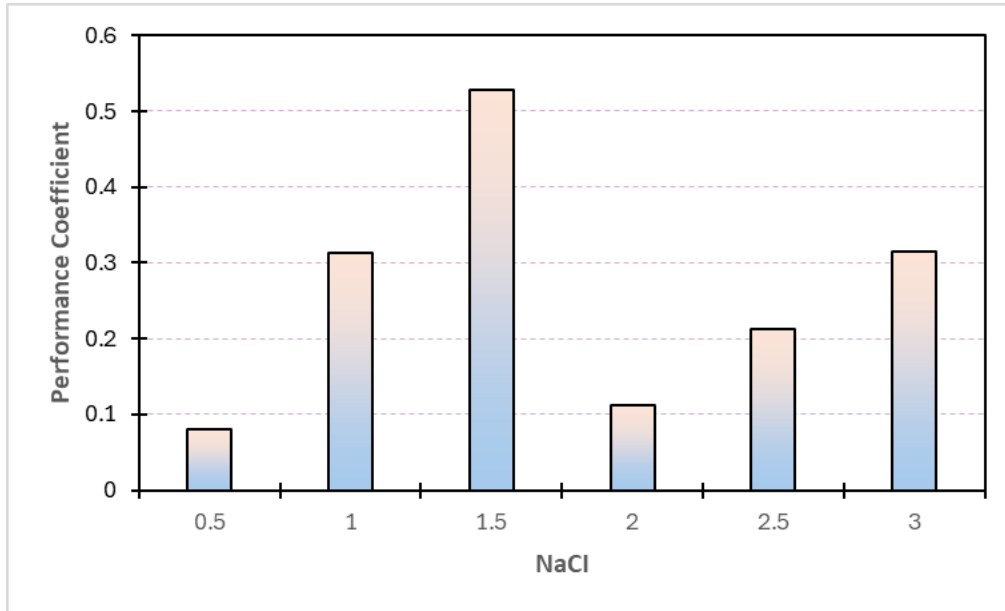
significant decline to 0.0171, representing a reduction of approximately 81%, while a further increase to 40 °C led to a slight additional decrease to 0.0129. This sharp drop suggests that temperatures above 30 °C induce thermal stress on electroactive microbial communities, disrupt biofilm stability, and reduce electron transfer efficiency. Additionally, elevated temperatures may enhance competing metabolic pathways and increase internal losses, thereby lowering overall system performance. These results confirm that 30 °C represents the optimal operating temperature for maximizing MFC efficiency under the tested conditions, while higher temperatures negatively impact performance.



**Figure 4.27** Effect of glucose concentration on the performance coefficient of soil-based microbial fuel cell

Figure 4.27 demonstrates the influence of glucose concentration on the performance coefficient of the soil-based microbial fuel cell (MFC), revealing a non-linear relationship between substrate availability and system efficiency. The highest performance coefficient was observed at 1 g glucose (0.4449), indicating optimal substrate conditions for microbial metabolism and efficient extracellular electron transfer. At lower concentration (0.5 g), the performance coefficient was significantly lower (0.1867), suggesting substrate limitation and reduced microbial activity. Increasing the glucose concentration to 1.5 g resulted in a sharp decline to 0.1214, likely due to the onset of competing metabolic pathways such as fermentation, which reduce Coulombic efficiency. At higher concentrations (2.5 g and 3 g), the performance coefficient increased again to

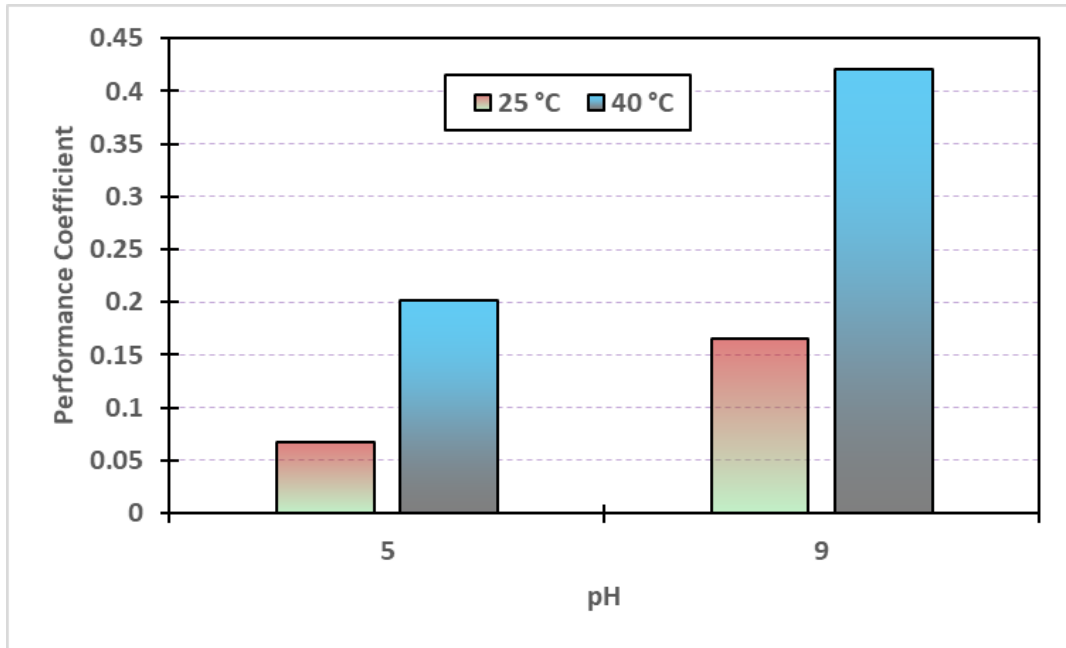
0.3872 and 0.3567, respectively, indicating partial recovery due to increased electron donor availability; however, these values remained below the optimal 1 g condition. This trend highlights that moderate glucose concentration provides the best balance between microbial activity and electrochemical efficiency, while both insufficient and excessive substrate levels negatively impact MFC performance.



**Figure 4.28** Effect of NaCl concentration on the performance coefficient of soil-based microbial fuel cell

Figure 4.28 depicts the effect of NaCl concentration on the performance coefficient of the soil-based microbial fuel cell (MFC), showing a clear optimum at moderate salinity. The performance coefficient increased significantly from 0.0810 at 0.5 g NaCl to 0.3125 at 1 g, reaching a maximum value of 0.5285 at 1.5 g, indicating that moderate ionic strength enhances electrolyte conductivity and reduces internal resistance, thereby improving electron transport. However, further increasing the NaCl concentration to 2 g resulted in a sharp decline to 0.1117, suggesting the onset of osmotic stress and inhibition of electroactive microbial activity. At higher concentrations of 2.5 g and 3 g, the performance coefficient partially recovered to 0.2121 and 0.3155, respectively, likely due to improved ionic conductivity, but remained below the optimal 1.5 g condition. These results demonstrate that while NaCl enhances MFC performance by improving charge transport, excessive salinity negatively affects microbial viability and biofilm stability, highlighting the importance of maintaining an optimal salt concentration for maximum efficiency.

Figure 4.29 explained the influence of pH and temperature on the performance coefficient of the soil-based microbial fuel cell (MFC), demonstrating that both parameters significantly affect system efficiency. At 25 °C, the performance coefficient increased from 0.0670 at pH 5 to 0.1649 at pH 9, indicating improved electrochemical performance under alkaline conditions. A similar trend was observed at 40 °C, where the performance coefficient rose from 0.2013 at pH 5 to a maximum of 0.4210 at pH 9, showing a substantial enhancement with both higher temperature and higher pH.

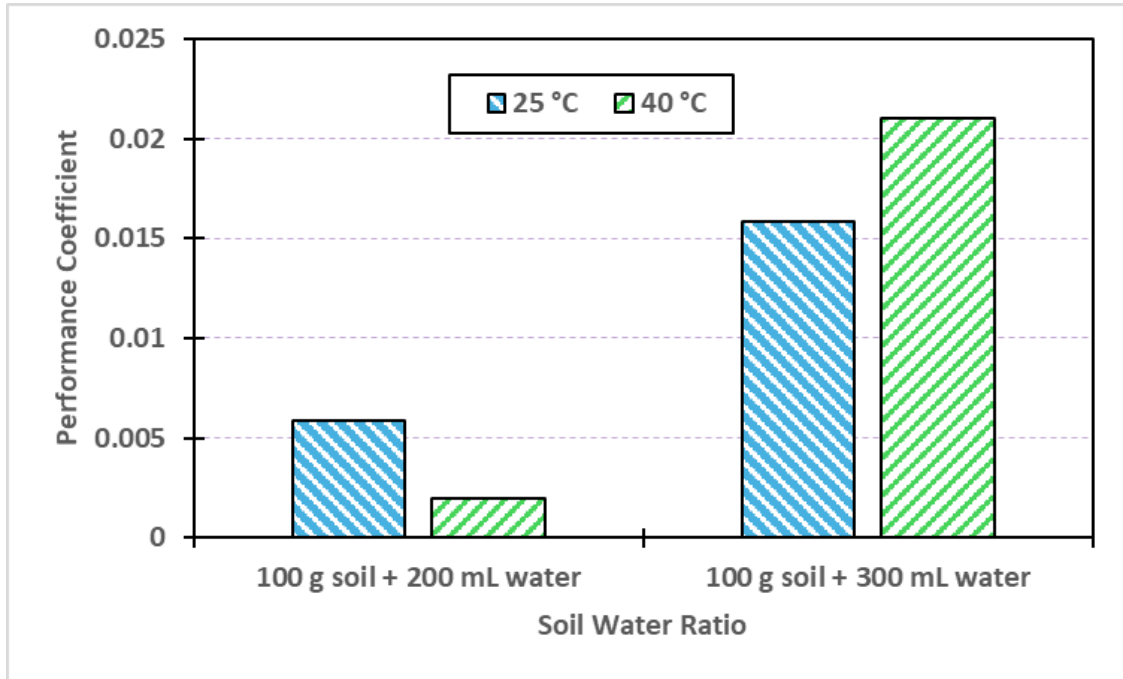


**Figure 4.29** Combined effect of pH and temperature on the performance coefficient of soil-based microbial fuel cell

The higher performance at 40 °C can be attributed to increased microbial metabolic rates and reduced internal resistance, while alkaline conditions (pH 9) likely improve ionic conductivity and electrode reaction kinetics. In contrast, acidic conditions (pH 5) result in lower performance due to proton accumulation, microbial inhibition, and increased internal resistance. Overall, the results indicate that the combination of elevated temperature (40 °C) and alkaline pH (9) provides the most favorable conditions for maximizing MFC performance.

The Figure 4.30 presents the effect of soil–water ratio and temperature on the performance coefficient of the soil-based microbial fuel cell (MFC), highlighting the importance of moisture content in system efficiency. At 25 °C, increasing the water volume

from 200 mL to 300 mL significantly improved the performance coefficient from 0.00585 to 0.01582, indicating enhanced ionic conductivity and reduced internal resistance due to better pore connectivity in the soil matrix. A similar trend was observed at 40 °C, where the performance coefficient increased markedly from 0.00197 at 200 mL to 0.02102 at 300 mL, representing the highest value among all tested conditions



**Figure 4.30** Effect of soil–water ratio and temperature on the performance coefficient of soil-based microbial fuel cell

The lower performance at 200 mL suggests limited proton transport and higher ohmic losses under insufficient moisture. Additionally, the higher temperature (40 °C) further amplified the effect of increased water content, likely due to improved ion mobility and faster electrochemical kinetics. These results demonstrate that adequate moisture is a critical factor for optimizing MFC performance, and that the combination of higher water volume (300 mL) and elevated temperature (40 °C) provides the most favorable conditions for maximizing efficiency.

#### 4.8 Results of ANOVA Analysis

One-way analysis of variance (ANOVA) was conducted to evaluate the effects of key operating parameters, namely temperature, glucose concentration, NaCl concentration, and pH, on the voltage output of the system. For each experimental condition, hourly voltage

measurements recorded over a 24-hour period were treated as repeated observations to improve the statistical robustness of the analysis. The ANOVA was performed using a significance level of  $\alpha = 0.05$ .

**Table 4.26** ANOVA results for the effects of operating parameters on voltage output

| <b>Parameter</b>      | <b>Conditions Tested</b> | <b>F-value</b> | <b>p-value</b>         | <b>Significance</b> |
|-----------------------|--------------------------|----------------|------------------------|---------------------|
| Temperature           | 25-50°C                  | 52.58          | $2.62 \times 10^{-30}$ | Significant         |
| Glucose concentration | 0.5-3 g                  | 21.11          | $4.5 \times 10^{-13}$  | Significant         |
| NaCl concentration    | 0.5-3 g                  | 14.23          | $1.83 \times 10^{-9}$  | Significant         |
| pH                    | 5, 7, 9                  | 13.62          | $1.04 \times 10^{-5}$  | Significant         |

The results presented in Table 4.26 demonstrate that all parameters investigated have a statistically significant effect on voltage output ( $p < 0.05$ ). Among these parameters, temperature exhibited the most pronounced influence, as indicated by the highest F-value ( $F = 52.58$ ) and an extremely low p-value ( $2.62 \times 10^{-30}$ ), confirming a very strong statistical significance. Glucose concentration also showed a substantial impact on voltage ( $F = 21.11$ ,  $p = 4.5 \times 10^{-13}$ ), followed by NaCl concentration ( $F = 14.23$ ,  $p = 1.83 \times 10^{-9}$ ) and pH ( $F = 13.62$ ,  $p = 1.04 \times 10^{-5}$ ). The relative magnitudes of the F-values suggest that temperature is the most influential parameter governing system performance, whereas pH has the least, yet still statistically significant, effect.

In addition to statistical significance, the optimal operating conditions were identified based on the highest average voltage values obtained under each parameter. The maximum voltage output was achieved at 25°C for temperature, 1 g for glucose concentration, 1.5 g for NaCl concentration, and 7 pH. These findings indicate that system performance is highly sensitive to variations in environmental and operational conditions, and careful optimization of these parameters is essential to achieve enhanced voltage generation. Furthermore, the use of time-resolved voltage measurements provides a more comprehensive representation of system behavior compared to single-point measurements, thereby increasing the reliability of the statistical evaluation.

#### 4.9 Statistical Analysis of the Effect of Temperature on Voltage Using ANOVA

The figure 4.31 illustrates the effect of temperature on voltage output, highlighting a non-linear relationship between the two variables. The highest voltage (~75 mV) was observed at 25 °C, indicating optimal microbial activity under ambient conditions.

As temperature increased to 30–40 °C, a significant decline in voltage was recorded, reaching a minimum around 35–40 °C (~13–15 mV). However, further increase in temperature to 45 °C and 50 °C resulted in partial recovery of voltage output, reaching approximately 32 mV and 41 mV, respectively.

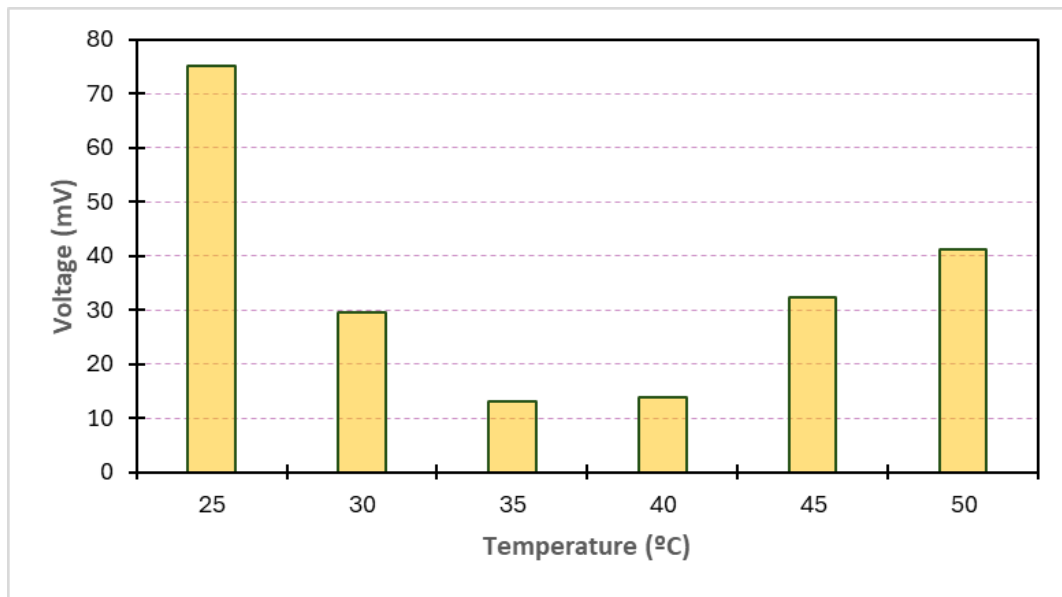


Figure 4.31 ANOVA for the effect to temperature

These trends suggest that while moderate temperature increases initially suppress system performance, higher temperatures may enhance microbial kinetics up to a certain threshold, consistent with the ANOVA findings that temperature is a highly significant factor influencing voltage generation.

#### 4.10 Statistical Analysis of the Effect of Glucose on Voltage Using ANOVA

The figure 4.32 presents the effect of glucose concentration on voltage output, showing a clear non-linear trend. The voltage increases from ~32 mV at 0.5 g to a maximum of ~50 mV at 1 g, indicating an optimal substrate concentration for microbial activity.



**Figure 4.32** ANOVA for the effect to glucose

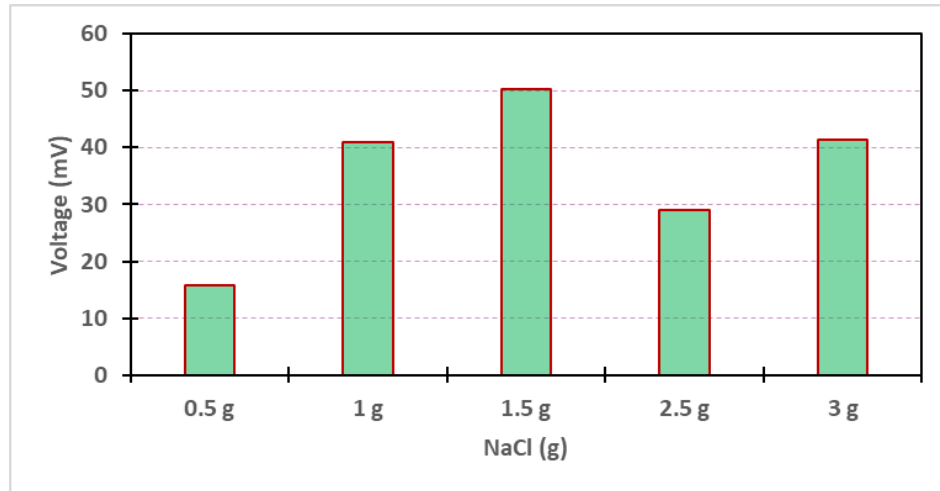
Beyond this point, a sharp decrease is observed at 1.5 g (~23 mV), suggesting substrate inhibition or metabolic imbalance. At higher concentrations (2.5 g and 3 g), the voltage partially recovers to ~46 mV and ~42 mV, respectively, but does not exceed the peak at 1 g. These results align with the ANOVA findings, confirming that glucose concentration significantly influences voltage output and that an optimum level exists for maximum performance.

#### 4.11 Statistical Analysis of the Effect of NaCl on Voltage Using ANOVA

The figure 4.33 shows the effect of NaCl concentration on voltage output, demonstrating a clear optimum behavior.

The voltage increases significantly from ~16 mV at 0.5 g to a maximum of ~50 mV at 1.5 g, indicating enhanced ionic conductivity and improved electron transfer. However, beyond this optimum, the voltage decreases to ~29 mV at 2.5 g, suggesting inhibitory effects due to excessive salinity. At 3 g, the voltage slightly recovers to ~42 mV but remains below the peak value at 1.5 g.

These results are consistent with the ANOVA analysis, confirming that NaCl concentration has a statistically significant effect on system performance.

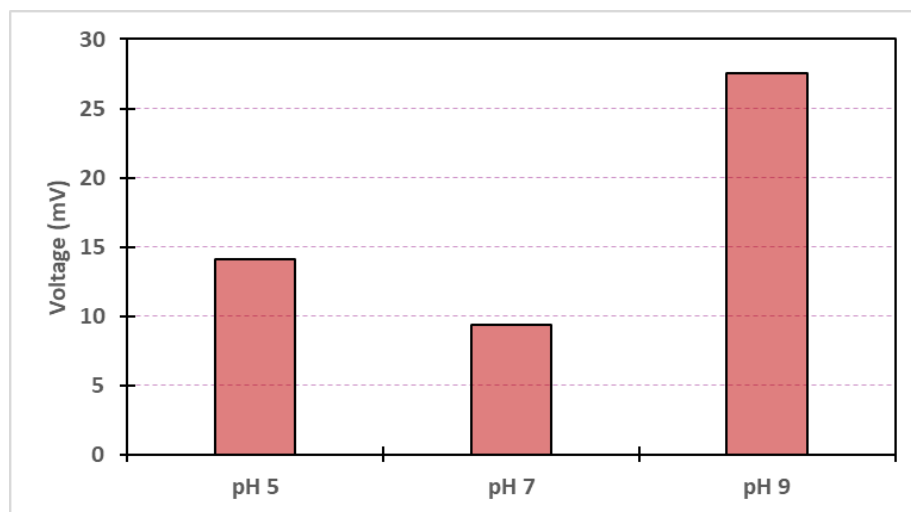


**Figure 4.33** ANOVA for the effect to NaCl

#### 4.12 Statistical Analysis of the Effect of pH on Voltage Using ANOVA

The figure 4.34 illustrates the influence of pH on voltage output, showing a clear dependence on the acidity/alkalinity of the system. The voltage decreases from ~14 mV at pH 5 to ~9 mV at neutral pH 7, indicating reduced electrochemical activity under neutral conditions.

However, a significant increase is observed at pH 9, where the voltage reaches ~27–28 mV, representing the highest performance among the tested conditions. This suggests that alkaline conditions favor microbial activity and electron transfer processes in the system. These observations are consistent with the ANOVA results, confirming that pH has a statistically significant effect on voltage generation.



**Figure 4.34** ANOVA for the effect to pH

### 4.13 Overall Comparative Assessment

In this section, all experimental cases that are studied in this work are compared based on the voltage value that are observed. Table 4.27 summarizes the best cases for each case. From the data shown in Table 4.27, the dependence of voltage generation in the SMFC is seen to be significantly influenced by environmental parameters. There is an optimal operating window for each parameter that contributes to the best voltage generation. When analyzing the impact of temperature, it should be noted that the maximal voltage (90 mV) was generated under baseline conditions with temperature being equal to 25 °C. This suggests that the best voltage generation requires a moderate temperature when stable microbial activity is achieved. According to the impact of substrate concentration, the voltage (160 mV) was highest when 1.5 g of glucose was used and 40 °C temperature. The obtained information proves that there is an optimal amount of substrates for microbial cells' metabolism and for extracellular electron transfer.

**Table 4.27** Summary of the best cases experimentally tested

| <b>Parameter</b>      | <b>Best Condition Tested</b> | <b>Highest Voltage reached</b> |
|-----------------------|------------------------------|--------------------------------|
| Temperature           | 25°C                         | 90 mV                          |
| Glucose concentration | 1.5g at 40°C                 | 160 mV                         |
| NaCl concentration    | 1g at 40°C                   | 100 mL                         |
| pH                    | 7 at 40°C                    | 950 mL                         |
| Water Content         | 300 mL at 25°C               | 426 mL                         |

Regarding electrolyte concentration, the voltage value (~100 mV) was higher when NaCl concentration was 1.0 g and temperature was 40 °C. This finding suggests that there is an optimal level of electrolyte that minimizes ohmic losses in SMFC operation. The joint influence of two parameters – pH and temperature – resulted in the most significant improvement of SMFC characteristics with the voltage being equal to 950 mV at pH 7 and

40 °C. Neutral pH allows to maintain active microbial enzymes, protons' mobility and proper functioning of biofilms in SMFC. Another important characteristic analyzed was water content. The best voltage (426 mV) generation was registered when 300 mL water was present and the temperature equaled 25 °C. Optimal moisture content provides good ionic transport and minimization of SMFC internal resistance.

Thus, the results obtained prove that voltage generation in SMFC is influenced by biological, electrochemical and environmental parameters with particular ranges of their values resulting in best voltage generation.

## 5. CONCLUSIONS AND RECOMMENDATIONS

This chapter presents a brief description of the main results obtained from the experimental study of the soil-based microbial fuel cell system. The study aimed to assess the effect of various factors such as temperature, pH, glucose concentration, sodium chloride solution addition, and volume of water on voltage production. The results demonstrate the intricate relationship between microbial activity, electrochemical reactions, and environmental factors in microbial fuel cells. Finally, suggestions on how to improve system stability and operational parameters, and how to improve bioelectricity production in microbial fuel cells based on soil media, are presented.

### 5.1 Conclusions

In soil-based microbial fuel cells, environmental parameters play a crucial role in governing microbial activity, electrochemical reactions, and overall system performance. Temperature was also identified as a parameter of significant influence. Under the 45hr test, moderate temperature levels of 35-45 °C resulted in better and more stable voltage outputs compared to both lower (25-30 °C) and higher temperature levels. Specifically, it was established that a temperature of 45 °C resulted in the highest sustained voltage output of 55 mV, whereas a temperature of 35 °C resulted in a voltage output of ~50 mV. On the other hand, the temperature of 50 °C was characterized by voltage fluctuations from 30 mV, reducing to ~16 mV. These findings confirm that excessive temperature levels lead to microbial stress, causing reduced long-term electrochemical stability, whereas moderate temperature levels enhance microbial kinetics and EET. The most important conclusions obtained from this work can be listed as follows:

- Regarding the experiment under a 1:1 soil/water ratio, it was established that the highest voltage outputs were recorded at a temperature of 25 °C, at which voltage peaks approached ~90 mV. Voltage outputs decreased at 30 °C, 35 °C, and even more at 40 °C. This indicates that optimal voltage outputs are dependent on temperature levels. These findings confirm that microbial adaptation, substrate, and internal resistance are significant factors in voltage output trends.
- Glucose concentration experiments revealed the existence of an optimum substrate range. Specifically, under a temperature of 25 °C, it was established that a concentration of 1.0 g resulted in the highest sustained voltage output of 66 mV,

whereas a concentration of 0.5 g resulted in substrate limitation, causing a significant reduction in voltage outputs from 20 mV to a final voltage of ~20 mV. On the other hand, concentrations of 2.5 g and 3.0 g resulted in excessive voltage peaks, characterized by significant voltage instability due to substrate competition and possible accumulation of intermediate metabolites. Similarly, at a temperature of 40 °C, excessive glucose concentration resulted in voltage fluctuations, causing reduced voltage stability.

- The experiments with sodium chloride (NaCl) showed that the relationship between salinity and voltage output is non-linear. At 25°C, moderate levels of NaCl (1.5 g) showed improved performance, whereas excessive levels of NaCl inhibited the performance of the MFC by suppressing the voltage output. At 40°C, the highest voltage output was achieved at the lowest level of NaCl (1.0 g), indicating that excessive levels of NaCl cause osmotic stress.
- The pH and temperature experiment showed that at neutral pH (7) and at elevated temperatures (40°C), the voltage output was extremely high, reaching up to 950 mV, whereas at both acidic (5) and alkaline (9) pH levels, the performance of the MFC was compromised, indicating that proton transport, biofilm formation, and microbial tolerance are critical factors in the performance of the MFC.
- The volume of water (moisture levels in the soil) was also a critical factor in the performance of the MFC, as the highest voltage output was achieved at the 25°C, 300 mL condition, indicating that excessive levels of moisture reduce the internal resistance of the MFC, whereas the decline in the voltage output after some time indicates that at high levels of moisture, the redox balance of the MFC is compromised.
- The overall results of the experiment show that the performance of the MFC depends on the balance between microbial, electrochemical, ionic, and environmental factors, as the performance of the MFC was optimal at moderate levels of temperature (35-45°C), pH (7), NaCl (1.0 g), glucose (1.0 g), and moisture levels (300 mL at favorable temperatures).

The results of the study reveal that an increase in voltage corresponds with improved biofilm maturation, increased transfer of electrons, and decreased internal resistance, while

a decrease in voltage relates to substrate depletion, microbial competition, osmotic stress, thermal inhibition, and mass transfer limitations. This understanding of the biological and electrochemical mechanisms involved in MFCs in soils offers a comprehensive knowledge base for future optimization strategies for these systems.

## **5.2 Recommendations**

Based on the experimental findings of this study, several recommendations are proposed to guide future research and support the advancement of soil-based microbial fuel cell technology.

- The MFC system should be scaled up to pilot-scale and industrial-scale levels to determine its technical feasibility, long-term stability, and economic viability under real operating conditions.
- A detailed life cycle assessment should be conducted to evaluate the overall sustainability of the MFC system, including material use, environmental impacts, and end-of-life considerations.
- Future work should explore integrating MFCs with wastewater treatment processes and hybrid bioelectrochemical systems, as such configurations can enhance both pollutant removal efficiency and energy generation.
- Further optimization of operating conditions is necessary to improve voltage output and overall system performance. This includes refining electrode materials, soil characteristics, moisture levels, and microbial activity.
- System integration studies, along with optimization, should be performed to show the potential of MFCs for real-world applications as a sustainable, cost-effective, and environmentally friendly energy technology capable of contributing to future green energy systems.

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