SIMULTANEOUS GENERATION OF ELECTRICITY AND WASTEWATER TREATMENT USING SINGLE CHAMBERED MICROBIAL FUEL CELL

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A thesis submitted to Bahria University, Islamabad in partial fulfillment of the requirement for the degree of B.S in Environmental Sciences

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DEDICATION

We dedicate this thesis to our families, who have been our rock throughout our academic journey. Their unwavering love, support, and encouragement have sustained us through the highs and lows of this challenging process. We are grateful for their patience and understanding as we have spent countless hours researching, writing, and revising. Their belief in us has fueled my determination to complete this thesis to the best of our abilities. We also dedicate this thesis to our mentors, who have provided invaluable guidance, feedback, and expertise. Their mentorship has shaped us both as a scholar and as a person, and we are grateful for their generosity in sharing their knowledge and time. Lastly, we dedicate this thesis to all those who are committed to advancing knowledge in their respective fields. May our collective efforts contribute to a better world for future generations.

ABSTRACT

Microbial fuel cells (MFCs) hold great promise as a renewable energy source that utilizes the metabolic activity of microorganisms to generate electricity. However, MFC performance is influenced by various factors, such as pH, mediator selection, and food source. This study aimed to investigate the impact of these variables on MFC power output. The results showed that pH is a crucial factor in determining MFC performance, with an optimal range required for maximum output. Increasing pH to 9.5 negatively impacted microorganism activity and led to a decrease in current output. Mediator selection also played a critical role in enhancing MFC efficiency, with a higher redox potential leading to increased power output. The study also found that food source selection is critical to achieving maximum power output, with easily degradable and sustainable sources improving MFC performance. The performance of MFC was also investigated by measuring water parameters such as BOD and COD and exploring the use of a mediator and bagasse as a food source. The addition of a phosphate buffer as a mediator resulted in a gradual increase in current output, reaching a maximum of 75.3 mV at week 4.5. The use of bagasse as a food source led to a high initial current output of 91.7 mV at week 1.5, likely due to the presence of easily degradable components. However, the current output decreased over time, reaching a low of 10.2 mV at week 4.5, as the microbial community depleted these nutrients. This paper suggests that pH, mediator selection, and food source are critical factors that must be considered when designing MFCs. By optimizing these variables, MFCs may be developed into a more efficient and sustainable source of energy that could help address global energy challenges.These results highlight the importance of developing new food sources for MFCs that can improve their efficiency and sustainability. These results represent an important step towards the development of more efficient and sustainable sources of energy generation, which is a crucial global challenge.

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CHAPTER 1

INTRODUCTION

As the world's growing population continues to expand and develop, so makes the demand for energy. Unfortunately, traditional energy sources including fossil fuels are limited and non-renewable, causing them to deplete at a very fast pace. This not only provides a tremendous problem for addressing future energy requirements but also has substantial environmental consequences. The production, delivery, and utilization of energy significantly influence both natural and human surroundings, ranging from changes in land use and the discharge of contaminants to the harmful impacts of fuel cycles such as coal and biomass. To overcome these concerns, it is critical to shift toward renewable energy sources such as fuel cells. Microbial fuel cells use chemical processes to transform the energy held in fuels into usable power, making them a safe and effective way to create electricity. Fuel cells have the potential to supply a reliable alternative to energy in the future, making them a possible solution to the world's energy dilemma.

1.1 Renewable Energy Sources and their Share in the Global Energy Mix

Renewable energy sources such as hydro, biomass, geothermal, solar, wind, and marine energies. Renewable energy sources are becoming increasingly popular as a sustainable alternative to traditional energy sources like coal, gas, and oil. These renewable resources, such as hydro, biomass, geothermal, solar, wind, and marine energies, are considered clean and green, emitting little or no harmful carbon monoxide, carbon dioxide, and sulfur dioxide (Okedu, K. E., A. Tahour, et al. 2020). In 2019, the global proportion of renewable energy in the total primary energy supply was 17.5%, according to the International Renewable Energy Agency (IRENA). Advanced renewables (excluding traditional biomass) made up 12.9% of the total (Okedu, K. E., A. Tahour, et al. 2020).

Biomass is the most significant renewable energy source, accounting for over 11% of the total world primary energy output in 2019 (Bhatia, S. K., A. K. Palai, et al. 2021). This includes both contemporary biomass (biogas and fuels) and traditional biomass (for cooking and heating, such as wood and animal manure). Hydropower, solar power, wind power, and geothermal energy are also major renewable energy sources, accounting for 6.7%, 2.3%, 3.9%, and 0.4% of the total world primary energy output in 2019, respectively (Bhatia, S. K., A. K. Palai, et al. 2021).

Although renewable energies have the potential to significantly contribute to the global energy mix, their adoption is not without challenges. Some renewable energy sources, such as solar and wind, are intermittent and not always available to generate power (Ellabban, O., H. Abu-Rub, et al. 2014). In addition, the resources and infrastructure required for the development of various renewable energy sources can be costly, limiting their widespread adoption. However, ongoing research and development in the renewable energy sector are helping to address these issues.

The renewable energy mix can vary greatly by region due to factors such as topography, climate, and economics. For example, hydropower is a significant source of renewable energy in Brazil, which has a suitable environment for hydroelectric power. China is also a significant producer of hydropower and traditional biomass energy. The United States and Europe have a higher adoption of solar and wind power, while traditional biomass is still a common energy source in underdeveloped countries, particularly in Asia and Africa (Demirbas, A., A. Sahin-Demirbas, et al.2004). However, traditional biomass can have negative impacts on the environment and human health, such as environmental pollution and deforestation.

Natural resource depletion is a pressing global issue that require immediate attention. The overuse and exploitation of resources such as water, fossil fuels, and minerals not only leads to their depletion, but also has negative impacts on the environment and economy. Governments and individuals must adopt sustainable policies and practices that prioritize resource conservation and responsible usage in order to address this problem. This can include the implementation of renewable energy sources, sustainable agriculture techniques, and active promotion of waste management and consumption management. In addition to these measures, addressing population growth and promoting economic development that is not reliant on resource depletion are also important strategies for addressing this issue. If action is not taken, the depletion of these resources could have severe consequences for future generations.

1.2. The Environmental, Social, and Economic Impact of Renewable Energy

1.2.1 Environmental Impacts

Renewable energy technologies can have both positive and negative effects on the environment. Positive impacts include reduced greenhouse gas emissions, conservation of natural resources, and improved air and water quality (Sayed, E. T., T. Wilberforce, et al. 2021). Negative impacts include the potential for noise pollution, habitat destruction, and negative effects on local ecosystems. It is important to assess the potential environmental impacts of renewable energy projects and put measures in place to reduce or mitigate these impacts wherever possible.

1.2.2 Social impacts of renewable energy

Renewable energy can have both positive and negative impacts on society. Positive impacts include the development of new employment and economic opportunities, as well as improvements in water and air quality, which can benefit public health. Negative impacts include the possibility of conflict with local populations and displacement of people as a result of the construction of renewable energy projects (Okedu, K. E., A. Tahour, et al. 2020). It is important to evaluate the potential social impacts of renewable energy projects and put measures in place to reduce or mitigate these impacts wherever possible.

1.2.3 Economic impacts of renewable energy

The potential for new jobs and economic opportunities is one of the main economic benefits of renewable energy. Renewable energy project development and operation can generate direct jobs in the renewable energy industry as well as indirect jobs in related industries such as manufacturing and construction. Additionally, the use of renewable energy can result in economic benefits by lowering energy costs for businesses and individuals, making them more competitive in the global economy (Okedu, K. E., A.

Tahour, et al. 2020). Renewable energy can also provide indirect economic benefits by reducing the negative externalities associated with fossil fuel consumption, such as air pollution and greenhouse gas emissions.

1.3 Potential of MFC in Pakistan

Microbial fuel cells (MFCs) have the potential to address energy shortages and increase energy security in Pakistan. MFCs can generate power from agricultural waste, which is abundant in Pakistan and can also be used to treat and purify wastewater, which would benefit both public health and the environment (Ullah, Z. and S. Zeshan 2020). MFCs can also generate electricity in remote areas where traditional energy sources are scarce. Pakistani researchers are currently working on developing and optimizing MFC technology, using locally available materials and microbes. However, MFC technology is still in its early stages of development and faces technological challenges before it can be widely implemented. MFCs can be a supplementary energy source, providing a stable and sustainable supply of electricity for small-scale applications with low energy demand, but may not be able to meet the energy demands of large-scale industrial operations that require large amounts of power (Javed, M. M., M. A. Nisar, et al. 2017). Pakistan has a significant renewable energy potential, including solar, wind, and biomass resources, but has not fully utilized these resources, with renewables making up only 4% of the overall energy mix (Wang, Y., L. Xu, et al. 2020). Solar and biomass energy in particular show great potential in Pakistan, but weak regulations and poor management have hindered the development of these technologies (Yaseen, M., F. Abbas, et al. 2020).

Energy security and environmental concerns are driving growing worldwide biomass usage, even in low-income nations like Pakistan. It is critical to understand the present energy system in different sections of the country in order to properly utilize local biomass reserves to fulfill future energy demands (Yaseen, M., F. Abbas, et al. 2020). Various programs and technologies are in the works to transition away from fossil fuels and minimize greenhouse gas emissions.

1.3.1 The main advantages of MFCs include

1. Efficiency: MFCs can operate at higher efficiencies than traditional fuel cells, meaning they can convert more of the energy in the fuel into electricity.
2. Cost: MFCs can be made using low-cost materials and manufacturing techniques, making them a potentially more affordable option for electricity generation.
3. Size and portability: MFCs are small and lightweight, making them easy to transport and install in various locations.
4. Flexibility: MFCs can be powered by a variety of fuels, including biomass, making them a flexible option for electricity generation.
5. Sustainability: MFCs can be powered by renewable fuels such as biomass, making them a more sustainable option for electricity generation. (Parkhey, P. and R. Sahu 2021).

1.4 Non-Renewable Energy Resources

Fossil fuels, including oil, gas, and coal, are currently the primary sources of energy for the world. These sources are non-renewable, meaning that once they are used up, they will not be replenished. The world currently has approximately 1688 billion barrels of proven oil reserves, 6558 trillion cubic feet of proven natural gas reserves, and 891 billion tons of proven coal reserves (Abas, N., A. Kalair, et al. 2015). These reserves are being consumed at rates of 0.092 billion barrels of oil, 0.329 trillion cubic feet of natural gas, and 7.89 billion tons of coal per day. However, the world's consumption of fossil fuels is increasing at a faster rate than the rate at which new reserves are being discovered, with an annual increase in consumption of 1.4 million barrels of oil, 4.5 billion cubic feet of natural gas, and 3.1 million tons of coal (Abas, N., A. Kalair, et al. 2015).

The use of fossil fuels has significant environmental impacts, as the burning of these fuels releases large amounts of carbon dioxide into the atmosphere. This contributes to the problem of climate change, which is caused by an increase in greenhouse gasses in the atmosphere (Güney, T. 2019). To address this issue, it is necessary to transition to more sustainable and renewable energy sources. Renewable energy sources, such as solar and wind power, do not produce greenhouse gasses when they are used and have the potential to provide a significant portion of the world's energy needs. However, the transition from fossil fuels to renewable energy sources will require significant initial investment and the development of new technologies (Güney, T. 2019).

1.5 Microbial fuel cell

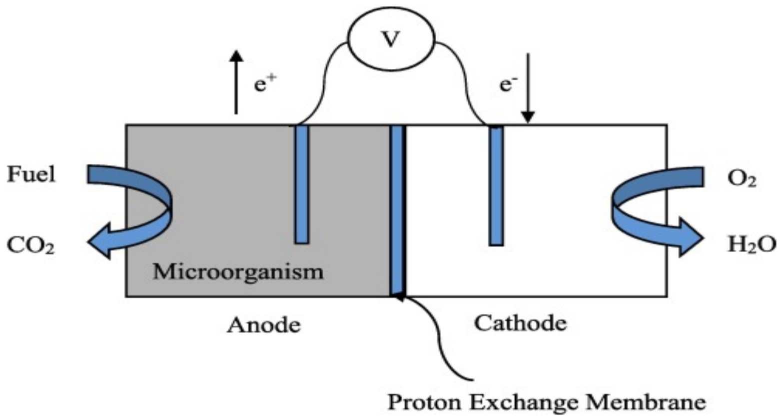


Figure. 1.1 Schematic diagram of simple MFC

In the transportation industry, the focus has switched to renewable energy sources and fuel cells due to the unsustainable nature and environmental effects of fossil fuels. In this investigation, the production of energy from electrons liberated during biological processes aided by bacteria is assessed (Obileke, K., H. Onyeaka, et al. 2021). Microbial fuel cells (MFC) are an environmentally benign way to produce energy while also cleaning wastewater. They can remove up to 50% of the chemical oxygen demand and have power densities of 420–460 mW/m2. The system generates electricity by using the power of bacterial metabolism (Obileke, K., H. Onyeaka, et al. 2021).

The idea of MFCs is not brand-new. The original theory was tested in 1910, but due to its low current density, weak power output, and reliance on electron mediators to transport electrons from the cell to the anode, it received little attention. Following a significant advancement in the field that revealed some microorganisms may be capable of transferring electrons straight to the anode, research into MFCs accelerated (Santoro, C., C. Arbizzani, et al. 2017).

A form of renewable energy technology known as microbial fuel cells (MFCs) uses microbes to produce power through metabolic processes. Due to its ability to remove up to 50% of the chemical oxygen requirement and power densities ranging from 420–460 mW/m2, MFCs have attracted interest as a technique to produce energy while also cleaning wastewater (Santoro, C., C. Arbizzani, et al. 2017). Bacteria are used in MFCs to oxidize both organic and inorganic materials and generate electricity. The bacteria's generated electrons go from the cathode (positive terminal) to the anode (negative terminal) over a resistor or under a load. A proton-selective membrane separates the cathode from the anode (Franks, A. E. and K. P. Nevin 2010).

Although MFCs have been theoretically possible since 1910, it wasn't until recent advances in the area that scientists learned which bacteria may transmit electrons straight to the anode, increasing efficiency and power production. MFCs have the potential to contribute a substantial contribution to the renewable energy industry by providing a potentially environmentally friendly method of producing power while cleaning wastewater. The capacity of MFCs to burn a range of organic resources as fuel, such as wastewater, food waste, and byproducts from forestry and agriculture, is one of its key advantages (Franks, A. E. and K. P. Nevin 2010). MFCs may utilize materials that would normally be thrown away, making them a sustainable choice for producing power.

MFCs have been utilized in a number of applications, including as generating electricity in underdeveloped nations, powering distant sensors and communication systems, and purifying sewage. For instance, MFCs have been used to run a meteorological station in a remote part of Canada where there are no conventional sources of electricity. Brewery wastewater has also been treated using MFCs, resulting in considerable energy savings and decreased greenhouse gas emissions. MFCs have been utilized in poor nations to deliver electricity to off-grid areas, enhancing quality of life and enhancing access to energy.
When employing acetate as fuel, the following common reactions take place at the anode and cathode:

Anodic reaction:

$CH_3COOH + 2H_2O \; microbe \rightarrow 2CO_2 + 8e^- + 8H^+$ ------------------(1)

Cathodic reaction:

$2O_2 + 8e^- + 8H^+ \rightarrow 4H_2O$ --------------------(2)

Overall reaction

$CH_3COOH + 2O_2 \; microbe \rightarrow 2H_2O + 2CO_2$ --------------------(3)

At the MFC's anode, the anodic process (equation 1) takes place. Acetate (CH_3COOH) and water (H_2O) are the reactants in this equation, whereas carbon dioxide (CO_2) and protons (H^+) are the products. The bacteria oxidize the acetate during this process, producing electrons that are then transferred to the anode. The proton-selective membrane allows the protons to be transported to the cathode.

At the MFC's cathode, the cathodic reaction (equation 2) takes place. In this equation, oxygen (O_2) and protons (H^+) are the reactants, while water is the product (H_2O). Protons and oxygen combine to create water molecules in this process. The external circuit allows the electrons that were moved from the cathode to the anode during the anodic reaction to flow to the cathode, completing the circuit.

The anodic and cathodic processes combine to form the overall reaction (equation 3). In this equation, the reactants are acetate and oxygen, while the products are water and carbon dioxide. This reaction shows the general process of generating electricity in an MFC, in which bacteria oxidize acetate and transport electrons to the anode, while oxygen and protons combine to produce water molecules at the cathode. The movement of electrons via the external circuit generates a current, which may be utilized to power a load or do labor (Zhou, M., J. Yang, et al. 2013). MFCs are a promising renewable energy technology with several possible uses. MFCs have the potential to contribute significantly to the renewable energy mix with more research and development.

1.5.1. Working of MFCs

Modern microbial electrochemistry terminology and technologies were categorized by Schroder. Faraday and capacitive mechanisms are the foundation of microbial electrochemistry (ME). Direct extracellular electron transfer and mediated extracellular electron transfer are the mechanisms of ME technologies, which occur in the anodic chamber. Fewer processes have been documented to occur in the cathodic chamber (Zhou, M., J. Yang, et al. 2013). The oxidation of a microbial biofilm of organic materials in the anodic chamber produces protons (H+) and electrons (e). In a subsequent reduction step, these ions are used to create hydrogen and methane. The anodic biofilm undergoes a forward reaction and has a catalytic effect inside the chamber. As a result, electrons are liberated from the anode's electrogenic biofilm and are guided to the cathode from an external circuit. In the cathodic chamber, anodic electrons decrease H2O while protons form the hydroxyl ion (OH) and hydrogen gas (H2) (Slate, A. J., K. A. Whitehead, et al. 2019). The metabolic capabilities and substrate utilization of the microbial species being employed are constant determinants of the anodic electron transfer rate, but further study is needed to understand how different MFCs interact with their substrates and how different microbial species interact with one another.

1.6 Types of microbial fuel cells

1.6.1. Mediator MFCs

The focus of mediator MFCs is on the transport of electrons toward the anode via bacterially mediated electron transfer (Obileke, K., H. Onyeaka, et al 2021). As depicted in Fig. 1, this form of MFC uses chemical mediators to assist in the electron flow to the anode, including neutral red, humic acid, anthraquinone-2, 6-disulfonate, and others. Logan did note that the typical mediators are neutral red, potassium ferricyanide, and methyl viologen (Logan, B. E., B. Hamelers, et al. 2006). The term "electroactive metabolites" refers to these chemical mediators. Because these mediators are expensive and harmful, Flimban et al. recommended that we need to find alternate strategies to enhance power generation and lower capital costs (Flimban, S. G., T. Kim, et al. 2018).. The viewpoint provided by Flimban et al. was appropriate for the treatment of wastewater.

For mediator MFC, anaerobic digestion is crucial due to the presence of oxygen that steals electrons, interfering with the mediator work because oxygen is less electronegative than other gasses (Flimban, S. G., T. Kim, et al. 2018). The mediator enters the cell during the transfer of electrons, receiving electrons before freeing and transferring them to the anode, which serves as the final electron acceptor. The mediator now deposits its electrons and is oxidized back to its original condition (Flimban, S. G., T. Kim, et al. 2018). According to the study, MFCs function at a high sustained level of physical activity because bacteria can generate their mediator or transport electrons directly to the electrode (Obileke, K., H. Onyeaka, et al 2021).

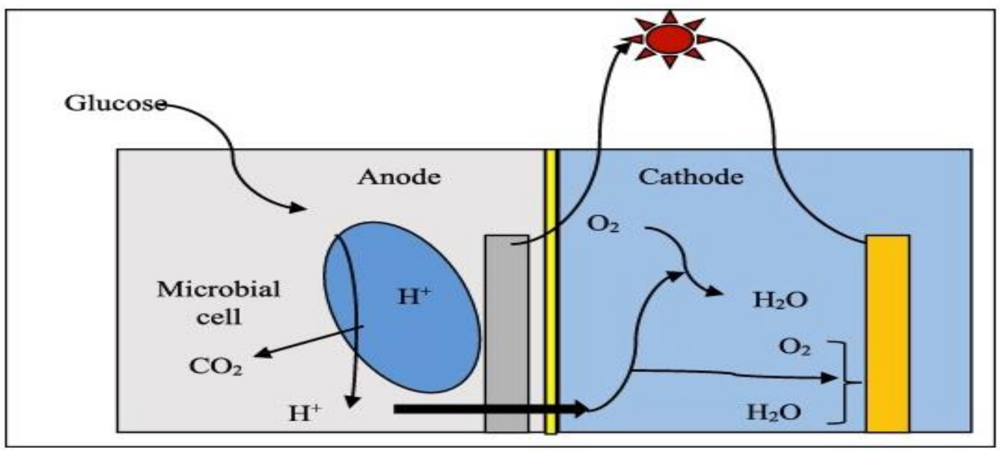


Figure.1.2 Schematic diagram illustrating the flow of electrons to the anode through the chemical mediator.

1.6.2. Mediator less MFCs

Microorganisms can be used to create electricity without the use of intermediaries. No additional external mediators are incorporated into the system in this kind of MFCS (S. Dharmalingam, et al 2019). The majority of wastewater bacteria carry electrons to electrodes in order to generate energy utilizing nanowires, which are long appendages (Logan, B. E., B. Hamelers, et al. 2006). Due to their non-toxicity and lower cost, mediator-less MFCs have an advantage over mediator MFCs. Oh, and Logan reported that the dissimilatory metal-reducing Clostridium butyricum is used to operate the majority of the mediator-less MFCs (Logan, B. E., B. Hamelers, et al. 2006).The presence of electrochemically active redox enzymes for effective transfer of electrons to the anode, fuel oxidation at the anode, the external resistance of the circuit, oxygen reduction at the cathode, and the transfer of proton to the cathode through the membrane that results in pH variation and also inhibits microbial activities are some factors to be taken into account for the mediator-less MFC (Flimban, S. G., T. Kim, et al. 2018). Additionally, certain plants such reed sweet grass, cordgrass, rice, tomatoes, lupines, and algae can provide energy directly to mediator-less MFCs. Plant microbial fuel cells are this method or setup. The mediator-less MFCs are therefore created as microbial electrolysis cells, soil based microbial fuel cells, and phototrophic biofilm microbial fuel cells (Paulraj, C. R. K. J., M. A. Bernard, et al. 2019).

1.6.3. Single-Chamber Microbial Fuel Cells (MFCs)

Single-chamber microbial fuel cells (MFCs) are devices that use microorganisms to convert the chemical energy present in organic matter into electricity. These MFCs consist of a single chamber that contains both the microorganisms and the electrode, and they operate by using bacteria to break down organic matter, such as wastewater, and generate electrons in the process (Logroño, W., G. Ramírez, et al. 2015).

In a single-chamber MFC, the microorganisms consume the organic matter in the wastewater and produce electrons, which are transferred directly to the electrode (Logroño, W., G. Ramírez, et al. 2015). The electrical current generated by the MFC can then be used to perform a variety of tasks, such as powering a pump or providing electricity to a remote location (Wu, Y., X. Zhao, et al. 2018).

Single-chamber MFCs have several advantages over other types of MFCs, including simplicity of design, low cost, and ease of maintenance. However, they may not be as efficient at generating electricity as other types of MFCs, and they may produce lower levels of COD removal, which is a measure of the organic pollutant load in wastewater (Wu, Y., X. Zhao, et al. 2018).

1.7 Applications of MFC for wastewater treatment

MFCs are a bioelectrochemical system that primarily uses electro-active microorganisms for the treatment of wastewater, the production of bioenergy, and other purposes. The finest Bioelectrochemical Systems (BES) systems for treating wastewater and other applications that depend on the electrogenic characteristics of bacterial populations or single strains are MFCs (Li, W.-W., H.-Q. Yu, et al. 2014).Wastewater serves as a substrate for bacterial development in this system. MFC technology's enormous potential for the treatment of water without the use of traditional techniques has drawn numerous engineers and research scientists from around the world. The byproducts of this process, which are anticipated to include bio-flocculants, bioplastics, bioelectricity, bio-hydrogen, methane, and many other value-added products created using wastewater as a substrate, are gaining increasing scientific attention (Li,W.-W., H.-Q. Yu, et al. 2014).

1.7.1. Wastewater treatment

Microbial fuel cells (MFCs) use microorganisms to treat wastewater by breaking down the organic matter present in the wastewater and generating electricity in the process. This process is known as anaerobic digestion, and it occurs in the absence of oxygen (Oh, S. T., J. R. Kim, et al. 2010).The microorganisms in an MFC are typically bacteria, such as Escherichia coli or Rhodopseudomonas palustris, which are able to consume organic matter and produce electrons as a byproduct of their metabolism. The bacteria consume the organic matter in the wastewater and transfer the electrons to an electrode, where they can be collected and used as an electrical current.

The efficiency of an MFC in treating wastewater depends on several factors, including the type and concentration of the organic matter in the wastewater, the type and density of the microorganisms used, the design and materials of the MFC, and the operating conditions of the system. In general, MFCs are able to achieve high levels of chemical oxygen demand (COD) removal, which is a measure of the organic pollutant load in wastewater (Oh, S. T., J. R. Kim, et al. 2010). MFCs have also been shown to have high power densities, which is a measure of the amount of electricity generated per unit of volume, and high coulombic efficiencies, which is a measure of the percentage of electrons that are transferred to the electrode.

Another example of using MFCs for wastewater treatment is the use of a single-chamber system, where the microorganisms and the electrode are contained in the same chamber. In this case, the microorganisms consume the organic matter in the wastewater and produce electrons, which are transferred directly to the electrode (Li, W.-W., H.-Q. Yu, et al. 2014). The electrical current generated by the MFC can then be used to perform a variety of tasks, such as powering a pump or providing electricity to a remote location. However, there is still room for improvement in the design and performance of MFCs, and research is ongoing to optimize the efficiency and effectiveness of these systems for wastewater treatment.

1.7.2. Bioremediation in Microbial Fuel Cells

Microbial fuel cells (MFCs) can be used for bioremediation, the process of using microorganisms to remove or neutralize pollutants from the environment. Electrotrophic microorganisms, which can obtain electrons from electrodes, are especially useful in MFCs for bioremediation (Verma, J., D. Kumar, et al. 2021). MFCs can be designed to remove heavy metals from wastewater, which is a significant concern due to the harmful effects of these metals on living organisms. Traditional methods of removing heavy metals from wastewater can be complex and difficult, making MFCs a promising alternative. MFCs have been shown to be effective in removing a variety of heavy metals, including copper, zinc, cadmium, antimony, lead, manganese, and iron (Verma, J., D. Kumar, et al. 2021). However, the effectiveness of different MFC designs in removing heavy metals may vary. Future research may involve the use of different MFC designs to improve heavy metal removal rates.

Objectives

1. To construct lab scale single chambered Microbial Fuel Cells.
2. To assess the effect of various chemical parameters on electricity generation by Microbial Fuel Cells.
3. To assess the effect of various chemical parameters on wastewater treatment by Microbial Fuel Cells.

CHAPTER 2

MATERIALS AND METHODS

2.1 Site Sampling

Two water samples were collected from the I-9 Treatment Plant on October 5th, 2022, between 11 am and 12 pm. The treatment plant, located in I-9/1, Islamabad, serves as the main sewage treatment facility for the city and is managed by the Capital Development Authority (CDA). The plant processes wastewater from both residential and industrial areas to make it safe for release into local streams and rivers. With a daily treatment capacity of 70 million gallons, the I9 Treatment Plant plays a crucial role in maintaining the environmental health of Islamabad.

2.2 Apparatus

The materials used in this study were specifically selected for use in single-chamber microbial fuel cells and included insulated copper wires, rod-shaped graphite electrodes with terminals, large-capacity 18000 ml beakers, and a CT Digital Multimeter model DT830D.

2.3 Sampling procedure

Two water samples, each with a volume of 10 liters, were collected for microbial fuel cell analysis. The first sample was taken from the aeration tank at the I9 Treatment Plant, while the second sample was collected from the direct discharge of untreated sludge for manure. Both samples were collected in plastic gallons, in accordance with the protocols established by the Science and Ecosystem Support Division and the Operating Procedure for Wastewater Sampling as outlined by the US Environmental Protection Agency. The samples contained equal portions of activated sludge and wastewater from the treatment plant. Within one hour of collection, the samples were transported to the laboratory of the Department of Earth and Environmental Sciences at Bahria University for analysis.

2.4 Microbial Fuel Cell Construction

In the study, four laboratory-scale microbial fuel cells (MFCs) were constructed. The MFCs were composed of electrodes connected to copper wires at one end of the terminal. The electrodes were partially submerged in the sample beakers to maintain anaerobic conditions and foster the growth of a bacterial film. The first experiment aimed to observe the impact of altering the pH of the MFC on its performance. MFC 1 served as the control sample, with no modifications made to the conditions within the MFC. MFC 2, on the other hand, had its pH altered using a NaOH solution to 9.5. This alteration allowed for the examination of any changes that occurred as a result of the change in pH and provided a comparison point to the control sample.

In the second experiment, the focus was on the impact of additional components on the performance of the MFCs. MFC 3 was constructed with the addition of a mediator, phosphate buffer, which was included to examine its effect on the performance of the MFC. The mediator HNO3, a strong oxidizing agent, has been shown to enhance the performance of microbial fuel cells. MFC 4 was constructed with the addition of bagasse as a feed for bacteria. This was done to investigate the potential for the MFC to utilize renewable materials as a source of energy for bacteria, and ultimately, enhance the performance of the MFC. These experiments aimed to provide a comprehensive evaluation of the construction and performance of microbial fuel cells. By comparing the control MFC to MFCs with altered pH and the addition of a mediator and feed material, the study aimed to gain a deeper understanding of the factors that impact the performance of these devices and their potential for use in wastewater treatment and electricity generation.

2.4.1. Incubation period

During the investigation, the MFC apparatus was kept in a laboratory at room temperature for a total of 8 weeks. The first 4 weeks were dedicated to the incubation period of MFC 1 and MFC 2, while the remaining 4 weeks were for MFC 3 and MFC 4. This incubation period was crucial in allowing the bacterial film to grow and the reactions in the MFCs to occur, leading to the evaluation of the performance of the microbial fuel cells

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Trends in Electricity Generation Using Microbial Fuel Cells

3.1.1. Current/voltage Measurements in Microbial Fuel Cells

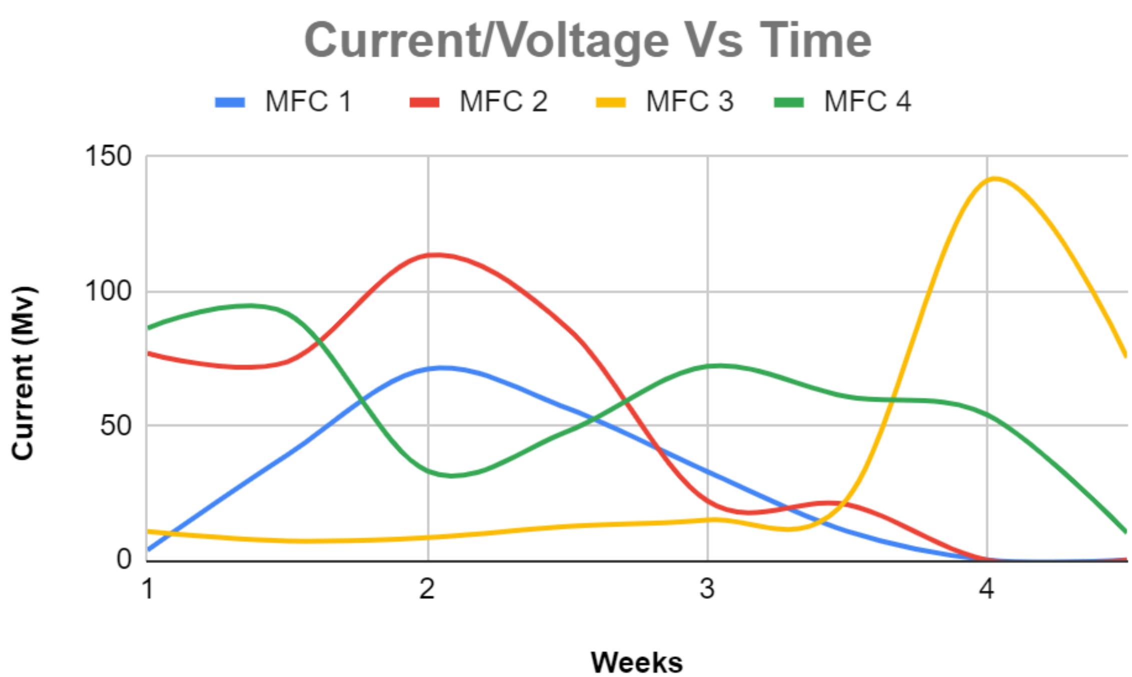


Figure 3.1 Current/voltage Measurements in Microbial Fuel Cells

The MFC 4 current readings showed that the addition of bagasse as a food source in MFC4 had the most positive impact on the MFC's performance. This may be because bagasse is a potential source of carbon and energy for microbial growth, which led to an increase in the population of microorganisms involved in the MFC. In contrast, the pH adjustment in MFC 2 initially resulted in an increase in current readings, but the benefits were not sustained over time. One possibility is that the adjustment led to a more favorable environment for the microorganisms to thrive, which resulted in increased electrical current production. For example, certain types of bacteria involved in MFCs are known to prefer slightly alkaline conditions. However, it's also possible that the pH adjustment caused an imbalance in the microbial community or led to other unintended consequences that ultimately limited the MFC's performance over time. The addition of a phosphate buffer in MFC 3 may have had a negative impact on the MFC's performance, possibly due to an imbalance in the microbial community or nutrient profile. Overall, the results suggest that the addition of a suitable food source may be a key factor in improving MFC performance.

3.1.2. Power Density Measurements in Microbial Fuel Cells

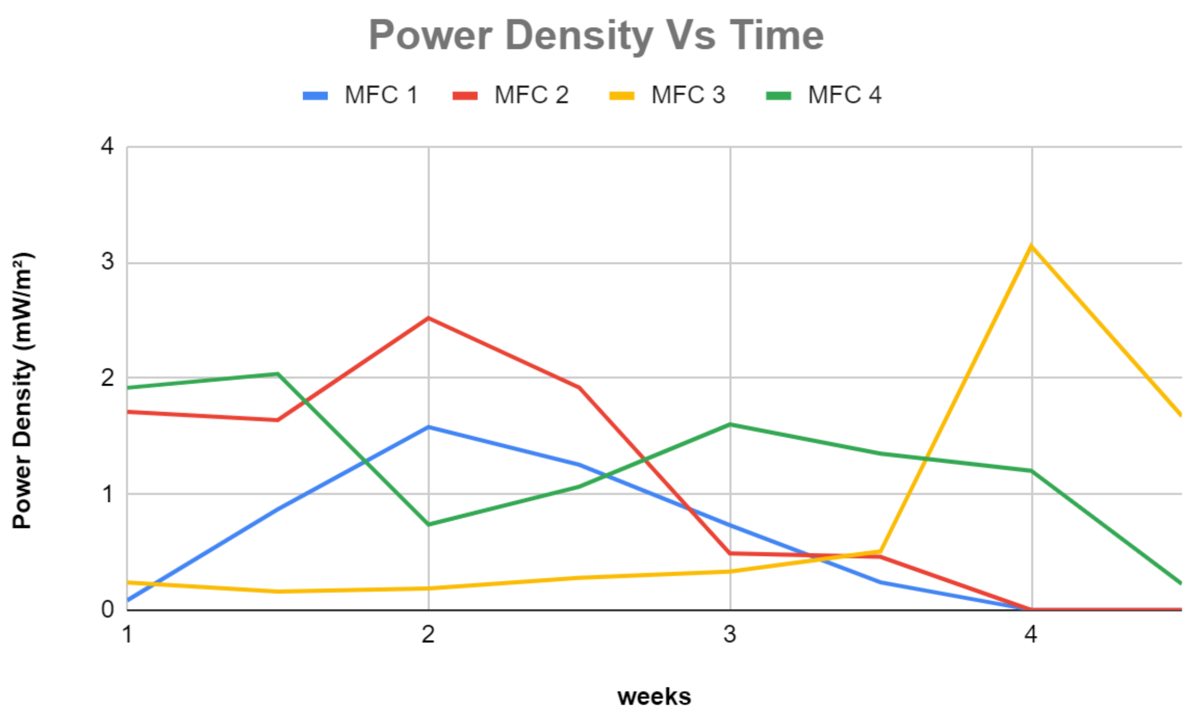


Figure 3.2 Power Density Measurements in Microbial Fuel Cells

The comparison of the power density readings from the four MFCs highlights the importance of adding a suitable food source to improve MFC performance. MFC 4 had the highest power density readings after the addition of bagasse. This could be due to bagasse being a potential source of carbon and energy for microbial growth, leading to an increase in the microorganism population involved in the MFC, resulting in increased power density. Followed by MFC 3, which had the second-highest power density readings after the addition of a phosphate buffer. MFC 2 had the third-highest power density readings after pH adjustment. The observed trend in the power density readings is consistent with the trends seen in the MFC current readings, providing further evidence that the addition of a suitable food source is crucial in enhancing MFC performance.

3.2 Wastewater Treatment Using Microbial Fuel Cells

3.2.1. Dissolved Oxygen Measured in Microbial Fuel Cells

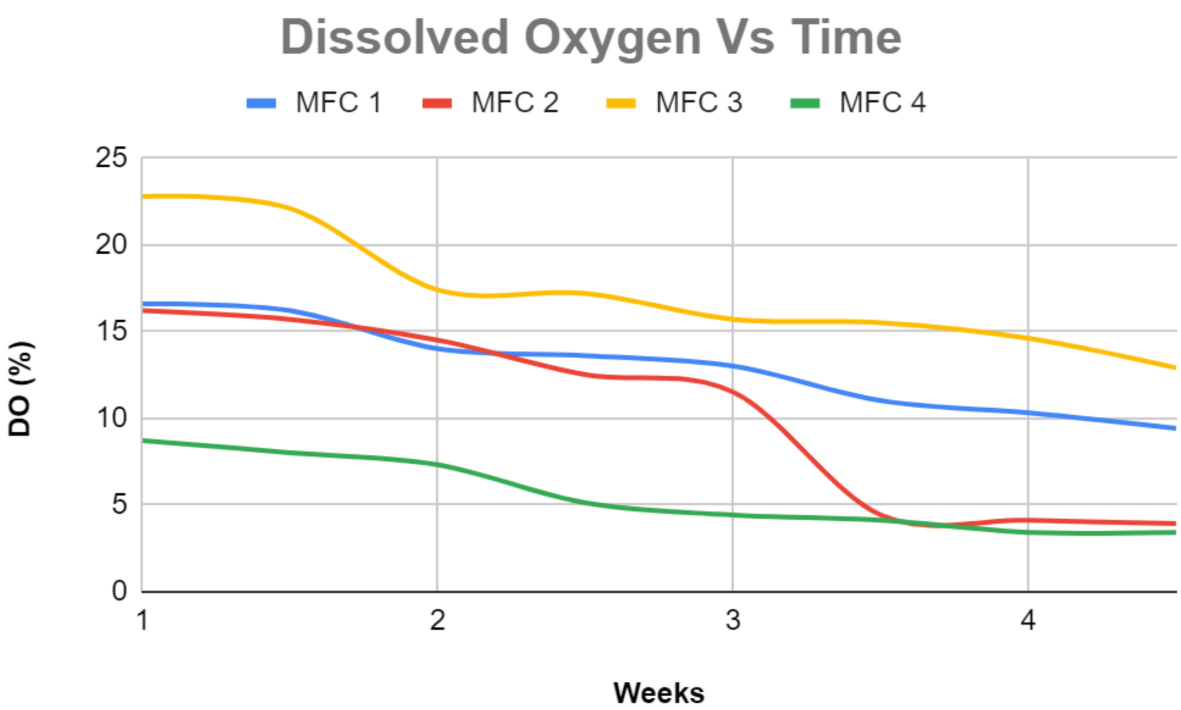
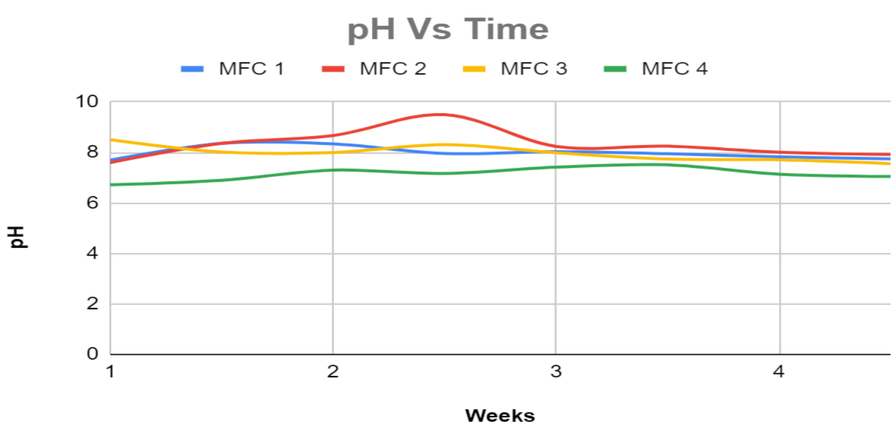


Figure 3.3 Dissolved Oxygen Measured in Microbial Fuel Cells

The dissolved oxygen (DO) data for the four MFCs suggest that the addition of a suitable substrate can influence the oxygen levels in the system. MFC 3 had the highest DO readings after the addition of a phosphate buffer, indicating that the added nutrient may have supported the growth of aerobic bacteria, leading to an increase in oxygen levels. In contrast, MFC 4 had the lowest DO readings, which may be due to the consumption of oxygen by the anaerobic bacteria involved in the breakdown of the added bagasse. MFC 2 had similar DO readings throughout the experiment, which could be attributed to the absence of a substrate that can support the growth of aerobic bacteria. The relationship between dissolved oxygen (D.O), bacterial growth, and current/voltage production is complex and depends on the specific conditions of the system. A general trend of decreasing dissolved oxygen levels over time for all four microbial fuel cells is observed. This trend may indicate that the microorganisms in the system are experiencing stress. In response to this stress, the bacteria may reduce their respiration, which ultimately results in a decrease in current/voltage production. In particular, MFC 2 and MFC 4 show a more pronounced decrease in dissolved oxygen levels compared to MFC 3. This could suggest that the addition of a carbon source to MFC 4 and the increase in pH in MFC 2 may be impacting the D.O levels in those systems. Respiration is one of the key metabolic processes that generate the electrical currents in microbial fuel cells. To prevent these fluctuations in current/voltage production, continuous addition of a carbon source and aeration can be helpful. The carbon source provides the microorganisms with the necessary nutrients to grow and produce electricity, while aeration ensures that the D.O levels remain within an acceptable range for the microorganisms to thrive.

3.2.2. pH Measured in Microbial Fuel Cells

Figure 3.4 pH Measured in Microbial Fuel Cells



The pH levels of the microbial fuel cells (MFCs) varied over the course of the experiment, with each MFC displaying a different trend. MFC 2 showed a steady increase in pH over time, ranging from 7.6 to 9.5. This increase in pH could be attributed to the addition of base, which helped to maintain a more alkaline environment in the MFC. MFC 3 initially started with a high pH of 8.51, likely due to the presence of the phosphate buffer. However, the pH gradually decreased over time and stabilized around 7.74. This could be due to the depletion of the buffer and the microbial activity in the MFC, which may have produced acidic byproducts. MFC 4 started with a relatively low pH of 6.72, which may be due to the addition of the sugarcane bagasse as a carbon source. As the microorganisms in the MFC metabolized the bagasse, they produced acidic byproducts, resulting in a decrease in pH over time. However, the pH in MFC 4 stabilized around 7.05 after four weeks. The reason behind eventual decrease in pH is due to acid production during anaerobic acid fermentation. When anaerobic acid fermentation takes place, organic compounds are broken down into simpler compounds such as acids, alcohols, and gasses in the absence of oxygen. This process results in the production of hydrogen ions (H+), which leads to a decrease in the pH of the system. However, the pH of the system can remain stable if the acid production is balanced by the buffering capacity of the environment, which can prevent a significant change in pH.

3.2.3. Biochemical Oxygen Demand Measured in Microbial Fuel Cells

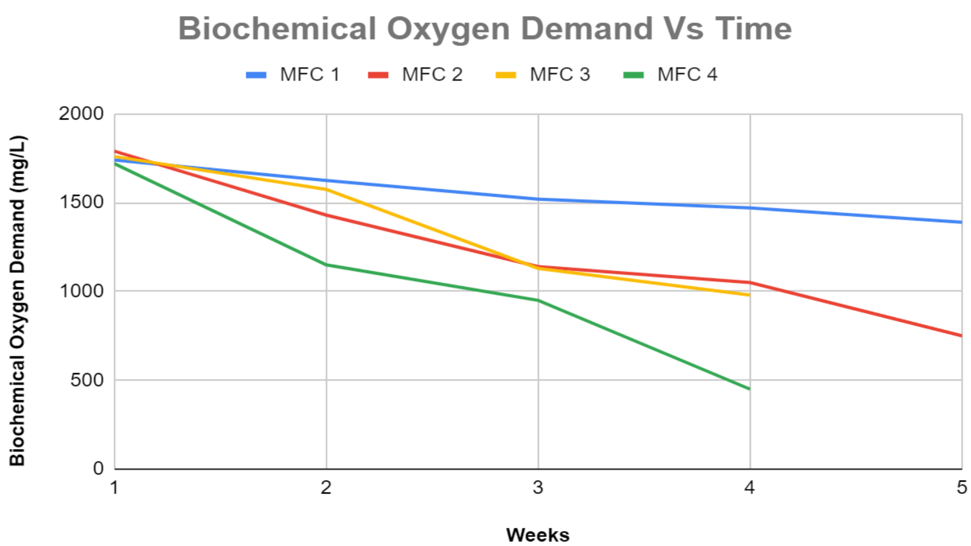


Figure 3.5 Biochemical Oxygen Demand Measured in Microbial Fuel Cells

The biochemical oxygen demand (BOD) is a measure of the amount of oxygen that microorganisms need to break down organic matter in water. In microbial fuel cells (MFCs), the microorganisms use the organic matter as a food source to generate electricity. Therefore, the BOD is an important indicator of the performance of MFCs, as higher BOD levels indicate the presence of more organic matter for the microorganisms to consume and produce electricity. The decreasing trends in BOD observed in MFC 2 and MFC 4 could be due to the depletion of the organic matter in the influent wastewater over time. As the microorganisms consume the organic matter, the BOD levels decrease, resulting in a decrease in current production. This is consistent with the previous readings where we saw a decrease in voltage/current production when the organic matter was depleted. MFC 3 showed fluctuations in BOD levels, which could be due to the presence of different types of organic matter in the influent wastewater. Some organic matter may be more easily biodegradable, leading to higher BOD levels, while others may be less biodegradable, resulting in lower BOD levels. However, despite the fluctuations in BOD levels, MFC 3 was still able to generate current/voltage, indicating that the microorganisms were able to utilize the organic matter for electricity production. The decrease in BOD over time is due to the growth of microorganisms that consume the organic matter in the wastewater as their energy source. As the microorganisms grow and reproduce, the available organic matter decreases, resulting in a decrease in BOD readings. The decreasing trend in BOD is also an indicator of the effectiveness of the MFCs in treating the wastewater. A decrease in BOD indicates that the wastewater is becoming less polluted and closer to meeting the required discharge standards. Hence, the readings over the weeks demonstrates the ability of MFCs to effectively treat wastewater and the potential for their application in sustainable wastewater treatment systems.

3.2.4. Chemical Oxygen Demand Measured in Microbial Fuel Cells

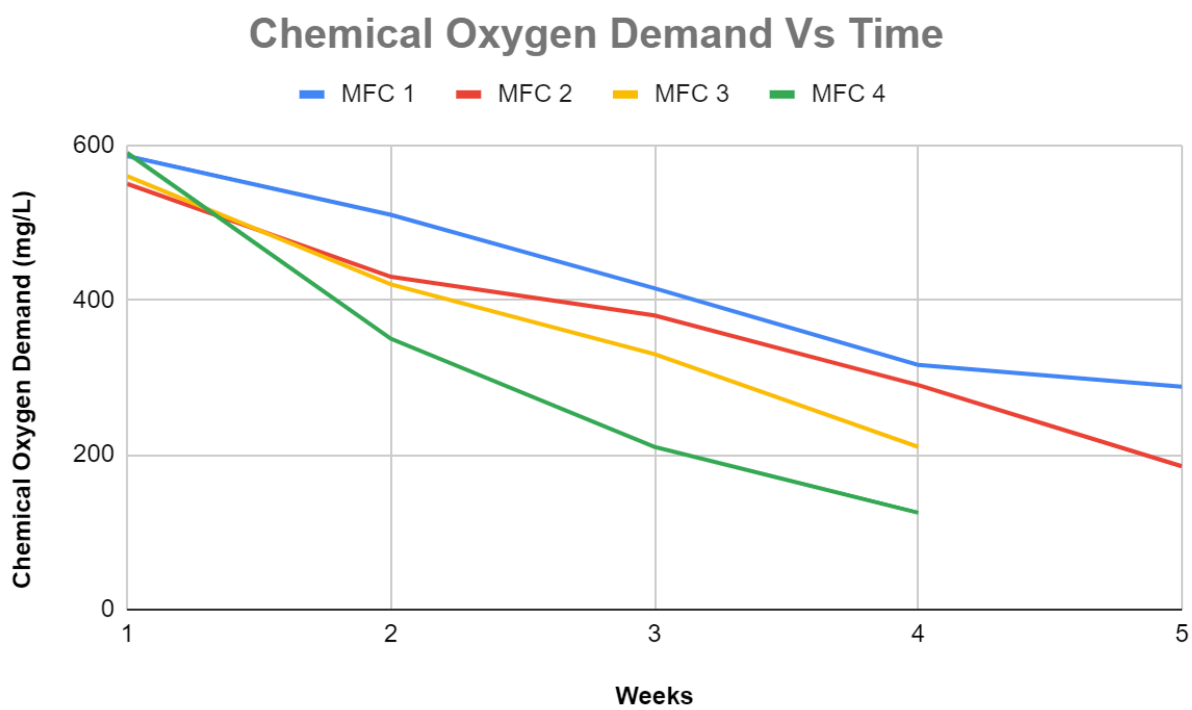


Figure 3.5 Chemical Oxygen Demand Measured in Microbial Fuel Cells

Chemical oxygen demand (COD) measures the oxygen required to chemically oxidize the organic matter. The results of the COD measurements for the four MFCs show a decreasing trend over the course of the experiment with the lowest COD values to be 185 mg/L, 210 mg/L, and 125 mg/L on Day 5, respectively. BOD and COD are typically used together to monitor water quality, and the ratio of BOD to COD can provide information about the type of organic matter present in the sample. This trend in decreasing COD concentration is consistent with the trend observed in BOD measurements for MFCs 2 and 4, indicating that the organic matter in the MFCs is being consumed by the microorganisms. However, it is important to monitor the COD and BOD concentrations regularly to ensure that the microorganisms have access to sufficient organic matter to maintain their metabolic activity and produce electricity. If the organic matter is depleted, the microorganisms may die off, and the performance of the MFCs will decrease.

3.2.5. Total Dissolved Solid (TDS) Levels Measured in Microbial Fuel Cells

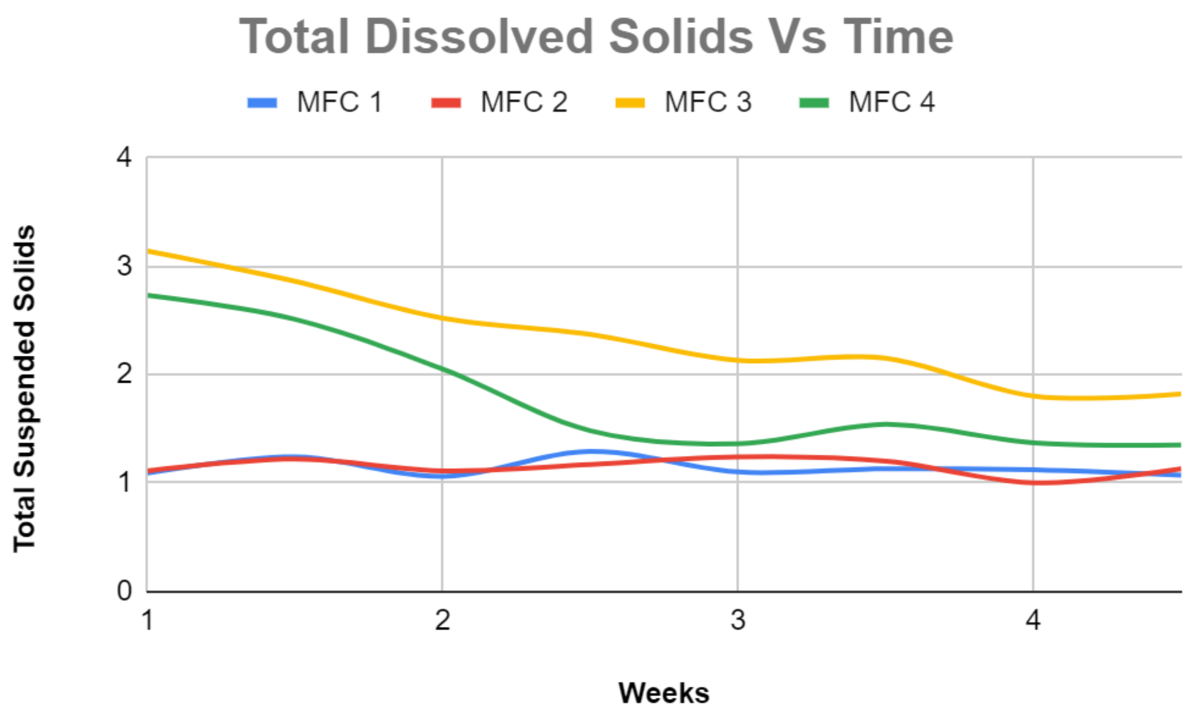


Figure 3.6 Total Dissolved Solid (TDS) Levels Measured in Microbial Fuel Cells

Total dissolved solids (TDS) are a measure of the amount of inorganic and organic substances dissolved in water. Overall, the trend in TDS for all MFCs shows a general decrease over time. MFC 3 had the highest TDS value on Day 1 with a reading of 3.14 ppt, which then gradually decreased over time to 1.80 ppt on Day 4. MFC 4 had the second-highest TDS value on Day 1 with approximately 2.73 ppt, which also decreased over time to 1.37 ppt on Day 4. MFCs 1 and 2 had lower TDS values than MFCs 3 and 4 on Day 1, with readings of 1.09 ppt and 1.11 ppt, respectively. The decrease in TDS observed in all MFCs over time is likely due to the microbial degradation of organic matter in the wastewater. As organic matter is broken down by the microbes, it is converted into other forms of inorganic matter that are not measured as TDS. The removal of inorganic and organic matter from the wastewater could potentially be used for energy production.

3.2.6. Total Suspended Solid (TSS) Levels Measured in Microbial Fuel Cells

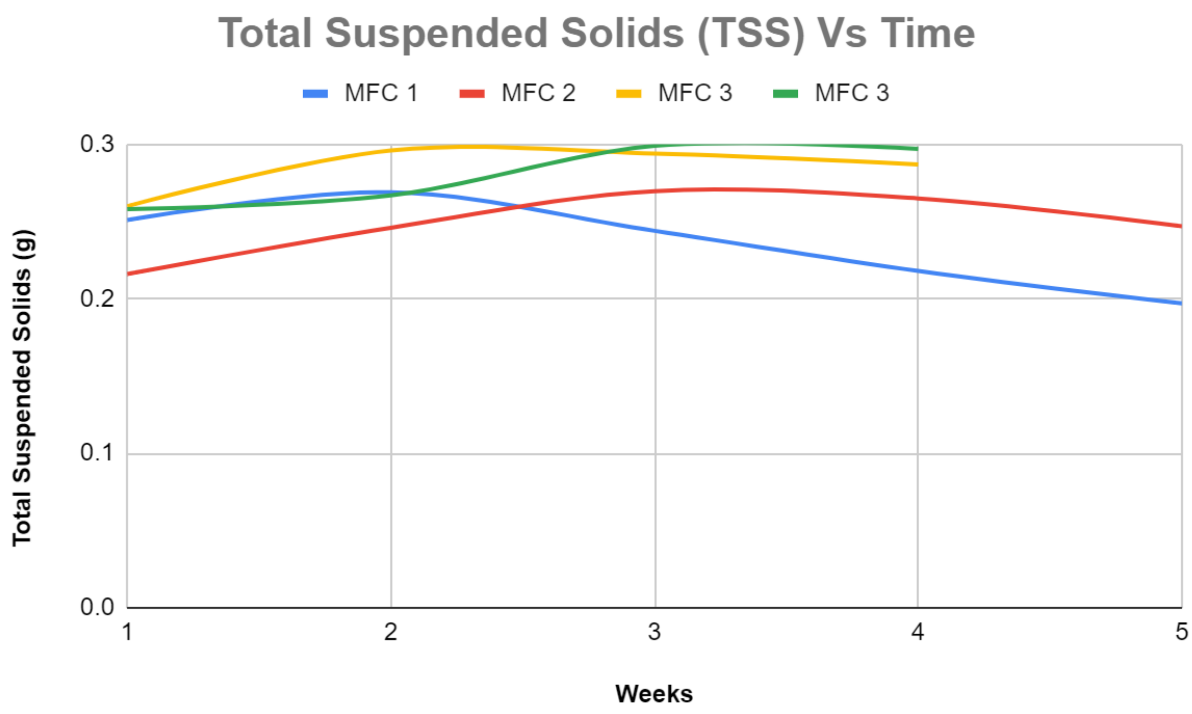


Figure 3.7 Total Suspended Solid (TSS) Levels Measured in Microbial Fuel Cells

Total suspended solids (TSS) refers to the amount of particles or materials that are suspended in the water and can be used as an indicator of the overall water quality. In the study, the trend for total suspended solids (TSS) in MFCs 2, 3, and 4 was monitored over a period of 4 days. On Day 1, the TSS value for MFC 2 was the lowest among the three MFCs, measuring at 0.216 mg/L. MFC 3 had a slightly higher TSS value of 0.26 mg/L, while MFC 4 recorded a TSS value of 0.258 mg/L. The following day, MFC 2 recorded a slightly higher TSS value of 0.246 mg/L compared to MFC 3's TSS value of 0.296 mg/L and MFC 4's TSS value of 0.267 mg/L. This pattern continued until Day 4 when MFC 4 had the highest TSS value of 0.297 mg/L among all MFCs, while MFC 3 had a TSS value of 0.287 mg/L, and MFC 2 recorded a TSS value of 0.265 mg/L. It is possible to speculate reasonings based on the experimental conditions. High levels of TSS can clog the electrode surface area, reducing the number of available attachment sites for microbial communities, which can adversely affect the system's performance. The readings suggest that MFC 2 maintained lower TSS values than the other MFCs on Day 1, which could indicate a better attachment of microbial communities to the electrode surface, leading to more efficient power generation. However, on Day 2, MFC 2's TSS value increased, which could suggest the detachment of microbial communities or the accumulation of additional suspended solids. It was also noted that induced pH increase could potentially lead to a decrease in the concentration of suspended solids as some particles may become less soluble or more prone to settling out. In MFC 3, a phosphate buffer could potentially act as a source of nutrients for microbial growth, leading to an increase in the TSS concentration. In MFC 4, bagasse was added, which is a material that can potentially increase the concentration of suspended solids due to the presence of fiber limiting the available electrode surface area and leading to reduced power output. Therefore, to optimize MFC performance, maintaining low TSS levels is crucial.

3.2.7. Electric Conductivity Measurements in Microbial Fuel Cells

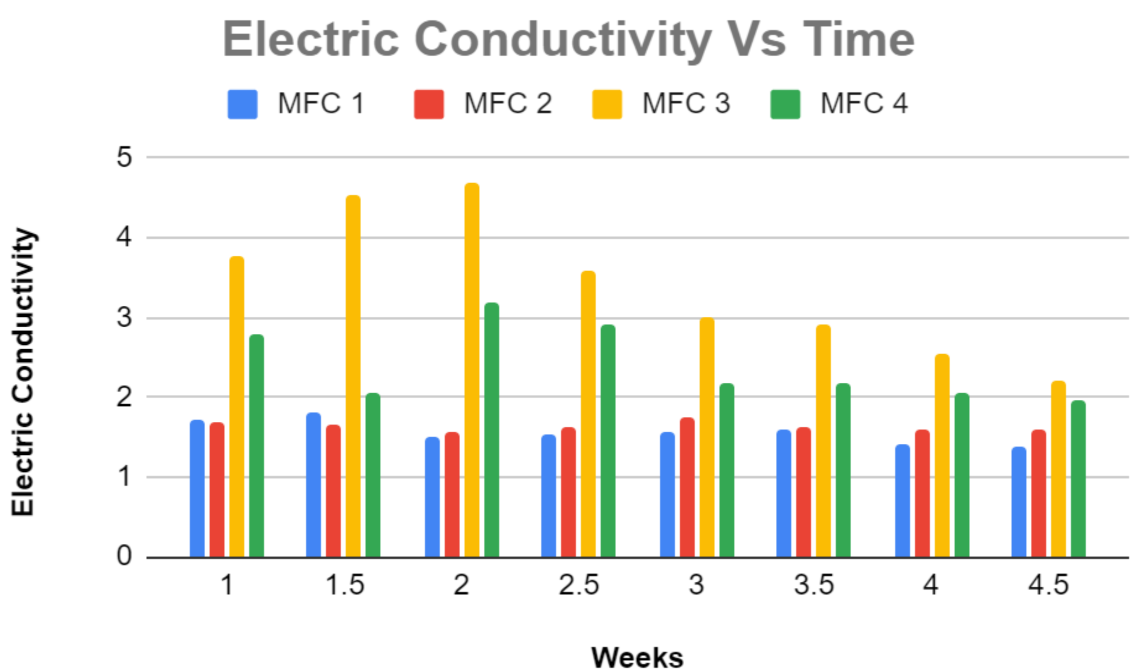


Figure 3.8 Electric Conductivity Measurements in Microbial Fuel Cells

Electrical conductivity (EC) is a measure of the ability of water to conduct an electrical current, which is directly related to the concentration of ions in the water. In the study, it is evident that MFC 3 consistently had the highest EC values among all MFCs throughout the study period. On week 1, MFC 3 had an EC value of 3.78 mS/cm, which was more than twice the EC value of MFC 2 and MFC 4 at 1.7 mS/cm and 2.78 mS/cm, respectively. This trend continued in the following week while in week 3, MFC 3's EC value dropped to 4.7 mS/cm, while MFC 4's EC value increased to 3.2 mS/cm, and MFC 2's EC value remained relatively stable at 1.55 mS/cm. Finally, in week 4, MFC 3 and MFC 2 had relatively stable EC values at 2.9 mS/cm and 1.64 mS/cm, respectively, while MFC 4's EC value decreased slightly to 2.06 mS/cm. The addition of phosphate buffer to MFC 3 and bagasse to MFC 4 may have led to an increase in the number of ions in the water, resulting in higher EC values. On the other hand, the increase in pH to 9.5 in MFC 2 may have caused a decrease in the number of ions, resulting in lower EC values. Overall, the trend in EC values suggests that MFC 3 was able to generate a higher concentration of ions in the water, which could potentially lead to higher electricity generation in the MFC.

3.3 Discussion

The present study shows how pH plays a critical role in determining the performance of microbial fuel cells, and that an optimal pH range is necessary to achieve maximum power output.The pH of the system was increased to 9.5, and the current output showed a decrease over time, with the lowest output of 0.05 mV observed at week 4.5. In contrast, a previous study by (Rojas-Contreras et al. 2019) revealed a similar trend, where increasing the pH to 9.0 led to a gradual decrease in current output over time after an increase in current output from 12.2 to 68.6 mV. The Li et al. study reported a relatively stable current output of 30-40 mV for the first 15 days used glucose and yeast extract (Rojas-Contreras et al. 2019), which could account for the differences in the observed trends.This finding is consistent with a study by (Rojas-Contreras et al. 2019), which revealed that the type of organic matter used as fuel can influence the performance of microbial fuel cells. One possible reason for the decrease in performance could be due to the effect of high pH on the activity of the microorganisms involved in the process of electricity generation. The microorganisms in a microbial fuel cell are responsible for breaking down organic matter in the fuel source and transferring electrons to the electrode, which generates an electrical current. At high pH values, the activity of microorganisms may be reduced, leading to a decrease in the rate of organic matter degradation and electron transfer.

Previous research has shown that the addition of a mediator can facilitate electron transfer between bacteria and the electrode, leading to increased power output. With the addition of a phosphate buffer as a mediator, the current output showed a gradual increase with a maximum of 75.3 mV at week 4.5. Comparing these findings to a study where a different mediator potassium ferricyanide was added to the anode chamber of a microbial fuel cell increasing the power output by up to 25 times which is 250 mV after 45 days (Zhang et al. 2007). The phosphate buffer serves as a chemical mediator that helps to shuttle electrons from the bacteria to the electrode. However, compared to the use of potassium ferricyanide as a mediator, phosphate buffers have a lower efficiency in terms of increasing the power output of MFCs. This is because potassium ferricyanide has a higher redox potential, which means it is more effective at accepting and donating electrons, leading to a greater transfer of electrons from the bacteria to the electrode (Zhang et al. 2007). It is important to note that the experimental conditions and set-up may differ between studies, which could account for the differences in results. Nonetheless, the present experiment provides evidence that the addition of a phosphate buffer mediator can contribute to an increase in current output in MFCs.

The study investigated the performance of a microbial fuel cell utilizing bagasse as a food source. The fluctuation in current output observed can be attributed to the nature of bagasse as a complex and heterogeneous substrate with varying levels of biodegradability. The initial high current output of 91.7 mV observed at week 1.5 may have been due to the presence of easily degradable components of bagasse, such as simple sugars, that were readily metabolized by the microbial community in the fuel cell. However, as the microbial community depleted these easily accessible nutrients, the current output decreased over time, reaching a low of 10.2 mV at week 4.5. The decrease in current output could also be attributed to the accumulation of fermentation by-products, such as organic acids, that can inhibit microbial growth and metabolism.

Furthermore, the heterogeneous nature of bagasse can result in uneven distribution of nutrients and microbial growth within the fuel cell, leading to variations in current output. On the contrary, (Kim et al. 2017) study found that the microbial fuel cell utilizing brewery wastewater as a food source exhibited a steady increase in current output, reaching a maximum of 469 mV after 22 days of operation (Kim et al. 2017). In comparison to other food sources, such as brewery wastewater, bagasse may not be as optimal for microbial fuel cell performance due to its lower biodegradability and variable nutrient content. However, the utilization of bagasse as a substrate in microbial fuel cells may still have potential as a sustainable means of generating electricity, especially if optimized for specific microbial communities and operating conditions.

In the present study, four microbial fuel cells (MFCs) were used to measure water parameters, including dissolved oxygen, biochemical oxygen demand (BOD), and chemical oxygen demand (COD). The average initial BOD and COD values for all MFCs were approximately 1550 mg/L and 480 mg/L, respectively. Over time, there was a decrease in both BOD and COD values in all MFCs. In contrast, the second study investigated the use of MFCs to treat molasses wastewater with different concentrations of COD. The results showed that the MFC system operated stably and achieved high COD removal efficiency of 88.5% at the end of the cycle (Bai, et al. 2022). Finally, a low-cost MFCs-BOD biosensor was used in the third study to determine BOD in sewage samples. The determination range was 50-500 mg/L, and the determination time was within 3 hours (Wang, et al. 2022), with a maximum voltage reaching around 550 mV. The results showed that the biosensor accurately determined the BOD content of sewage water samples (Wang, et al. 2022).

The presented results show that the average DO concentration in the MFCs was 7.8 mg/L, which is lower than the optimal range for aerobic bacteria that typically populate the anode of MFCs. The lower DO concentration may be due to the limited diffusion of oxygen into the biofilm or the activity of anaerobic bacteria in the chamber, which can consume oxygen (Santos, et al. 2003). The lower DO concentration in MFCs can limit the activity of aerobic microorganisms and result in the development of anaerobic conditions, which can negatively affect the performance of the MFC. Despite the lower DO concentration, it can be observed that the highest current was generated when the pH was between 7.7 and 8.67, and that the DO concentration was around 16.6 to 13 percent. In contrast, another study optimized a mediator-less MFC in terms of various operating conditions, including DO concentration. The study found that the highest current was generated at a pH of 7 and that the DO concentration was around 6 mg/L at the DO-limited condition (Santos, et al. 2003). While the two studies have different focuses and methods, both emphasize the importance of monitoring DO concentration in MFCs and how it affects their performance**.**

The present study had an average TDS concentration of 500 mg/L, which is higher than the optimal range for MFC operation. This higher TDS concentration may be due to the presence of organic matter, salts, and minerals in the wastewater used as a feedstock for the MFCs. High TDS concentrations can lead to fouling of the electrode surface and decreased electron transfer rates, negatively impacting MFC performance. On the other hand, results of the double-chamber MFC study showed that the optimum voltage output and power generation were achieved at TDS concentrations of 20 g/L and 5 g/L (Adelekan, et al. 2014) respectively, indicating the importance of maintaining an appropriate TDS level for optimal MFC performance. The current results showed variability in the effectiveness of different MFC strategies in reducing total suspended solids (TSS), with MFC 2 showing the lowest TSS values and MFC 3 and MFC 4 showing increases in TSS values. In comparison, a previous study operated MFCs on primary effluents for more than 400 days, achieved 65-70% chemical oxygen demand (COD) removal and 50% suspended solids reduction (Bajracharya, et al. 2013).

The study also investigated the electrical conductivity (EC) of the MFCs. Results indicated that the EC values in the MFCs were higher than those reported in other studies, with an average of 2000 μS/cm. The higher EC values in this study may be attributed to the presence of charged ions and compounds in the wastewater, which can increase the conductivity of the solution and affect MFC performance. On the contrary, wood-based biochars were used as MFC electrodes to enhance the electrical conductivity of MFC (Srikanth, et al. 2014). This study did not report EC values, but the use of biochar electrodes presents a potential solution to reduce the cost and carbon footprint of MFCs, while also offering agronomic benefits from waste disposal (Srikanth, et al. 2014). Despite the higher EC, the MFCs still showed promising results in terms of COD removal and suspended solids reduction. These results demonstrate the technical viability of MFC technology in effectively removing pollutants from wastewater and recovering energy from waste (Bajracharya, et al. 2013).

Conclusion

1. In conclusion, the construction and operation of four small-scale microbial fuel cells (MFCs) using activated sludge and wastewater as the feedstock, and graphite electrodes connected with copper wires as the output detectors, proved to be a successful method for studying the potential of MFCs in electricity generation and wastewater treatment.
2. MFC performance was evaluated based on various parameters. Substrate addition showed a positive impact on current and power density, but depletion of nutrients can decrease output. pH adjustment should be monitored to avoid affecting microbial community balance and current sustainability.
3. Utilizing a good substrate for bacteria in MFCs can lead to increased power production. MFC 4 showed that using bagasse as a carbon source resulted in a maximum power density that was approximately 7 times higher than that of the control experiment without any carbon source. This indicates that bagasse has potential as a renewable and sustainable energy source for MFCs.

Recommendations

1. While bacteria are commonly used in microbial fuel cells, other types of microbes such as archaea and fungi can also be used. Experimenting with different types of microbes can lead to improved efficiency and output of the microbial fuel cell.
2. Microbial fuel cells can use a variety of organic substrates as fuel and mediator, including glucose, acetate, and wastewater. Experiment with different substrates to determine which ones produce the highest energy output.
3. The design of the anode and cathode can have a significant impact on the performance of the microbial fuel cell. Experiment with different materials, surface areas, and configurations to optimize the design.
4. Electron transfer from the microbes to the electrode can be a limiting factor in the performance of microbial fuel cells. Strategies such as using conductive materials or adding electron shuttles can enhance electron transfer and improve energy production.

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ANNEXURE

Sample Site

Figure of aeration tank

Figure for Sample Collection

Figure  of microbial fuel cells

Figure of Multi-meter used for taking readings

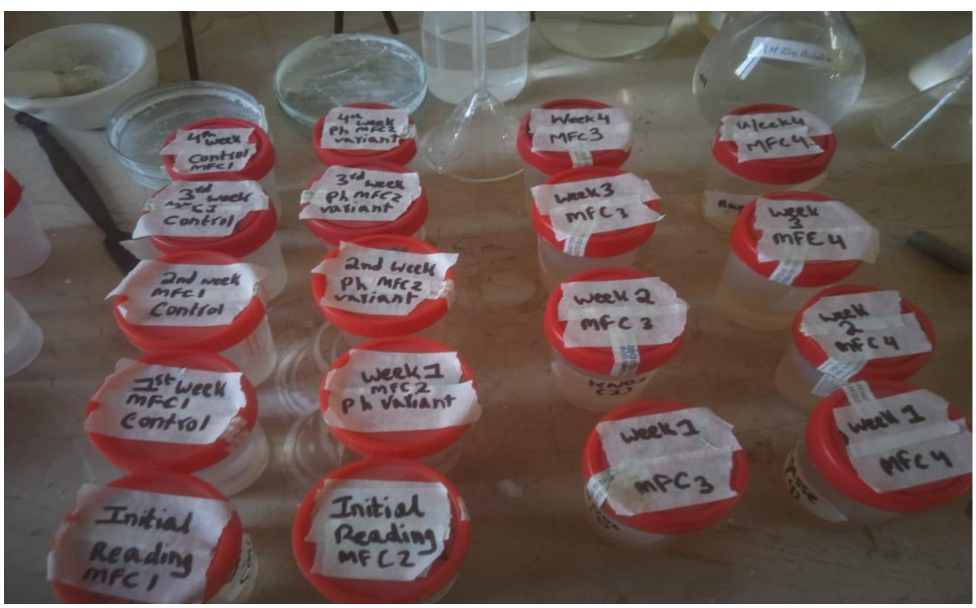

Figure of BOD COD Sample