Jimma University

Jimma Institute of Technology

Faculty of Civil and Environmental Engineering

Department of Water Supply and Environmental Engineering

Environmental Engineering Post Graduate Program

Development of microbial fuel cell for electricity generation using human wastes

By
Tensay Kifle

A thesis submitted to the School of Post Graduate Studies of Jimma University in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering.

September, 2021
Jimma, Ethiopia

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Advisor: Prof., Dr.-Ing. Esayas Alemayehu

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September, 2021

Jimma, Ethiopia

APPROVAL SHEET

We, as thesis advisors, hereby certify that we have read and reviewed Tensay Kifle Habtemariam thesis, Development of microbial fuel cell for electricity generation using human wastes, which was completed under our supervision. We recommend that it can be submitted as fulfilling thesis requirement.

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As a member of board examiner of MSc. thesis final defense examination, we certify that we have read, evaluate thesis prepared by Tensay Kifle Habtemariam and examined the candidate. We recommend that, the thesis could be accepted as fulfilling the thesis requirements for the degree of masters of Science in Environmental Engineering.

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DECLARATION

I, Tensay Kifle, hereby declare that the research work on ‘Development of microbial fuel cell for electricity generation using human wastes’ for the MSc degree at the Jimma University is my original work and the research has not presented for award of any degree either in Jimma University or any other university.

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This thesis has been submitted for examination with our approval as university advisor and program chairperson.

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(Co-advisor)

ACKNOWLEDGEMENTS

First of all, I would like to thank the Almighty of God for his assistance; not only in my study but also throughout my entire life. I wish to express my profound gratitude and indebtedness to my advisors Pro.-Dr.–Ing. Esayas Alemayehu and Mr. Chali Dereje (M.Sc) for introducing the present topic and for their inspiring guidance, constructive criticism and valuable suggestions throughout the work of this study work.

Next, my thanks go to Seifu Kebede Environmental Engineering Chair holder, for technical and scientifically assistance in my education and thesis work. I would also express my gratitude to the staffs of the department of Hydraulic and water resource engineering department of Wolkite University for their heart full guidance and the support they have provided me. In addition, my thanks go to Mr. Yared Temesgen and Amsayawu Genet (Hydraulic and water resource Engineering department of Wolkite University) who gave me technical assistance up to the end of my thesis work.

My sincere thanks to all my friends and seniors who have patiently extended all sorts of help for accomplishing this study work. Finally, it was impossible to complete this study without the understanding of my family, especially my Uncle Mr.Abebe Habtemariam Soresa

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ACRONYMS and ABBREVIATIONS

AD	Anaerobic digestion
BES	Bio-Electrochemical Systems
BOD	Biochemical Oxygen Demand
BOD_5	Five days Biological Oxygen Demand
CH_4	Methane
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
FC	Faecal Coliform
GHG	Green House Gas
H_2S	Hydrogen Sulphide
H_2O	Water
JiT	Jimma Institute of Technology
JU	Jimma University
MC	Moisture Content
Mg/L	Milligram Per Liter
MFC	Microbial Fuel Cell
NH_3	Ammonia
NH_4	Ammonium
OCV	Open Circuit Voltage
OM	Organic Matter
pH	Power of Hydrogen
TC	Total Coliform TK Total Potassium
TOC	Total Organic Carbon
TOD	Total Oxygen Demand
TN	Total Nitrogen
TP	Total Phosphorus
TS	Total Solid
UNEP	United Nation Environmental Program

VS	Volatile Solid
WW	Wastewater
WKU	Wolkite University
WW	Wastewater

ABSTRACT

A microbial fuel cell (MFC) is a bio-electrochemical device that generates electricity by harnessing the natural metabolisms of microbes. The sustainable use of our resource is critical to overcoming the problem of climate change ever increasing global energy and rising shortage of fossil fuel. In recent years the use of fossil fuel, especially oil and gas has increased resulting in global energy crisis one of way to solve the ongoing global warming problem is to use green bioenergy. In the current situation energy crisis is a growing problem throughout the world, which necessitates the creation of alternative energy sources that generate less carbon dioxide and benefit the ecosystem, like the use of wastewater best solution for such challenge. The study's objectives were investigated a laboratory-based experimental work. The capacity of microbial fuel cells and the type of substrate employed were evaluated using experimental research designs.The quantity of electric current produced by wastewater during treatment was measured using an experimental approach. The cross-sectional methodology was used to inspect the capability of each three type of substrates power generation capability during the research design. Maximum Voltage output or OCV (open circuit voltage) values of 118.93 mV, 144.84 mV, and 89.76 mV are attained when the resistance is infinite for MFC1, MFC2, and MFC3, respectively. The maximum voltage production achieved in blackwater substrate it generated 144.84mV and the smallest generated from graywater it was 89.76mV.The MFC that employed graywater as a substrate produced the least amount of electricity of the three, but it was the most stable. COD reduction was highest in Blackwater waste, at roughly 87.94%, compared to 65.83 % and 80.22 % for urine and graywater waste, respectively and BOD_5 *removal of substrate urine, blackwater and graywater are 67.79%, 91.35% and 28.89% respectively value in the BOD reduction also blackwater substrate attained the highest reduction. This Study was discovering materials for MFC; the desire for cheaper electrode materials is creating chance MFC technology from being implemented outside of the lab. Material for the electrodes Metals such as aluminum and steel can boost power generation, and their widespread use would result in lower cost of materials. The power productions of electrodes depend on type of substrates and surface area of electrode so that MFC2 produced high yield of voltage. Generally a based on laboratory data shown that in the research use the microbial fuel cell the best option to solve problem of energy shortage for rural community.*

Keywords:

Anaerobic, Bacteria, Electricity, Electrode, Microbial Fuel Cell, Power, Substrate, Voltage, Wastewater

CHAPTER ONE

1. INTRODUCTION

1.1.Background of the Research

Our society is still looking for new and better ways to generate energy that is both affordable and renewable. A microbial fuel cell (MFC) is a bio-electrochemical device that generates electricity by harnessing the natural metabolisms of microbes(Li, 2013).The sustainable use of our resource is critical to overcoming the problem of climate change ever increasing global energy and rising shortage of fossil fuel. In recent years the use of fossil fuel, especially oil and gas has increased resulting in global energy crisis one of way to solve the ongoing global warming problem is to use green bioenergy. Microbial Fuel Cell (MFC) is a promising device that turns chemical energy into electricity and eliminates contaminants from wastewater using microorganisms' catalytic action(Naina Mohamed *et al.*, 2020).

Ethiopia, as a country with a high population growth rate of roughly 2.6% per year, should be able to use any type of waste produced by human activities to generate alternative energy. Human excreta can now be used in the development of MFC technology (faeces). As a result, Ethiopia's large population should be viewed as a positive factor(Andriani *et al.*, 2015). Electrical energy is one sort of energy that is commonly employed in daily human activities, while other types of energy are used for lighting, cooking, and manufacturing. The availability of electrical energy determines the level of welfare of a people in a certain area. Electricity consumption requirements can be used as a metric for gauging community well-being(Ansori *et al.*, 2019).

Microorganisms can biologically breakdown organic wastes and nutrients, simulating natural system change. Biological treatment might take place in either an aerobic or anaerobic setting. Microorganisms devour dissolved and colloidal organic materials in wastewater in both conditions(Cynthia, 2014).

Microbial fuel cell is rapidly growing, eco-friendly and green technology. Microorganisms are used in this technology to convert chemical energy stored in the biodegradable part of organic substance into electric current while treating the waste(Roy *et al.*, 2017).

Microbial fuel cells have emerged as a promising but challenging technology in recent years. MFC is regarded as a promising sustainable technology for meeting rising energy demands, particularly when wastewaters are used as substrates, as they can generate electricity and treat wastewater at the same time, potentially offsetting the operational costs of wastewater treatment plants(Logan, 2005). Bacteria can be used in MFCs to generate electricity while also biodegrading organic materials or waste.

Scientists and researchers began searching for ways to extract energy from renewable natural resources in this era of rising energy demand and continuous depletion of fossil fuels, not to mention their well-known environmental impact(Khaloufi and Elasli, 2019). On a human scale, these sources are usually inexhaustible, renewable, and a viable alternative to the rather than use of fossil fuels. Covering the world's energy needs is no longer the only concern; another problem has emerged, namely global warming. Burning fossil fuels emits greenhouse gases, primarily carbon dioxide, into the atmosphere, raising global temperatures due to the greenhouse effect. Fossil fuels contain a lot of stored energy, and it takes millions of years for them to be formed again after they've been used. As a result, it is critical to find alternative green options before they are exhausted(Tharali *et al*, 2016).

One of today's top priorities is ensuring clean water for future generations with renewable energy. Wastewater treatment methods and innovations are currently generating a lot of research interest. Scientists recognize the value of wastewater treatment for a variety of purposes, including supplying safe drinking water, agricultural uses such as irrigation, and, most notably, ensuring safe waste water management to the ecosystem. However, these technologies necessitate a sizable budget for a process that also necessitates electricity(Khaloufi and Elasli, 2019).

Excreta, such as human feces and urine, are used directly to provide good electricity and plant nutrients to agricultural soil. These items typically do not include industrial chemical contaminants that could prevent municipal wastewater from being reused, but they need be treated to lower human pathogen levels to a safe level(Sch, Stenstr and Control, 2005). Systems that separate or divert urine and feces are now available as alternatives to the traditional wastewater system, allowing for more effective fertilizer utilization. By avoiding mixing the fractions in areas where there is no piped sewerage, nutrient utilization and sanitation can be enhanced(Schönning, 2001). The situation is similar in poor countries with low hygienic standards. The goal will be to minimize the incidence of illnesses by implementing sanitation as a whole, including the introduction of new alternatives, in conjunction with other interventions such as clean water supply, treatment and storage, and hygiene/health education with power supply of usage of MFC(Sch, Stenstr and Control, 2005).

People currently live and raise their in much polluted environments in many Ethiopian cities, towns, and rural areas. The most polluted and disease-ridden ecosystems are urban and peri-urban areas. Lack of proper excreta disposal facilities and inadequate solid waste collection and disposal are to blame for much of this pollution, which leads to high rates of sickness, starvation, and death(K faris & Alemayehu, 2002).Because of the probable existence of high quantities of disease-causing organisms, exposure to untreated feces should always be considered hazardous; concentrations vary depending on the abundance of disease-causing organisms in a given community. Bacteria, viruses, parasitic protozoa, and helminths are among the species found(WHO and UNEP, 2019).

Sustainability in wastewater management involves not only protection of human health and the environment, but also efficient and effective long-term water management, minimization of energy requirements, and closing the loop on natural resource cycles.

Microbial fuel cells (MFCs) have the potential to generate electricity while also being totally self-sustaining. Low-cost systems are possible, because to advances in MFC research, however full-scale systems for the poor world market have yet to be built. Over one billion people in poor and middle-income nations do not have access to sanitary facilities that isolate excreta from human touch, and over 2.6 billion of them defecate in the open(UN-Water, 2021).

Microbial fuel cells carry the potential as a long-term solution to meet the growing energy demands of developing countries while also protecting the environment(Gude, 2016a).

1.2.Statements of the Problem

Microbial fuel cells use biodegradable materials to create energy in the presence of microorganisms. Wastewater contains a large amount of organic matter that can be oxidized in MFCs to produce electricity. In the current situation lack of renewable energy technologies easily available in local market and energy crisis is a growing problem throughout the world, which necessitates the creation of alternative energy sources that generate less carbon dioxide and benefit the ecosystem, such as the use of wastewater. Because of depleting resources and the contribution of these fuels to the accumulation of carbon dioxide in the atmosphere, continued use of petroleum-sourced fuels is now generally regarded as unsustainable. Microbial fuels cell that are renewable and carbon neutral are important for environmental and economic sustainability. Sustainable energy sources are essential for achieving energy security and combating climate change. Human being all over the world must be work to create safe environment so that looking for eco-friend energy frameworks in which renewable energy sources can play a key role, allowing them to transition to a more stable, efficient, and sustainable energy route.

Electricity in Rural Ethiopia villages has not been connected to the electricity grid that is powered by Ethiopia electrical power (EEP). Ethiopia currently has a 45 percent electricity penetration rate, with decentralized solutions providing access to 11 percent of the population; however the majority of Ethiopians use expensive kerosene for their primary light source, with solar lights/rechargeable lamps as the second-most used light source(Pappis *et al.*, 2021).

The worldwide community must be searching for alternatives to meet the global energy demand due to the depletion of fossil fuels, the quantity of waste, the effects of climate change, and the exponential rise of human population(Md Khudzari *et al.*, 2018). Water, nutrition, and universal education are all critical components of growth. These other businesses, on the other hand, would fail without widespread electrification. Obtaining the progress needed for the country's development.

1.3.Objective of the Research

1.3.1. General Objective

The main goal of this study is to develop a microbial fuel cell for electricity generation from human wastes.

1.3.2. Specific objectives

- To determine physiochemical and biological analysis of wastewater before and after treatment.
- To investigate how much current produce from wastewater the different type’s substrates.
- To analysis of the electrode material effect of electric current generation.

1.4. Research Question

1. What are the variation of physiochemical and biological characteristic of wastewater in treatment?
2. How much electric current produce from the wastewater sample?
3. Why do electrode materials influence the electricity production?

1.5. Significance of the Research

A microbial fuel cell is a renewable device that uses organic wastes to generate power or merely to purify water. The purpose of this study is typical rural and pre-urban area which would eventually help provide energy to the house's heaters and electrification. Focusing on this research is primarily motivated by the socio-environmental impact. The consumption of energy is growing, so it's critical to keep looking for alternatives far apart with fossil fuels.

As biogas digesters, microbial fuel cells (MFCs) can generate energy from waste. They're not like biogas digesters in that they are built to produce electricity without producing any intermediate gas. MFCs use bacteria to transform the biochemical energy in organic matter into usable electrical energy, allowing them to produce electricity anaerobically from organic matter. MFCs have been identified as a promising technology for use in developing countries because of their ability to use waste as a fuel source and to treat wastewater without the use of electricity. Organic materials such as human waste have all been successfully used as MFC substrates and are widely available in rural and urban Ethiopia.

MFCs can be stacked to increase voltage, or an energy harvesting system can be used, but a minimum voltage must be achieved to operate an energy harvesting system, and a necessary power output must also be attained for the electricity to be usable from in this study laboratory output. As a result, the emphasis of this study was on the possible use of a basic household MFC one made entirely of low-cost or locally available materials.Growing the development of renewable energy while lowering costs, energy use, land use, and waste generation (sludge) are this type of the technologies that can help solve these problems and lead treated wastewater to conservation. Biogas, biomass, fertilizers, and compost are some of the technologies that can help solve these problems and lead treated wastewater to conservation. Reduced greenhouse gas emissions, high efficiency, versatility in installation and service, production of renewable energy resources, reduced demand for foreign oil, and enhanced environmental quality are all advantages of microbial fuel cells and to influence policy maker to bring solution for energy crisis.

1.6. Scope of the Research

The scope of the study is limited to the study of microbial fuel cell for generating of electricity using of human waste as input. This research was included three types of substrate such as urine, blackwater and graywater and for each three microbial fuel cell was studied. The study focused to evaluate the stated study objectives Development of microbial fuel cell for electricity generation using human wastes. The studied first analysis physiochemical and biological each substrate before and after treatment of wastes and second analysis electrochemical analysis of MFCs of each type of substrates in the study include voltage, electric current, current density power, power density and resistance of MFCs. Data were prepared for study groups that involved through laboratory experiments. Analysis was conducted to interpret and evaluate the study variables and instruments. The key finding from this study was used to establish development of microbial fuel cell for electricity generation using human wastes serving electricity rural community of Ethiopia.

1.7. Limitations

The difficulties encountered in this study included a lack of biological measurement equipment in laboratory, which resulted in the failure to identify the type of bacteria that play an important role in the decomposition of organic matter in microbial fuel cells. Another impact of the study was the study's scheduled study period, which was the study's main limitation.

1.8. Organizations of the study

Thesis of the document was organized from five chapters. Chapter 1 has focused on introduction, statements of the problems, general and specific objectives, and research questions, significance of the study and the scope of the study. Chapter 2 focused on literature review related with the study. Chapter 3 was focused on methods and materials used which includes area of the study, data collection and data processing. Chapter 4 was about results and discussions and chapter 5 focused on the conclusions and recommendation.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. History of Microbial Fuel Cell

Despite the fact that MFC research has been condensed over the last decade, Dr. M. C. Potter, a professor at the University of Durham in the United Kingdom, discovered in a series of experiments in 1911 that the bacteria E.coli can produce electricity when placed in an organic environment with platinum electrodes. His research resulted in the creation of a primary microbial fuel cell. Dr. M. C. Potter might also demonstrate how variables like temperature, concentration, and nutrient medium affect the amount of "electricity produced." As a result of his exhaustive experiments, he was able to reach a maximum voltage of 0.5mV, which was previously unheard of, making him the first scientist to show that real bacteria would result in a current(M.C.Potter, 1911). Professor Barnet Cohen succeeded in producing approximately 35mV and two milliampers of current exactly two decade later, in 1931 to be able to build such high potential and relatively good current Professor Cohen aligned and linked a number of microbial fuel cell in sequence(Flimban *et al.*, 2019).

Microbial Fuel Cells, or MFCs, are a technology that can help with a variety of issues. By using wastewater from homes and businesses center to produce electricity, we can save money on fuel and improve the quality of our power(Bose *et al.,* 2019). MFCs have the many benefits as a renewable energy technology such as MFCs can convert any type of biomass into energy, MFCs have a high efficiency in converting biomass energy to electricity, Microorganisms can be used as catalysts in MFCs instead of precious metals, MFCs have been extensively studied in terms of energy recycling as of late(Wang *et al.*, 2019).

Biological wastewater treatment methods take advantage of the coordinated behavior of microorganisms. Engineers need to consider the microbial community structure and how it responds to changing environmental conditions in order to evolve and optimize biological systems(Ferrera and Sánchez, 2016).

Table 2.1 History of MFC

No	Scientist	Nationality	Year	Contribution
1	Luigi Galvani (Physician and physicist)	Italy	1790	When he saw a twitching of an isolated frog limb after putting a brief electrical discharge through it, he coined the term bioelectricity.
2	Potter,M.C.,(Professor of botany at the University of Durham)	Durham ,UK	1910	The ability of organisms to create voltage and deliver current was demonstrated. Using platinum electrodes, researchers discovered electrical energy in cell cultures of E. coli and Saccharomyces cerevisiae.
3	Cohen, B.,	Cambridge, UK	1931	A batch of biological fuel cells produced more than 35mV, according to the author.
4	Rohrback, G. H., Scott, W. R. and canfield, J. H		1962	Invented the first biological fuel cell, which employed Clostridium butyricum as a biological material to produce hydrogen through glucose fermentation.
5	Allen, R. M. & Bennetto, H. P. from kings college in London, UK	London, UK	1993	Improved biological fuel cells using diverse microorganisms to improve both electron transfer efficiency and reaction rate using mediator systems were developed and proven.
6	Chang, I. S., Moon, H., Bretschger, O., Jang, J. K., Park, H. I., Nealson, K. H. & Kim, B. H. from Korean institute of science and technology (KIST)	South Korea	2006	It was discovered that certain electrochemically active bacterial species transfer electrons to electrodes without the use of mediator molecules.

(Flimban *et al.*, 2019)

2.2. Electrochemical Fuel Cell

Fuel cells produce electricity using an electrochemical mechanism that converts the energy held in a fuel straight into Direct current electricity. A fuel cell, like a battery, creates electricity directly from a chemical reaction, but requires reactants that are continuously supplied, as in an engine. Fuel cells are particularly appealing since they generate electrical energy without combusting fuel(Tawil *et al.*, 2008).

Fuel cell is an electrochemical technology that switches electricity through an electrochemical process using a hydrogen-rich fuel and oxygen. It is made up of two electrodes and an electrolyte that allows H^+ to pass through. A fuel cell is a small electrochemical "factory" that uses fuel to make energy. A fuel cell, like a factory, will keep producing product (electricity) as long as raw materials (fuel) are available. A fuel cell and a battery differ significantly in this regard. While both rely on electrochemistry to function, a fuel cell does not use any energy when it operates and produced electricity(O'Hayre, 2018).In a conventional combustion engine, fuel is burned, releasing heat. Consider the simplest example, the combustion of hydrogen:

$$H_2 + 1/2O_2 \xrightarrow{\qquad\qquad} H_2O \quad (1)$$

2.2.1. Fuel Cell Advantages

Fuel cells and main batteries have some similarities because they are both electrochemical energy conversion devices that rely on electrochemistry to work their magic. Fuel cells, in fact, combine many of the benefits of both engines and batteries(O'Hayre, 2018).

2.3. Types of fuel cells

The type of electrolyte is the most important distinction between fuel cell types. While all fuel cells use the same electrochemical principles, the type of ions that pass through the electrolyte determines the working temperature range. Furthermore, operating temperatures impose constraints on material physiochemical and thermo mechanical properties(Zuzul, 2017).

2.3.1. Alkaline Fuel cell

An alkaline solution (potassium hydroxide in water) is used as an electrolyte, as the name suggests. It was one of the first technologies for producing electrical energy and water in space that was developed and employed by the US space program. Non-precious metals are used for the anode and cathode, and there are numerous alternatives(Najmi, 2018).

With the introduction of anionic exchange membrane fuel cells (AEMFCs), the world of alkaline-based fuel cells has taken a giant step toward replacing traditional liquid electrolyte alkaline fuel cells (AFCs) (Ferriday and Middleton, 2021).

2.3.2. Polymer Electrolyte Fuel Cell (PEFC)

An ion exchange membrane (fluorinated sulfonic acid polymer or other similar polymer) that is an efficient proton conductor serves as the electrolyte in this fuel cell. Because water is the only liquid in this fuel cell, corrosion issues are low. Because the membrane must be hydrated, water management in the membrane is crucial for optimal performance. The fuel cell must function in settings where the by-product water does not evaporate faster than it is produced(Hall, 1987).

A polymer electrolyte membrane (PEM) fuel cell was adapted and enlarged into a stack using stirred tank reactor architecture. At various feed flow rates, the stack's steady-state and transient behaviors were investigated. Individual cell voltages differed due to non-uniform reactant distribution. Current and voltage instabilities were identified. Low feed flow rates, which could be attributed to a variety of factors(Tawil *et al.*, 2008).

2.4. Bio-Electrochemical System

The distinction between conventional electrochemical systems and bio-electrochemical systems is that the latter uses microorganisms as catalysts in addition to organic matter or wastewater as a fuel and energy source. These systems can be divided into three groups: Enzymatic Fuel Cells, Electrolysis Cells, and Electrogenesis Systems. They use two electrodes, the cathode and the anode, to extract energy from sludge or wastewater(Khaloufi and Elasli, 2019).Municipal, industrial, and animal wastewater wastes $1.5 * 10^8$ MWh each year, according to estimates. It is critical to recover at least a portion of this energy in order to approach a circular economy. MFC is a biotechnology that converts and recovers energy using microorganisms in an anaerobic environment(Cheng, 2009).

Microbial Fuel Cells (MFCs) are bio-electrochemical systems that transform chemical energy from organic substrates into electricity. This is due to the electrogenic bacteria' unique metabolic activity(Nenov *et al.*, 2017). The performance of MFC in terms of bioelectricity generation was assessed by measuring voltage output and power densities(Teoh *et al.*, 2020).

2.5. Types of Microbial Fuel Cell

2.5.1. Single Chamber Microbial Fuel Cell

The anode and cathode are both contained in a single chamber in this form of MFC. The anode is separated from the cathode by PEM and is either far or near it. Internal ohmic resistance can be reduced by reducing interelectrode distance, according to one theory(Singh and Kalia, 2017). However, severe issues such as microbial adulteration and reverse oxygen transport from cathode to anode occur normally in SCMFCs. Simpler and more cost-effective designs are proposed by SCMFCs. These MFCs typically just contain an anodic chamber, with no requirement for air in the cathodic chamber(Singh and Kalia, 2017). A cathode is directly linked to a PEM in a hydrogen fuel cell, allowing air and oxygen to react at the electrode. This principle is utilized to build a single chamber MFC in which the anodic chamber is connected to a porous air exposed cathode separated from one another by a gas diffusion layer or a PEM, resulting in passive oxygen transfer to the cathode(Flimban *et al.*, 2019).

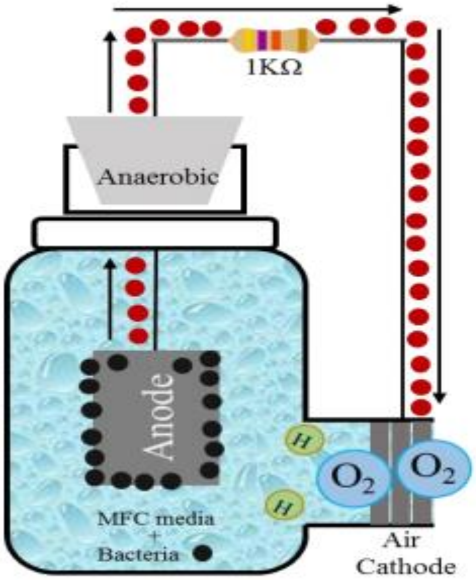
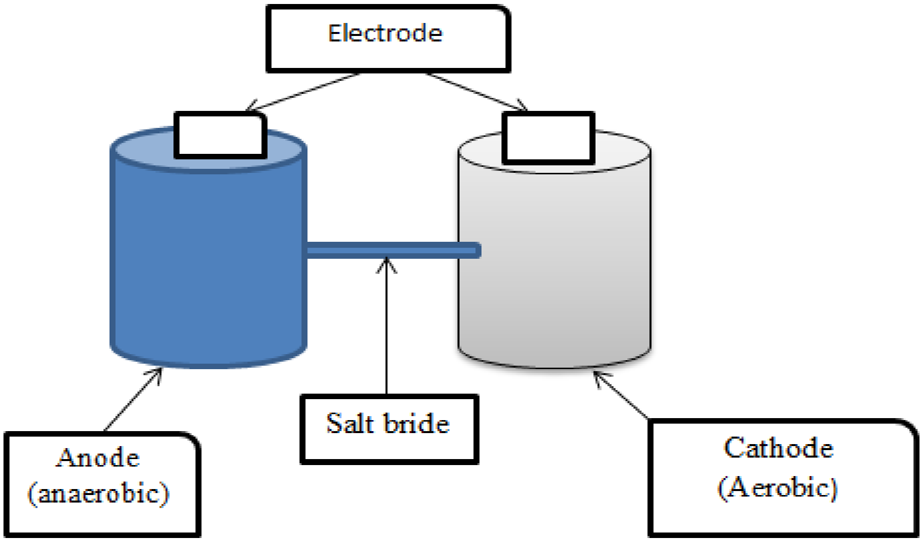


Figure 2.1 The Single chamber microbial fuel cell**(Flimban *et al.*, 2019).**

2.5.2. Double chamber

The simplest design of all MFCs is the double-chamber MFC and widely used in most research of MFC. One bottle (of various types) serves as the anode, while the other serves as the cathode, separated by PEM in a common design. In two-chamber MFCs, energy is typically generated by a predetermined medium (or substrate) in the anode and a determined catholyte solution in the cathode(Singh and Kalia, 2017). Anodic and cathode chambers are separated by a PEM in double-chambered fuel cells, which allows proton transport from the anode to the cathode while inhibiting oxygen diffusion into the anode. As a result, this setup is frequently utilized to clean wastewater while also producing electricity. Both the anode and the cathode are independent compartments that are joined by a proton exchange membrane (PEM), which serves primarily as a proton transfer medium to complete the circuit between the two chambers. This completes the reaction and prevents oxygen or oxidizers from diffusing from the cathode(Flimban, S.G.A., Ismail, I.M.I., Kim, T., Oh, 2019).



(Flimban *et al.*, 2019)

Figure 2.2 Dual chamber of MFC

2.5.3. Up Flow Chamber MFC

A U-shaped cathode inside the anode chamber was created for an up flow microbial fuel cell (UMFC) system(He *et al.*, 2006). Because of the substrate imbalances, the vertical cascade stacked MFC system may experience voltage reversal. However, because the electrolyte fluid flow was primarily gravity driven without pumps, the cascade stacked MFC can be employed as a low-cost option. Extra pumps are frequently needed to deliver substrate to horizontally stacked MFC systems(Flimban *et al.*, 2019).

2.5.4. Stack MFC

It is a structure in which fuel cells are stacked to make a fuel cell battery. This type of architecture has no effect on each cell's individual Columbic efficiency, but it raises the entire battery's output to be equivalent to conventional power sources. These can be stacked either in series or in parallel. Both are important and have excellent power efficiency, allowing them to be used as a power source. The series and parallel connections of the stack provide more voltage and current, respectively; as a result, the required voltage, current, and power in electronic devices can be met. Because MFCs can be connected in both series and parallel circuits, they are referred to as stacked MFCs(Flimban *et al.*, 2019). Terracotta cylinders were used to assemble individual MFCs(Gajda, Greenman and Ieropoulos, 2020).

2.6. Mechanism Microbial Fuel Cell

2.6.1. Oxidation-Reduction Reaction in Living Organism

This study focuses on the organic matter redox reactions and how they lead to antimicrobial activity. It examines what types of reactive oxygen species are produced, how they are produced, and where they are likely to end up within the phagosome, as well as the role of various oxidative reactions in microbial killing(Winterbourn and Kettle, 2013).

Use of microbial extracellular electron exchange process for catalyzing oxidation and reduction at electrode environment in MFC also described microbial electrocatalysist. The two compartments are normally isolated by a proton or cation trade layer and are electrically interconnected through an outer circuit with a resistor or burden.

Anaerobic substrate oxidation by microorganisms produces carbon dioxide, protons, and electrons. The protons are moved to the cathode chamber through a separator. The electrons are moved first to the anode and afterward stream to the cathode by means of an outside circuit consequently(Kalathil, Patil and Pant, 2018).

2.6.2. Microorganism

Detritus from plants and animals, as well as root exudates, provide vital energy and nutrients to soil microbial and faunal populations. Bacteria and fungi account for nearly all of the biomass in most soils, where they interact with a mix of micro- and macro-fauna in complex food-web systems that control the turnover of organic matter and nutrients in the environment.

In the ecosystem, there are several microorganisms that have different capabilities for decomposing organic carbon fractions such as cellulose, lignin, hemicelluloses, chitin, and lipids. Decomposition is primarily a microbial-mediated process, although its rate and extent are regulated by environmental factors such as soil temperature, moisture, oxygen, and other factors(Khatoon *et al.*, 2017).

To understand mineralization, respiration, and growth of heterotrophic microbes, microbial ecologists must understand the chemical composition of detrital organic material, since all of these microbial processes are heavily reliant on the compounds and elements present in organic material(Kirchman, 2013).

Table 2.2 Components found in household wastewater

Component	Of special interest	Environmental effect
Microorganisms	Pathogenic bacteria, virus and worms eggs	Risk when bathing and eating shellfish
Biodegradable organic materials	Oxygen depletion in rivers and lakes	Fish death, odours
Other organic Materials	Detergents, pesticides, fat, oil and grease, colouring, solvents, phenols, cyanide	Toxic effect, aesthetic inconveniences, bioaccumulation in the food chain
Nutrients	Nitrogen,phosphorus, ammonium	Eutrophication, oxygen depletion, toxic, effect
Metals	Hg, Pb, Cd, Cr, Cu, Ni	Toxic effect, bioaccumulation
Other inorganic materials	Acids, for example hydrogen sulphide, bases	Corrosion, toxic effect
Thermal effects	Hot water	Changing living conditions for flora and fauna
Odour (and taste)	Hydrogen sulphide	Aesthetic inconveniences, toxic effect
Radioactivity		Toxic effect, accumulation

(Khatoon *et al.*, 2017)

2.6.2.1.Exoelectrogens

Electroactive bacteria come in a wide variety of forms and have been studied for their ability to pass electrons and participate in bioelectricity generation in MFC systems. Including the first bacteria to display self-mediated properties to the most recent extremophiles, a wide range of bacteria have been highlighted for their abilities as biocatalysts(Pinto, 2017). MFC is a biotechnology that converts and recovers energy using microorganisms in an anaerobic environment. Unlike Anaerobic digestion of biogas, MFC belongs to the Bio-Electrochemical Systems (BESs) sub-discipline, which has the benefit of producing a direct electrical output. Exoelectrogens bacteria are used, which are capable of closing their respiratory electron chain on the electrode surface(Cheng, 2009).

2.6.3. Anodic Side Chamber

The anodic chamber is an important component of the MFC since it is where microorganisms assist in the degradation of biomass in the chamber. An electron acceptor is the anode electrode, which is a vital component in the electron transfer process. Various parameters, such as electrode material, bacteria, and proton transfer membrane, influence the MFC's operation and efficiency.

The anodic electron transfer mechanism is one of the most prominent systems. So much effort is being put into optimizing the system in order to achieve the greatest results. The most significant features for materials as anode electrodes are electrical conductivity, chemical stability, corrosion resistance, high surface area, high mechanical strength, and low cost(Chhazed, Makwana and Chavda, 2019).

2.6.4. Cathodic Chamber

An MFC's performance is mostly determined by the cathode. The cathode should have a high redox potential and features that allow for easy proton transfer. Commercially accessible carbon electrodes were initially the most commonly utilized, with graphite being the most popular due to qualities such as a large electrochemical window, low residual current, recyclability, reusability, and sufficient electrical conductivity.

Apart from graphite, cathode materials are similar to carbon-based anode materials such as carbon veil, carbon cloth, carbon rod, and carbon paper, as well as metal-based materials such as nickel and titanium. However, when these materials were employed as a rod or single sheet, productivity was reduced because they lacked a larger surface area, which is required for improved microbial activity increase(Chhazed, Makwana and Chavda, 2019).

2.6.5. Proton Exchanging Membrane

As the component through which proton transfer occurs, the membrane is important to the MFC's performance. The anodic and cathode chambers are physically separated by membranes. Although it has been discovered that membrane-less single-chamber MFCs have a greater power density, an increase in oxygen and substrate diffusion reduces the MFC's columbic efficiency(Chhazed, Makwana and Chavda, 2019). MFCs can be transformed into microbial electrolysis cells (MECs) for hydrogen production by delivering a higher voltage to reduce protons in the cathode(Sevda *et al.*, 2015).

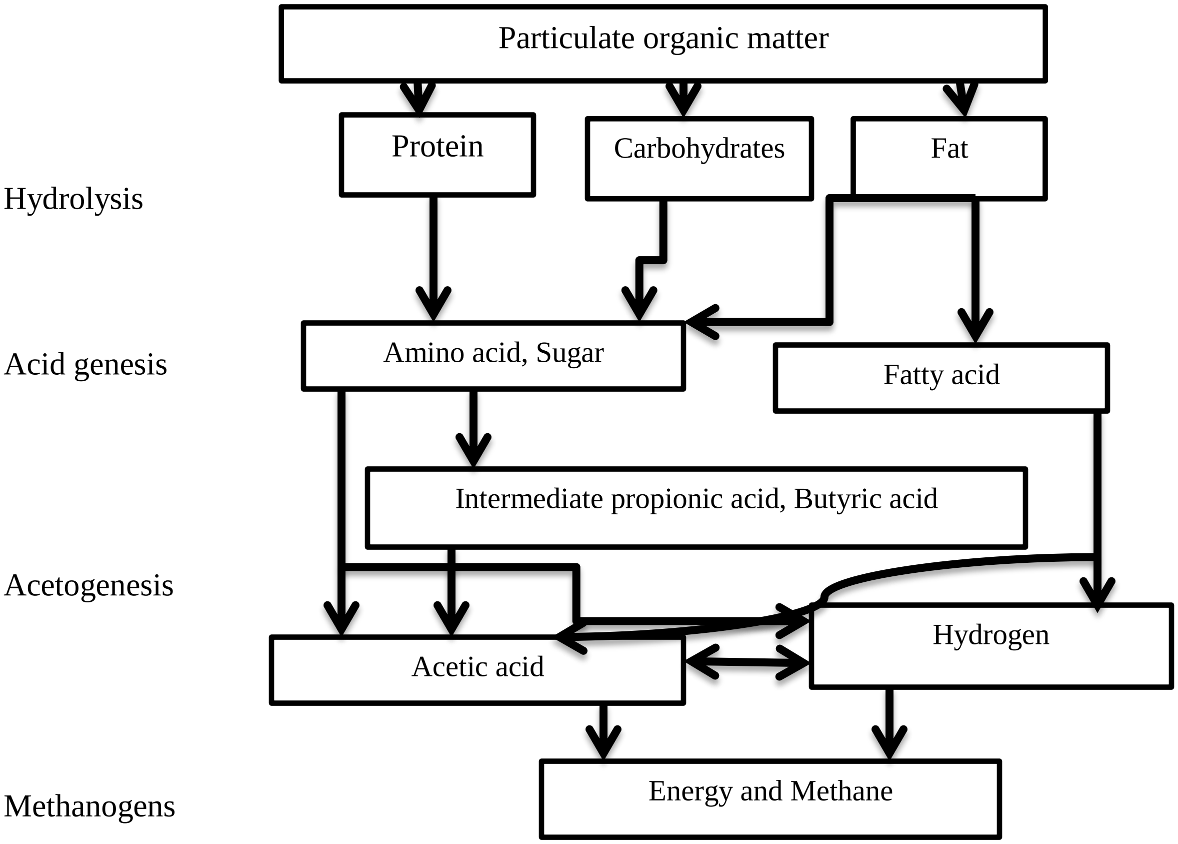
The ability of the proton exchange membrane to absorb water is directly proportional to the MFC's ability to generate electricity. The migration coefficient of the proton is larger than that of water when the water content of the membrane is high, and the conductivity is likewise higher.

As a result, the membrane's conductivity improves as its water absorption increases(Fan, Shi and Xi, 2020). Microbial Fuel Cells (MFCs) rely heavily on proton exchange membranes, but their expensive cost has long been a barrier to their commercialization(Ghasemi *et al.,* 2012).

2.6.6. Substrate

One of the most crucial components of a microbial cell is the substrate. It is a source of nutrients and consequently energy for microbes to carry out their metabolic respiratory activities. The cell's efficiency and performance are determined by the substrate's current and power densities, as well as Faraday's efficiency (Columbic Efficiency). Organic substrate can usually be employed as pure compounds for experimental purposes to measure the efficiency of any organic matter. Complex organic molecules present in wastewater, on the other hand, are useful because they provide an overview of the MFC's uses, which primarily include power generation and wastewater treatment(Khaloufi and Elasli, 2019). Human urine provides about 80% of the nitrogen, 50% of the phosphate, 10% of the chemical oxygen demand (COD), and significant amounts of sulfate and potassium to municipal wastewater, despite accounting for just 1% of the total volume(Wang *et al.*, 2017).

The type of organic substrate has a distinct effect on the MFC's performance. In comparison to glucose (single sugar) and sucrose for the same organic load, acetate is the most efficient substrate (double sugar). Despite the fact that the trends were usually comparable, the patterns of voltage and power generation over time for fermentable and nonfermentable substrates were very different(Ullah and Zeshan, 2020). Sludges from wastewater treatment are complex substrates that are rich in organic carbon, nutrients, and energy.Bomb calorimetry was used to determine the energy content of wastewater sludge(Gude, 2016b).



(Khatoon *et al.*, 2017)

Figure 2.3 Flow chart of anaerobic digestion

2.7. Microbial Fuel Cell Technology's Advantages

The usage of MFC technology has a variety of advantages. The following are some of the advantages of MFC: energy production (heat, light, electricity). In addition, organic wastes can be converted into high-quality fertilizer, and hygienic conditions can be improved by reducing diseases, worm eggs, and flies. In addition, the workload in firewood collecting and cooking has been reduced, mostly for women. Environmental benefits include the conservation of trees, soil, water, and air, as well as the reduction of CO_2 and CH_4 emissions from garbage disposal sites, which helps to mitigate global warming. As a result, if the conditions are favorable, MFC technology can make a significant contribution to conservation and development.

2.7.1. Economic Advantages

MFC is more cost-effective than other treatment approaches when looking at the entire life cycle. It can also stand for kerosene, diesel fuel, and maybe wood or charcoal. Energy supplies for both rural and urban communities its low capital with local product material. This is the equivalent of a few liters of wastewater. Because bio-slurry is used, it can improve soil productivity. As a result, there will be savings on chemical fertilizers and/or more money from greater agricultural yields, as well as a significant reduction in the workload of women seeking for firewood(Aberra and Fufa, 2016).

MFCs have a significant economic impact. To begin with, water treatment plants necessitate the use of energy in a very costly procedure. In the United States, for example, the cost of wastewater treatment is estimated to be around $30 billion per year, and this figure is rising. MFCs can be used to recover the energy required to maintain and supply water treatment plants, allowing them to become self-sufficient. The MFC then produces electricity while removing the contaminants from the effluent(Khaloufi and Elasli, 2019).

2.7.2. Health Advantages

Eye irritation, lung problems, asthma, dizziness/headaches, and respiratory tract infection are among the ailments caused by smoking. Typhoid, paratyphoid, cholera, and dysentery bacteria (in one or two weeks), hook worm, and bilharzias are among the main species eliminated in MFC plats, when sludge reacts with tapeworms and roundworms, they die altogether. In addition, when latrines are connected to MFC component, MFC improves family sanitation, makes cooking easier and cleaner, and creates greater hygiene(Amani, Nosrati and Sreekrishnan, 2010).

2.7.3. Environmental Advantages

Environmental challenges related to water sanitation are not limited to underdeveloped countries; they are among the most basic human and environmental needs worldwide. Environmental pollution in surface and ground water bodies is primarily caused by wastewater sources(Gude, 2016b). MFCs have a direct positive environmental impact. They give an alternative to using fossil fuels as a source of energy, reducing carbon dioxide emissions and greenhouse gas emissions while also producing power. MFCs also play a key role in the water-treatment industry, where they help to solve sanitation and water scarcity issues. The use of microbial fuel cells to regulate ecosystems has many advantages: Significantly decreases greenhouse gas (GHG) emissions, removes odour, creates sanitized compost and nutrient-rich liquid fertilizers, maximizes recycling benefits, and prevents land fertility degradation due to overuse of chemical fertilizers. Organic fertilizers derived by MFC wastewater treatment contain three times more nitrogen than the finest compost created through open or no-air digestion, reducing local deforestation and improving climate change monitoring strategies, according to research solve by MFC(Aberra and Fufa, 2016).

2.7.4. Implications for Society

The socio-environmental impact of an MFC is the most significant. The well-being of humans is dependent on the well-being of nature and the environment in which they live. MFCs use wastewater that would otherwise be discharged into the environment for a variety of purposes, including energy generation. In Ethiopia, the present goal is focused on renewable energies, which includes rural areas. MFC are Electric and heaters, which are usually used in rural areas and are the most inefficient, due to using fuel as energy source. As a result, the locals rely on burning wood for heat. As a result, MFCs can address societal challenges that are directly tied to electricity scarcity(Khaloufi and Elasli, 2019).

2.7.5. Technological Implications

MFCs are a relatively new technology that is still being studied. Extensive study is still being carried out, merging expertise from several domains and yielding excellent and promising findings. MFCs contribute to the continuing renewable energy research. Because the functioning concept of an MFC is totally natural, it is equally vital to recognize that MFCs open the way for new discoveries inspired by nature to contribute to technological growth(Khaloufi and Elasli, 2019).

2.7.6. Implications for Political

Sustainability is a topic that is being debated more and more these days, not just in Ethiopia but around the world. It is also a factor in big political decisions because it affects future generations. Ethiopia, in particular, is reliant on imports to meet its energy demands, and with global fossil fuel stocks dwindling, the country is moving toward allocating large expenditures to renewable energy. MFCs are a relatively new technology; however they align with the country's environmental goals(Khaloufi and Elasli, 2019).

2.7.7. Implications for Ethics

It is our responsibility to practice environmentally friendly behavior. Small actions, like large ones, should be recognized and cherished. The primary impetus for this study is the constant rise in environmental difficulties as a result of industrialization and technological growth, which is a double-edged sword. While human actions are necessary for our survival, it is also critical to be aware of the environmental impact we have. MFCs are compliant and justified from an ethical standpoint. They also symbolize the fight against climate change and the future of sustainability(Khaloufi and Elasli, 2019).

CHAPTER THREE

3. METHODS AND MATERIALS

3.1. Sampling Area

The study was conducted in Wolkite University is located in South Nation Nationality People Regional state (SNNPR) in Guraghe zone in cheha woreda sub-city Gubere town and separate woreda in south-western Ethiopia. Wolkite town found in the administrative center of the Gurage zone of the Southern Nations, Nationalities and Peoples' Region (SNNPR), this town has a latitude and longitude of 8°17′N 37°47′E and an elevation between 1910 and 1935 meters above sea level.

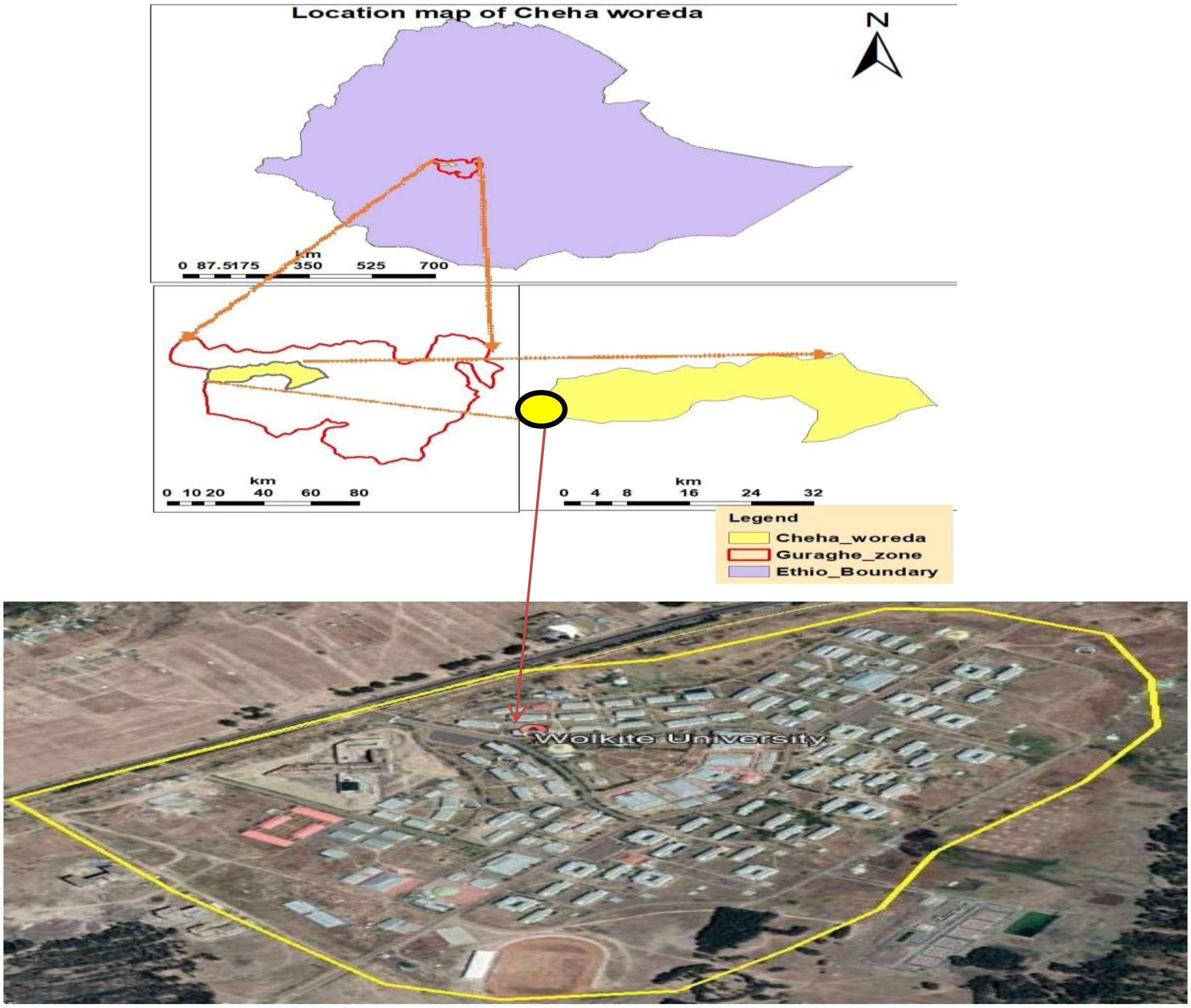


Figure 3.1 Map of study area

3.2. Materials

3.2.1. Plastic Bottles

Plastic bottles are low-cost, lightweight, and long-lasting materials that may be easily molded into a number of products for a variety of uses. For this study plastic bottle are hold capacity 2000ml of wastewater samples for each chamber of the microbial fuel cell. First, a two-chamber MFC was chosen because it is easier to manipulate and more adaptable than a single chamber MFC. To alter and experiment with different electrodes, it is more convenient to do so in a double chamber MFC. Have been utilized plastic kitchen food containers and water bottle with a 2L capacity for both the anode and cathode chambers. Plastic is less expensive and widely available than most ceramics, including glass which can break and cause leaks.

Figure 3.2 Two Liter plastic container

3.2.2. Electrode

Electrodes are crucial components in electrochemical systems. Some of the most significant materials from both dual chambers of MFC (metals) electrodes have been used. The electrode materials of choice are metals and their alloys. For this study have been used metal electrodes which are aluminums and stainless steel so those electrodes with greater qualities than their counterparts, and electrode material development trends.

3.2.2.1. Anode Electrode

Some significant elements must be considered while choosing an anode material; the material should then be: For large-scale applications, it is cost-effective, high conductivity, high porosity, non-corrosive, and bacterial growth-friendly.

Different materials can fit this criterion, and it was able to test three of them. Aluminum is usually employed as the anode in most studies because it is extremely conductive, non-corrosive, and porous, therefore it is characterized by its non-brittleness, which is its main advantage. Steel plates or rods, which have a high conductivity, have also been employed in various investigations.

Have been employed and tested aluminum and steel for our MFCs, with corresponding surface areas are the same 32 cm^2. The latter three ingredients were accessible in the chemistry lab; for the electrodes, purchased a huge metal mesh from a hardware store in Wolkite town.

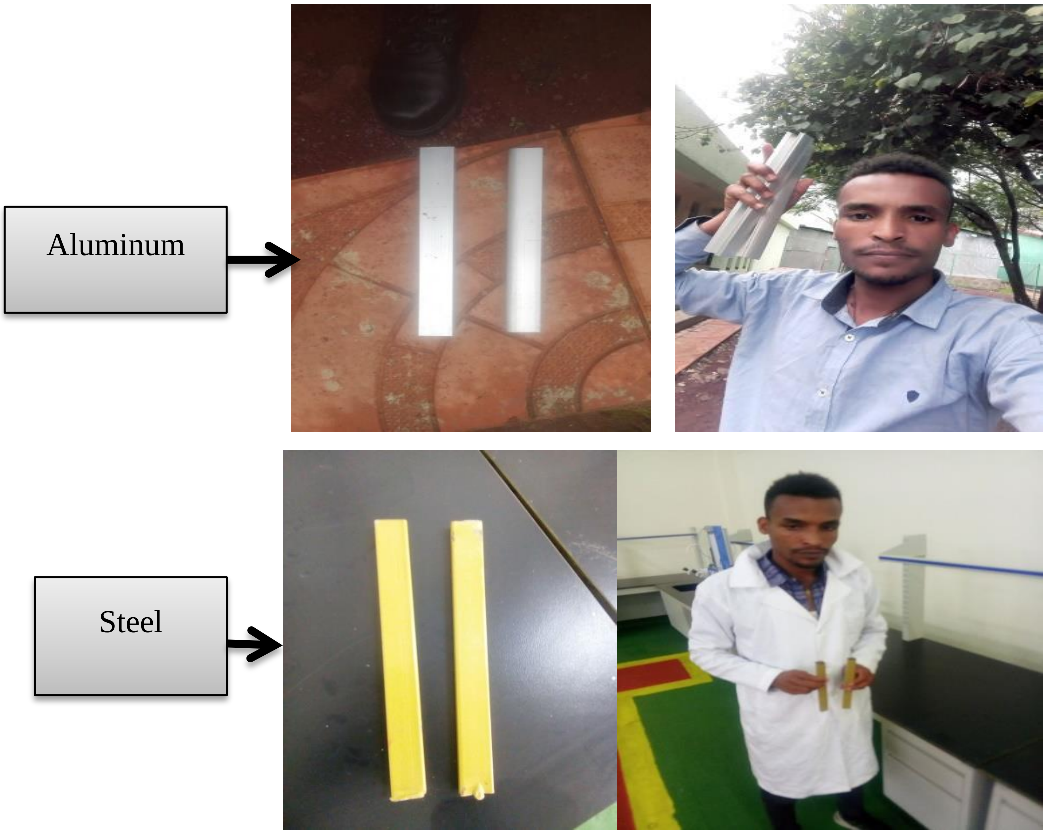


Figure 3.3 During preparation of aluminum and steel electrode materials

3.2.2.2. Cathode Electrode

The cathode, like the anode, must have two main properties: conductivity and non-corrosion. Steel sheets and aluminum can thus be used in place of the anode. The only difference in this research without application of the chemical a catalyst, but another study show that usually platinum, is used frequently to boost the reduction reaction at the cathode in MFC digestion. Typically scientists were utilizing a standard carbon cathode with platinum on one side in contact with water and the other in contact with air. It did not use any catalysts in studied since it is difficult to get on the market and requires specific expertise in materials coating to manufacture in the lab. As a result, the materials used as cathodes in our series of studies are aluminum and steel.

3.2.3. Salt Bridge

A salt bridge is an important component in an MFC because it keeps the anode and cathode separated. This is important because water in the cathode contains dissolved oxygen, and it wants to keep the anode anaerobic. Furthermore, it must allow for spontaneous proton migration from the anode to the cathode. As a result, selecting a membrane is not as simple as it appears. When it employ a solid, the cathode and anode are effectively separated, but protons are unable to migrate. Another issue for the membrane is preventing other substances from passing through, such as electrons or the substrate.

The anode and cathode processes are separated by the microbial fuel cell (MFC) membrane, which prevents oxygen from entering the anode chamber while allowing selective ion transport between the anode and cathode. Because of its strong proton conductivity, Nafion 117 is the most commonly used material. The biggest downside is the high cost ($1400/m^2) of the property(Khaloufi and Elasli, 2019). As a permeable membrane, it utilized a cotton rope (1m long) purchased from a curtain shop in Wolkite town and twisted multiple times (end length of 25cm) for each MFCs.

With a volume of 2000 ml plastic bottles and a diameter of 15 mm PVC pipe was used to connect both the cathode and anode chambers for MFC. Each pipe has 15mm holes for solution addition as well as salt bridge inlets. To prevent air from entering the anode side, the holes were sealed with a tiny cup.

3.2.4. Copper Wire

Copper wire is used for the external circuit that connects the cathode and anode, as in this study. The copper wires were attached to the electrodes with electric tape on both electrodes side chambers of the MFC.

3.2.5. Extra material

Finally, a glue gun was being required to join the MFC's various components. Furthermore, ‘Parafilm' was employed to reduce salt bridge leaks. The salt bridge was also covered with a variety of tapes, primarily electric tape. Finally, for data collection, we employed a digital multimeter. The many different varieties of glassware used in an analytical laboratory are one of the first things that most people notice when they go into one. Each piece of glassware is designed to serve a specific purpose. Glassware commonly encountered in an analytical laboratory includes have been used the following: Beaker, Graduated Cylinder, Pipet, Burets, Flask and Bottle.

In every work of laboratory, a pH meter is one of the most common pieces of analytical equipment. A pH meter is a device that determines the acidity or basicity of a sample by measuring its pH. Because changes in pH can have a major impact on the success of many treatment techniques, measuring pH is critical have been used for this study.

Spectrometer is devices that analyze the different parameter. A Spectrometer measures a sample's absorbance/transmittance. Chemical reagents are used to react with the substance to be measured. The various materials in the sample determine have been used. Dissolved oxygen and conductivity tests were performed on digital spectrometer.

Balances have been used to weigh solids analysis objects like dry chemicals, filters, and crucibles of wastewater samples.

Samples that would degrade if kept at ambient temperature prior to analysis can be kept in the refrigerator. COD, microbiological, organic, and other analyses are some examples. Temperature measurements are made with a thermometer used, which can have a substantial impact on waste sample several treatment operations in microbial fuel cell.

Crucibles are used to keep the sample contained while it is being heated on a burner. Ovens are used to bake chemical reagents or samples in order to dry them out or remove unwanted elements from them. A desiccator is a glass container with a tight-fitting lid. The desiccator is used to keep samples dry by filling it with desiccant. Typically, a sample is dried in an oven and then placed in a desiccator to cool before being weighed or going through further processing.

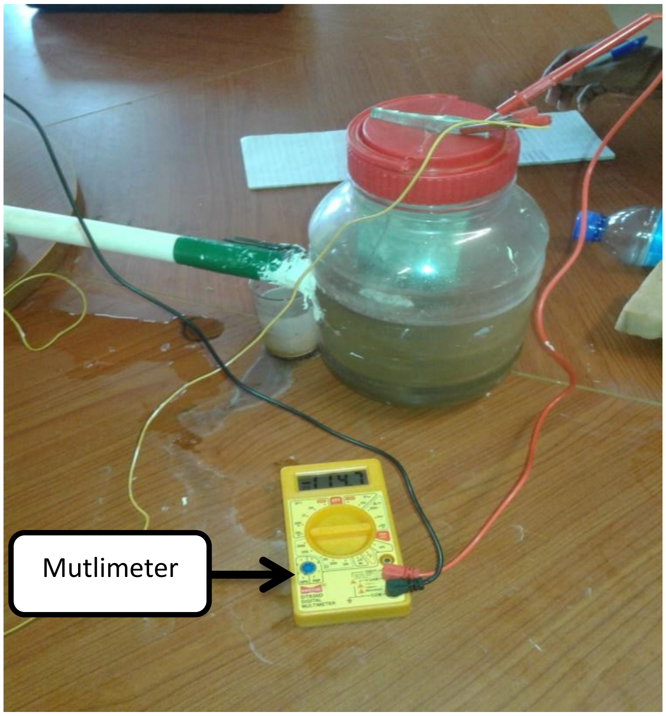


Figure 3.4 Figure Mutlimeter

3.2.6. Substrate

Study was tested three distinct substrates for microorganisms: urine, blackwater, and graywater. All of the sludge was collected from a deep sewage on campus; however the sludge contained more water than the real substrate. As a result, the sludge used in this research was gathered from various areas of the Wolkite University compound. 2L was measured and utilized for all substrates.

3.3. Study Design

The study's objectives was investigated a laboratory-based experimental work. The capacity of microbial fuel cells and the type of substrate employed were evaluated using experimental research designs. The quantity of electric current produced by wastewater during treatment was measured using an experimental approach. The cross-sectional methodology was used to inspect the capability of each type of substrate's power generation capability during the research design.

The MFC is made up of main four parts:

I. Anode: The bacteria and organic debris are kept in an anaerobic condition in the anode chamber.

II. Cathode: Container containing a conductive water solution

III. Proton-exchange membrane: Salt Bridge is a proton-exchange membrane that divides the anode and cathode and allows protons to flow between the two chambers.

IV. External circuit: permits electrons to enter the cathode and serves as a conduit for them to go through when they are extracted out of the anode's solution.

As part of their digestive process, bacteria in the anode chamber produce protons and electrons by oxidation. Microbial Fuel Cells (MFCs) are well-known for their ability to transfer chemical energy from organic substrates into electricity.This is due to the so-called Electrogenic bacteria' unique metabolic activity.Anode and cathode are connected by an external circuit and split into compartments by a proton exchange membrane in a conventional Microbial Fuel Cell(Nenov *et al.*, 2017).

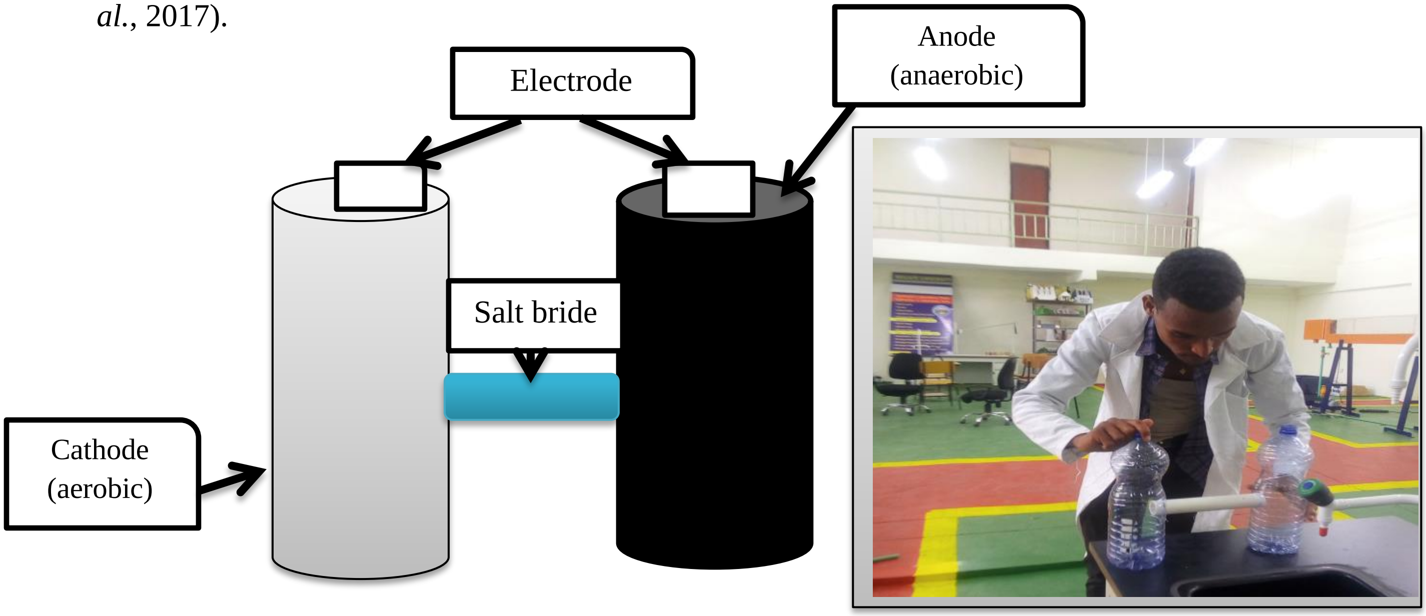


Figure 3.5 Figure diagram of MFC system setup.

An MFC can be divided into four major components, namely, an anaerobic anode chamber, an aerobic cathode chamber, and separator connecting the two chambers, which has to guarantee proton exchange. Anode chamber provides all the necessary conditions for the growth and the electron extraction from microorganisms. The chamber is fed with growth media named as anolyte, microorganisms, and an electrode that acts as the anode. The oxidative microbial metabolism in this chamber produces protons and electrons. The metabolic reactions are not allowed to proceed to completion and the intermediate electrons are drawn from the cell to produce electrical work. Electrons are transferred by microbes to the extracellular acceptor (anode) and flow to the cathode through a resistor, producing electricity.

Figure 3.6 Laboratory built of dual chamber of microbial fuel cell

The following are the procedure has been used to construct the double-chamber MFCs:

Step 1: A salt bridge is first created by soaking a 1m cotton rope in a very saturated salt solution for 2 hours at 95°C, then allowing it to absorb the fluid overnight (24 hours). Before being taped with two different types of tape, the rope was twisted multiple times. Both ends of the salt bridge were left exposed

Step 2: When using fire, prepare two holes in both plastic containers for the salt bridge to be inserted into. Ensured that there were no microscopic gaps by utilizing the glue gun to apply hot glue across the salt bridge

Step 3: Two more holes were incised at the top of the cathode container, one for the conducting wire and the other for the aquarium air pump shaft. Initially, an air pump was employed, but all of the findings were obtained without it.

Step 4: After the compartments have been set up, the electrodes are formed of aluminum by folding the mesh numerous times to obtain a surface area of 32 cm^2 (8*4), then securing the folds with paper clips. (Another stainless steel electrode had been made previously.)

Step 5: Copper wires are joined to the electrodes for both electrodes using a glue gun and electric tape.

Step 6: The electrodes were placed in their compartments while the anode chamber was sealed and the cathode was left aerated. After that, both wires were connected to an optical multimeter, which was used to detect the electric potential and other characteristics.

Step 7: Sludge is placed in the anode chamber and water is placed in the cathode chamber until the anode chamber is closed. Both water and sludge should cover the salt bridge and electrodes.

3.4. Sample Size and Sampling Procedure

3.4.1. Sample size

Samples were collected by purposive sampling technique from three wastewater source. For the sample selection important criteria were considered: Main source of wastewater stream in Wolkite University compound have taken samples from different polluting sources like waste disposal sites, and sewer line different manhole were considered.; Elevations, Longitude, and altitudes of selected sample sites were determined on the field using a GPS model 60.

First, it has chosen a two-chamber MFC because it can be easily manipulated and more flexible than the single chamber one. It wants to change and try different electrodes and for that reason, it is more convenient to do so in a double chamber MFC. For both the anode and cathode chambers, we used kitchen food containers made of plastic with a volume of 2L each (fig 3.5). Plastic is cheaper than most ceramics including glass that can be subjected to cracks and therefore leakages.

Figure 3.7 During sample collection of Blackwater Wolkite University compound manhole

Figure 3.8 During the sample collection of the Graywater Wolkite University compound from dorm and washing area.

Figure 3.9 Figure 3.9 Health adult urine sample collected from WKU chemistry staffs

Table 3.1 The Sample point of the substrate

Sample code	Name of the location	Size of sample in liter	GPS Reading			
			Easting	Northing	Elevation (m)	Time and date
MFC1(Urine)	WKU Chemistry lab staff	2 liter	36^0 49	$8^0$40	1911	8:30AM- 8:45Am 30/3/2021
MFC2(Black water)	WKU Block7manhole	2 liter	36^0 49	$8^0$59	1918	11:22 AM- 11:58AM 30/3/2021
MFC3(Gray Water	WKU Block 4 student bath room	2 liter	36^0 49	$8^0$39	1916	1:22 PM- 1:58 PM 30/3/2021

3.4.2. Sampling procedure

Wastewaters samples were collected various hours in the days. This was to ensure that the wastewaters had not been disturbed much through bacterial growth which can affect the temperature and content of total dissolved solids. All plastic bottles were cleaned with warm water and soap then rinsed with distilled water three times. Wastewater samples for microbial analysis were collected with 1000ml plastic bottles and holding in black box to prevent bacterial contamination. Wastewater samples were taken from Wolkite University compound found in SNNPR Ethiopia samples are in well-mixed typical samples. To limit sample fluctuation, the

WW was collected for one day in a row, in the morning, midday, and evening. The volume of WW collected in the morning, midday, and evening was then combined to create a single sample of wastewater for that day. Then after that day, the samples were collected brought into laboratory. The collected samples were maintained in the refrigerator at 4°C to avoid any changes in the results during the experiment.

MFC, suitable fittings, and other measurement devices were prepared before to collecting samples from each site. During the experiment, the sample was prepared, different parameters were tested and recorded (pH, Conductivity, TS, VS, Turbidity, and voltage), several mixes were prepared, and ultimately the experimental data were collected. At a temperature range of 22 – 40 °C, the pH of the solution (slurry) was adjusted over the course of the production time to a standard pH (5-8).

3.5. Sample Preservation, Measurement and Analysis Processes

3.5.1. Sample Preservation

The samples were put at 4 degrees Celsius for one day in their original water-based suspension. Prior to the trials, sludge samples were mixed together and left to acclimate at room temperature before inoculating the entire connected stack (3 MFCs) with 2000ml of sludge added through the MFC inflow and allowed to flow down

For each parameter, the maximum holding duration was retained until the beginning of the laboratory measurement process. Almost all compounds that are being examined must be maintained if the analysis cannot be completed immediately. A qualified laboratory was offering you with the essential types of sample bottles, as well as the required preservative, when you need to collect a sample. The maximum holding time was kept and performed based on the WHO/UNEP, 2016 standard protocol.

3.5.2. Analysis of Wastewater Sample

The data obtained from laboratory experiment was analyzed and summarized in to tables and graphs by using Microsoft office excel spreadsheet and different formulas. A variety of analytical procedures have been utilized in the laboratory. Five of the most common techniques used in a WW lab include ion specific analysis, gravimetric analyses, spectrometric analyses, titration analyses, and volumetric studies. In most ion specific analyses, an electrode and a voltmeter are utilized. Typically, the electrode is ion-specific, detecting only the ion of interest. The signal from the electrode is picked up by the multimeter, which is a millivolt meter. The meter's millivolt value rises in proportion to the sample concentration. In ion specific analyses, pH, voltage, and D.O. are commonly utilized. After a sample has been submitted to an analytical method, gravimetric analyses are utilized to determine its mass or change in mass.

A spectrometer used the ability of various substances to absorb or transmit different wavelengths of light to create a measurement. The amount of analyze in the sample determines how much light of a certain wavelength is transmitted or absorbed. To change or enhance light transmittance or absorbance, a reagent and/or indicator are frequently added to the sample. A titration analysis is a method of analyzing a sample by adding a specified amount of a standard solution to it. The concentration of analyze in the sample is proportional to the amount of standard solution added. The overall volume of the sample and the volume of analyze in the sample were compared using volumetric analysis. This method is used in several substrate tests.

3.6. Study Variables

3.6.1. Dependent Variable

To determination of electric current generating for each type of substrate anaerobically wastewater treatment to develop microbial fuel cell.

3.6.2. Independent Variable

The independent variables exist in development of microbial fuel cell research account here are below list.

First one is substrates microbial fuel cell (MFC) technology, which uses bacteria as a biocatalyst to convert diverse substrates from renewable sources into electricity, has the potential to be developed as an alternative energy source. The MFC substrate has benefits in wastewater treatment. MFC has been the subject of research to improve its electricity output.

Second one electrode in microbial fuel cells (MFCs), which use microorganisms as catalysts to oxidize organic (inorganic) matter and transform chemical energy into electricity, rely heavily on electrode materials to determine their performance (e.g., power production) and cost. The implications of current advancements in anode/cathode materials on various practical wastewater treatment processes are discussed in this research.

3.7. Data Collection Process

A sample must first be obtained before it can be analyzed while it may seem self-evident, the significance of gathering a representative sample cannot be emphasized. If the sample isn't representative, the analysis isn't only worthless; it could be misleading, leading to unneeded and potentially harmful treatment changes. In order to meet the thesis objectives, this procedure incorporates both secondary data (desk) and primary data (laboratory investigation) for the collection of relevant data. It includes the techniques used to achieve the theme. The deskwork comprises a review of modeling publications, books, and past work.

3.8. Data Quality Assurance

These laboratory experiment purposes are to generate accurate data. Because this data may be analyzed by experimenters, the laboratory must verify that the data it reports is accurate and that it can be proven to be such. To ensure that the data provided is reliable, the laboratory must create a Quality Assurance (QA) plan and follow its guidelines. On different days in laboratories, different analysts perform the same analyses. Each analyst must follow the same approach to ensure that the results obtained by different analysts are similar. Similarly, these established rules ensure that the analysts employ the same methodology on a daily basis. The best method to ensure that the laboratory produces reliable data is to carefully train all analysts in the correct operational protocols.

The data quality was ensured through triplicate sample analysis and replication (the average plus or minus was reported) of the samples in operational procedures for quality purposes, and software (excel software) was utilized for data reporting. To verify the results' credibility, adequate quality assurance procedures and measures were implemented. Data quality assurances were thoroughly evaluated, and triple measurements were taken to ensure data quality. Samples were taken three times and measurements were done three times alone to reduce error, and the average value was used for both field and laboratory measurements. While conducting the analysis, the quality of the data was ensured by tripling it and averaging the results.

3.9. Dissemination Plan

Before being disseminated, the study's findings will be offered for review to see if there are any issues. Following that, attempts will be made to open to relevant bodies for distribution. Publication in recognized national and international journals will also be considered.

CHAPTER FOUR

4. RESULT AND DISCUSSION

The goal of this part is to evaluate the performance of a variety of microbial fuel cells constructed in Wolkite University's chemistry laboratory. This section evaluates seven key parameters: characterizing wastewater, electric voltage generation, polarization curves, power density curves, fuel cell internal resistance, and columbic efficiency of each MFC and electrode influence in microbial fuel cell. It's worth mentioning that all MFCs are evaluated under identical external settings (room temperature and pressure). Study were tested the microbial fuel cells over the same time period, leaving them exposed to no external resistance for 24 hours for month.

4.1. Characterizing Wastewater

4.1.1. Prior to digestion in MFC raw wastewater is characterized.

Graywater, blackwater, and urine are all extremely different in terms of volume, quantity, and quality. Urine has the highest nutritional concentration, and its isolation allows for recovery from a much less volume. Despite that graywater makes up the bulk of domestics trash, it is rather clean and hence appropriate for reuse. Light graywater, which does not include kitchen wastewater, has very low particle, organic content, and nutritional levels. Blackwater contains the most organic matter, making it ideal for energy recovery. Urine, blackwater, and graywater quality are summarized in tabular form the shown findings, with further details provided later in this chapter.

Table 4.1 Source and primary contaminant human waste product for the study has taken.

	Urine	Blackwater	Graywater
Source	Toilet, urinals (with or without flush water)	Toilet (with flush water), kitchen sink, dishwasher	Non-kitchen sinks
Contaminant	Nutrients, pharmaceuticals, hormones, salts	Solids, organic matter, pathogens, nutrients	Personal care products, detergents

Graywater accounts for the bulk of domestic wastewater, but because it is largely uncontaminated, it can be reused without further treatment. Kitchen wastewater and brown water contain the most organic elements that can be converted to energy (feces, flush water). Urine has a high concentration of nutrients in a small amount of liquid.

Table 4.2, 4.3 and 4.4 shows the physiochemical and bacteriological parameters of the wastewater utilized before treatment in the investigation, as well as the experimental results. As a result, the value list in tabular form here are below three different substrates.

On average, an adult produces 0.8-1.5 Liter of urine each day, whereas a toddler produces around half that amount. Water makes up 95% of the mixture, with dissolved salts accounting for 5%. Food determines the quality of urine output per capita, yet scientifically established design values have emerged. While urine makes up only 1% of total residential wastewater, it contains 50-80% of total nutrients (75-80% nitrogen, 50-55 percent phosphorus, and 70 percent potassium), as well as the majority of pharmaceuticals and their metabolites. Adults are principally responsible for the elimination of macronutrients (nitrogen, potassium, phosphorus, and sulfur(S. Bakhri, 2015).

Table 4.2 Composition of urine wastewater before digestion

Nọ	Parameter	Unit	Value	WHO Standard	Ethiopian Standard	Remark
1	pH	-	6.3	6-8	5.5-9	Acceptable
2	TS	mg/l	385	≤50	≤120	Out of range
3	VS	mg/l	213	≤50	≤100	Out of range
4	BOD_5	mg/l	208	≤90	≤100	Out of range
5	COD	mg/l	600	≤200	≤250	Out of range
6	DO	mg/l	3.9	4-8	2-8	Acceptable
7	TK	mg/l	2740	≤1100	1100	Out of range
8	TP	mg/l	1600	≤500	≤500	Out of range
9	TN	mg/l	8830	700-2000	≤2000	Out of range
10	TC	Col/100ml	$215*10^4$	$\leq 50*10^4$	$\leq 50*10^4$	Out of range
11	FC	Col/100ml	$98*10^4$	$\leq 20*10^4$	$\leq 10*10^4$	Out of range
12	Conductivity	µS/cm	19067	≤11000	≤11000	Out of range

Col = colonies

In this research, blackwater is defined as wastewater from kitchen sinks and feces. Brownwater refers to the excrement part (together with flushing water and toilet paper). Brownwater has a high organic and sediment content, pharmaceutical and hormone residues, pathogens and indicator microorganisms in high concentrations, and lower nutritional loading than urine. Toilet paper generates BOD_5, TS and COD, and its cellulose component makes it difficult to breakdown.

Along with its high organic content, kitchen sink effluent is frequently combined with brownwater (relative to other graywater streams). Food residues, cleansers (detergents, drain cleaners, bleach, etc.) and oils/fats are all found in kitchen sink and dishwasher effluent. It is one of the most little polluted graywater streams (VS, COD and BOD).

The fact that it contains the greatest amount of nutrients and pharmaceutical/hormone residues (more than half) while being the smallest in volume is the main motivator for urine source separation. Blackwater (feces and kitchen wastewater) contains high levels of organic and nutritional content, as well as sediments, bacteria, and pharmaceutical/hormone residues. Graywater is the lowest polluted of the three streams yet contributes the most to total volume. The most detergents and personal care items are found in light graywater, which is also low in nutrients and pathogens. It's also low in organic content because it's not made with kitchen garbage(S. Bakhri, 2015).

Table 4.3 Composition of black water before digestion

Nọ	Parameter	Unit	Value(mean)	WHO Standard	Ethiopian standard	Remark
1	pH	-	6.7	6-8	5.5-9	Acceptable
2	TS	mg/l	3982	≤50	≤120	Out of range
3	VS	mg/l	1231	≤50	≤100	Out of range
4	BOD_5	mg/l	902	≤90	≤100	Out of range
5	COD	mg/l	1600	≤200	≤250	Out of range
6	DO	mg/l	2.56	4-8	2-8	Acceptable
7	TK	mg/l	1112	≤1100	≤1100	Out of range
8	TP	mg/l	500	≤500	≤500	Out of range
9	TN	mg/l	1388	700-2000	≤2000	Acceptable
10	TC	Col/100ml	$513*10^4$	$\leq 50*10^4$	$\leq 50*10^4$	Out of range
11	FC	Col/100ml	$317*10^4$	$\leq 20*10^4$	$\leq 10*10^4$	Out of range
12	Conductivity	µS/cm	27894	≤11000	≤11000	Out of range

Col = colonies

≤ less or equal

The wastewater from non-kitchen sinks, laundry, and showers is called to as graywater in this study. This is known as "mild graywater" in the scientific community. When compared to other graywater sources, “dark graywater” comprises kitchen sinks, which are the most polluting. 40-60% of the pollution load is contributed by kitchen wastewater (VS, COD, BOD, total oil, and active substances). Graywater's physical and chemical quality varies and is based on its source, according to the lab experiment. This is due to the fact that the quality of cleaning and bathing products, the number of people in a household, and other sink disposal procedures and personal behaviors all have an impact on quality(S. Bakhri, 2015).

When split from urine, graywater is low in particles and nutrients, while blackwater wastewater has a BOD of 902 mg/L and a COD of 1600 mg/L. The variation of these values in relation to the source is clearly seen. Cleaning goods, shampoo/soap, perfumes, and cosmetics have limited biodegradability and can include a lot of micropollutants. Surfactant (detergent) concentrations vary as expected based on graywater sub-stream. Dishwashers and washing machines can provide a high Phosphorus loads if phosphorus is included in detergents; however there is a widespread movement to remove phosphates from detergents. Graywater may also contain significant levels of heavy metals (from plumbing) and salts (from detergents). Pathogens are generally lower in light graywater (as compared to all other home sources), however fecal indicator bacteria and skin/mucus pathogens eliminated during a bath/shower can be present for microbiological bacteria of concern in graywater). If cloth diapers are washed in the same machine as the rest of the laundry, fecal contamination is almost certain to occur; hence this should be factored into the treatment system's design. Graywater flow unpredictability and temperature changes are important factors to consider when designing a treatment system.

Graywater study is a rapidly growing topic, tanks to water reuse applications; hence there is a wealth of knowledge available. The majority of the qualitative data available is for mixed graywater, although a growing number of researches are distinguishing between “light” and “dark” graywater. Apart from quality, there is a wealth of information about treatment and reuse.

Table 4.4 Composition of Graywater before digestion

Nọ	Parameter	Unit	Value	WHO Standard	Ethiopian standard	Remark
1	pH	-	7.4	6-8	5.5-9	Acceptable
2	TS	mg/l	56	50-90	≤120	Acceptable
3	VS	mg/l	37	≤50	≤100	Acceptable
4	BOD_5	mg/l	45	≤90	≤100	Acceptable
5	COD	mg/l	900	≤200	≤250	Out of range
6	DO	mg/l	3.9	4-8	2-8	Acceptable
7	TK	mg/l	5564	≤1100	≤1100	Out of range
8	TP	mg/l	1352	≤500	≤500	Out of range
9	TN	mg/l	564	≤700-2000	≤2000	Acceptable
10	TC	Col/100ml	$6.7*10^4$	$\leq 50*10^4$	$\leq 50*10^4$	Acceptable
11	FC	Col/100ml	$4*10^4$	$\leq 20*10^4$	$\leq 10*10^4$	Acceptable
12	Conductivity	µS/cm	12271	≤11000	≤11000	Out of range

Col = colonies

4.1.2. After the Digestion in MFC, Effluent is characterized.

Table 4.5, 4.6 and 4.7 summarizes the physicochemical and bacteriological parameters of the wastewater feedstock's following digestion in MFC. The found values were wastewaters after digestive in MFC as tables described.

4.1.2.2.Change in urine quality during storage

Urine from a healthy person is usually consistent and germ-free. However, bacteria may be present in the collection system or via cross-contamination with excrement once urine has been redirected and stored. Urine's high concentration of biodegradable organic compounds may act as a food source for aerobic or anaerobic microorganisms, resulting in urea hydrolysis and the associated consequence.

The principal contributors to the transformation of urine after it has been discharged, redirected, and stored are microbial urea hydrolysis, mineral precipitation, and ammonia volatilization. By catalyzing the hydrolysis of urea to ammonia and bicarbonate, urea-hydrolyzing bacteria have the largest impact on the modification of urine quality. Prior to this transformation, roughly 85% of the nitrogen in urine is fixed as urea and around 5% as ammonia, however after urea hydrolysis, 90 percent of the nitrogen is fixed as ammonia. The effects of this shift include a quick rise in pH from around 6 to 9, ammonia volatilization (if the urine is not in a closed storage tank designed to prevent volatilization), and precipitation.

The choice of urine treatment/reuse technologies is influenced by a change in pH and subsequent precipitation. First, because up to 33% of total ammonia is volatile, there will be ammonia losses and odor difficulties when stored pee is transported and applied to the ground (the buffer capacity is so high that acid addition to prevent this is uneconomical). A change in phosphorus concentration, which is a good indicator of precipitation, would be another concern. Undiluted urine contains 30% soluble phosphorus in the solid phase of the precipitates, although this fraction rises with dilution. Calcium and magnesium concentrations limit phosphorus precipitation, and preserved urine normally contains all calcium and magnesium. Because the partitioning of phosphorus into soluble and solid phases is crucial when considering alternative recovery strategies, the hardness and volume of flushing water are elements to consider in urine collecting selections. Calcium and magnesium addition is another promising phosphorus recovery strategy(S. Bakhri, 2015).

Table 4.5 Summarizes the physicochemical and bacteriological parameters of the urine after digestion

Nọ	Parameter	Unit	Value	WHO Standard	Ethiopian standard	Remark
1	pH	-	6.8	6-8	5.5-9	Acceptable
2	TS	mg/l	156	≤50	≤120	Out of range
3	VS	mg/l	78	≤50	≤100	Acceptable
4	BOD_5	mg/l	67	≤90	≤100	Acceptable
5	COD	mg/l	205	≤200	≤250	Acceptable
6	DO	mg/l	4.2	4-8	2-8	Acceptable
7	TK	mg/l	1031	≤1100	≤1100	acceptable
8	TP	mg/l	464	500	500	Acceptable
9	TN	mg/l	1923	700-2000	≤2000	Acceptable
10	TC	Col/100ml	$134*10^4$	$50*10^4$	$50*10^4$	Acceptable
11	FC	Col/100ml	$54*10^4$	$20*10^4$	$10*10^4$	Acceptable
12	Conductivity	µS/cm	9645	11000	11000	Acceptable

Col = colonies

Full data of blackwater substrate laboratory output found appendix c.

Table 4.6 Summarizes the physicochemical and bacteriological parameters of the blackwater.

Nọ	Parameter	Unit	Value	WHO Standard	Ethiopian standard	Remark
1	pH	-	7.1	6-8	5.5-9	Acceptable
2	TS	mg/l	1256	≤50	≤120	Out of range
3	VS	mg/l	874	≤50	≤100	Acceptable
4	BOD$_5$	mg/l	78	≤90	≤100	Acceptable
5	COD	mg/l	193	≤200	≤250	Acceptable
6	DO	mg/l	4.8	4-8	2-8	Acceptable
7	TK	mg/l	636	≤1100	≤1100	Acceptable
8	TP	mg/l	230	≤500	≤500	Acceptable
9	TN	mg/l	879	700-2000	≤2000	Acceptable
10	TC	Col/100ml	$302*10^4$	$\leq 50*10^4$	$\leq 50*10^4$	Acceptable
11	FC	Col/100ml	$125*10^4$	$\leq 20*10^4$	$\leq 10*10^4$	Acceptable
12	Conductivity	µS/cm	10092	≤11000	≤11000	Acceptable

Col = colonies

Data of the blackwater substrate laboratory output are found appendix d more detail result.

Table 4.7 Summarizes the physicochemical and bacteriological parameters of the graywater after digestion

Nọ	Parameter	Unit	Value	WHO Standard	Ethiopian standard	Remark
1	pH	-	7.2	6-8	5.5-9	Acceptable
2	TS	mg/l	38.2	≤50	≤120	Acceptable
3	VS	mg/l	21.6	≤50	≤100	Acceptable
4	BOD$_5$	mg/l	32	≤90	≤100	Acceptable
5	COD	mg/l	178	≤200	≤250	Acceptable
6	DO	mg/l	4.2	4-8	2-8	Acceptable
7	TK	mg/l	2674	≤1100	≤1100	Out of range
8	TP	mg/l	489	≤500	≤500	Acceptable
9	TN	mg/l	332	700-2000	≤2000	Acceptable
10	TC	Col/100ml	$0.13*10^4$	$5\leq 0*10^4$	$5\leq 0*10^4$	Acceptable
11	FC	Col/100ml	$0.04*10^4$	$\leq 20*10^4$	$\leq 10*10^4$	Acceptable
12	Conductivity	µS/cm	4515	≤11000	≤11000	Acceptable

4.2. Electrochemical Analysis

4.2.1 Electric voltage production

The three sets of MFCs were tested for maximum voltage output in laboratory experiment such as urine, blackwater, and graywater at ambient temperatures ranging from 22±10°C. For all three sets, the voltage output was measured using a calibrated multimeter (Model No 8NF6R) across a

1000 ohm resistor at regular intervals of 24 hours until the output voltage dropped to zero already for month.

In study developed MFC model, the blackwater substrate produces the maximum voltage output when all three substrates are run under the identical environmental conditions. The voltage change for blackwater substrate is significantly more powerful than for other substrates, and electricity generation practically reduced after 13 days, which is much less time than both urine and graywater, which have been taken to generate maximum voltage.

The MFC that employed graywater as a substrate produced the least amount of electricity of the three, but it was the most stable. COD reduction was the lowest in urine waste, at roughly 65.83 %, compared to 87.94 % and 82.22% for blackwater and graywater waste, respectively and BOD_5 removal of substrate urine, blackwater and graywater are 67.79%, 91.35% and 28.89% respectively so that the value maximum BOD_5 removal happened in blackwater substrate and the lowest occurred in graywater substrate.

4.2.1.1.Urine substrate voltage produced

The maximum voltage output of the MFC employing urine waste is 118.93 mV, which is achieved on the sixteen day (384hrs.). After 16 days, the rate of electricity generation was significantly slowed. At the end of the experiment, the pH was increased from 6.3 to 6.8 and COD levels were reduced from 600 to 205 mg/L on average. Here below interpret in excel graph analysis of verses per day shown on next page.

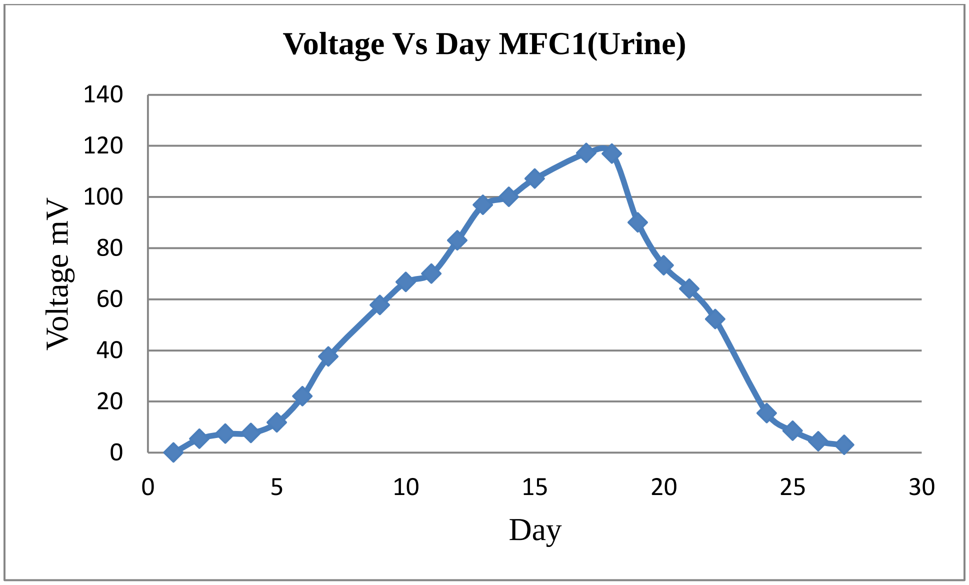


Figure 4.1 Urine substrate voltage produced

4.2.1.2.Blackwater substrate voltage produced

The highest voltage output of the MFC utilizing blackwater 144.84 mV, which it was reached on the 13 days (312 hrs.). After 13 days, the rate of electricity generation had significantly slowed. On the eleventh day, the pH was raised from 6.1 to 7.1. COD levels were reduced from 1600 mg/L to 193 mg/L on average while BOD removed 91.35% from microbial fuel cell treatment.

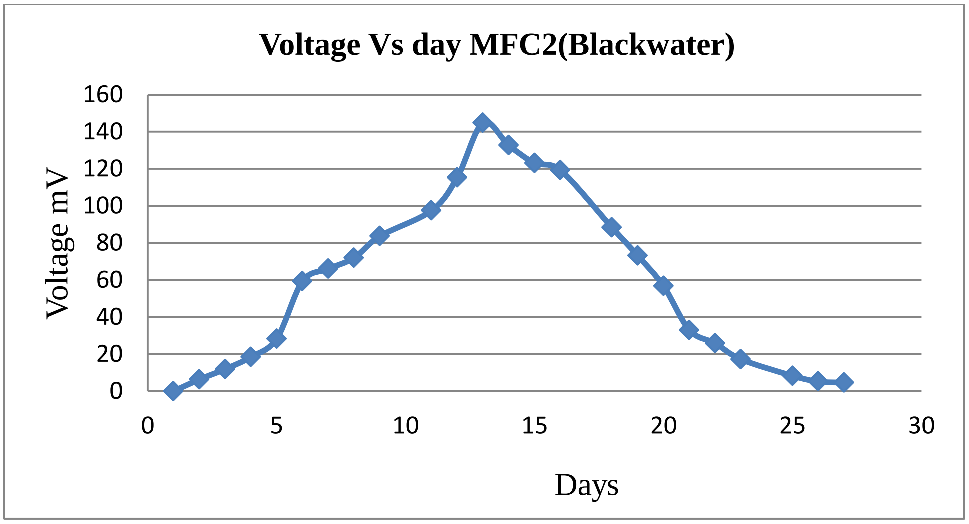


Figure 4.2 The blackwater substrate voltage produced.

4.2.1.3.Graywater substrate voltage produced

The maximum voltage output of the graywater MFC is 89.76 mV, which it reached on the eighteen day (432 hours). The electrical production was maintained for over a month. The pH was reduction from 7.4 on the first day to 7.2 at the conclusion of the experiment. COD elimination was reduced from 45 mg/L to 32 mg/L.

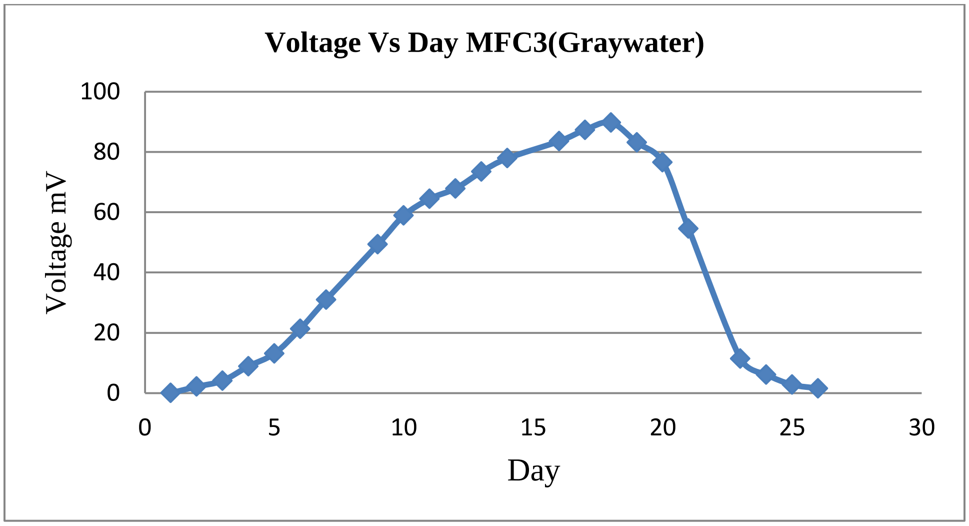


Figure 4.3 The graywater substrate voltage produced.

4.3. Polarization Curve

A polarization curve is used in electrochemistry to depict current density as a function of voltage (the electric potential of the electrodes). A wide range of external resistances is linked to the external circuit to form a polarization curve. As a result, as the load changes, the fuel cell's voltage changes, meaning that the current changes as well(Khaloufi and Elasli, 2019).

It utilized the identical set of resistances for all MFCs, recording the voltage levels at each one. This was done with the help of a multimeter. The current was calculated using Ohm's law. The current density is then normalized by the electrode surface (the anodic surface), keeping the unit of (mA/cm^2). Utilized the excel graph tool to create a curve with the proper linear fit, which illustrated below graph. **Ohm law** $V=I\times R$ (2)

Where I=Current

R=Resistance

All three the microbial fuel cells are generated polarization curve by using excel tool graph.

Graphing tools in Excel were used to create polarization curves for urine of the microbial fuel cell.

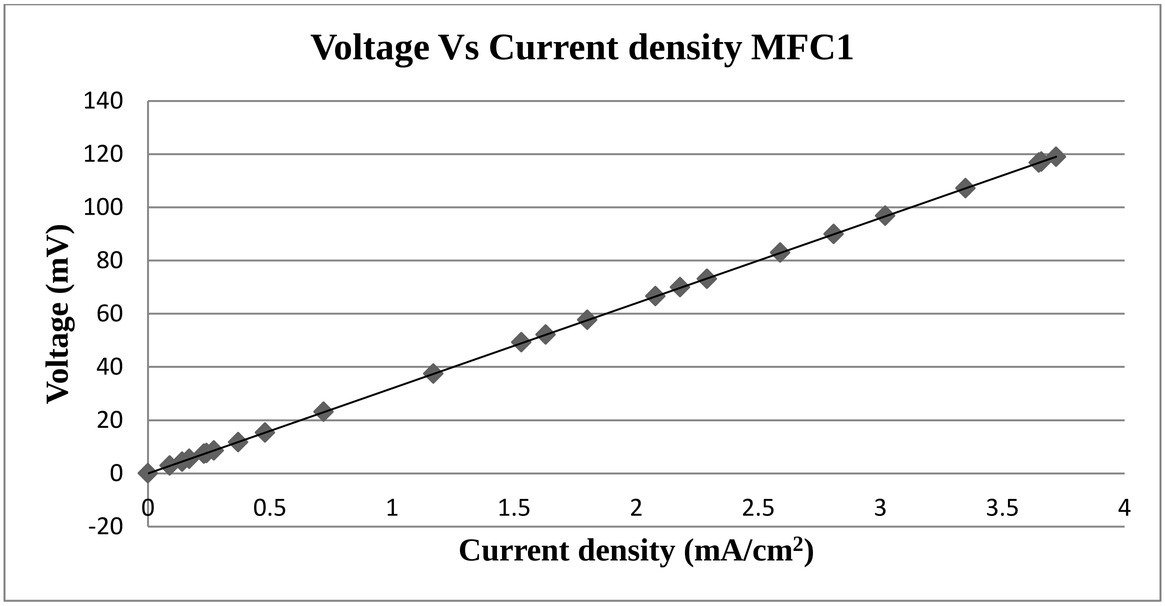


Figure 4.4 Polarization curves for urine of the microbial fuel cell (MF1).

Graphing tools in Excel were used to create polarization curves for blackwater of the microbial fuel cell.

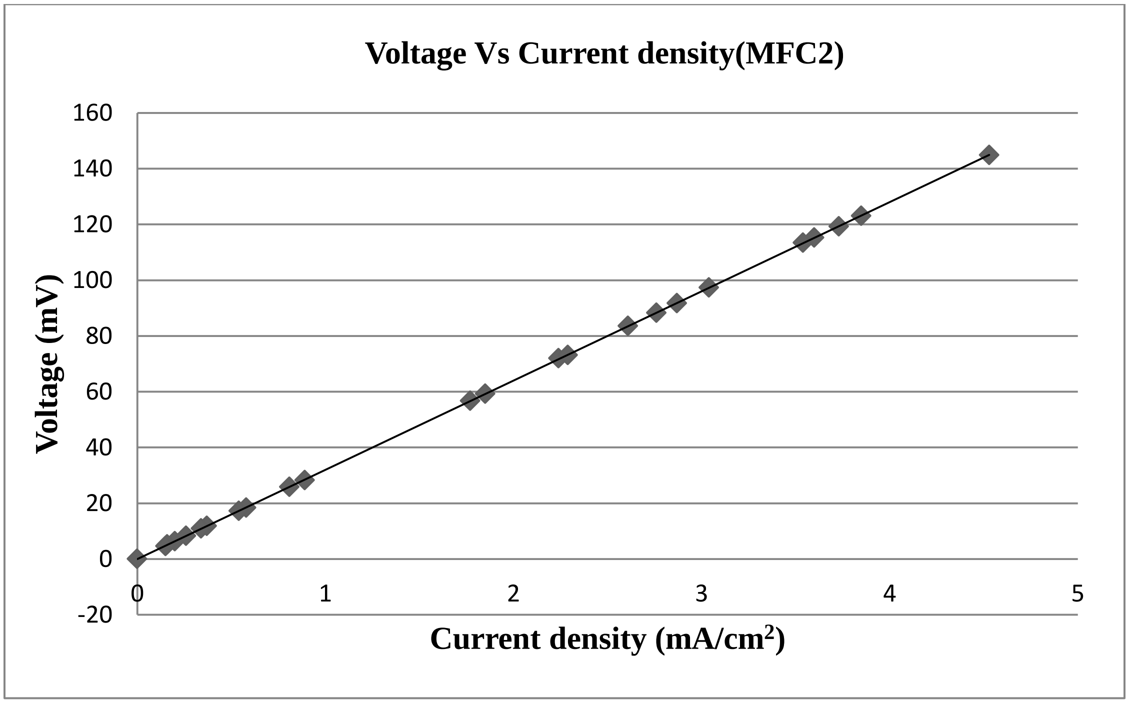


Figure 4.5 Polarization curves for blackwater of the microbial fuel cell (MFC2).

Graphing tools in Excel were used to create polarization curves for graywater of the microbial fuel cells.

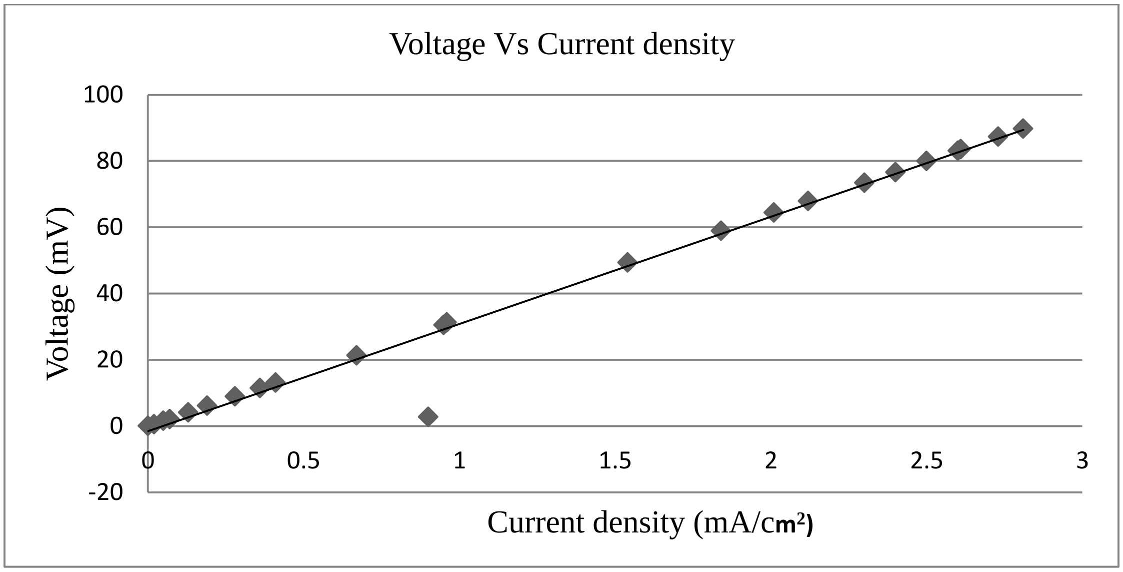


Figure 4.6 Polarization curves for graywater of the microbial fuel cell (MFC3).

As a function of current density, these graphs show how well microbial fuel cells maintain the electric potential. The three polarization lines show that there are linear relationships. Each graphic depicts a linear region all MFC with a constant voltage drop for all MFCs. Maximum Voltage output or OCV (open circuit voltage) values of 118.93 mV, 144.84 mV, and 89.76 mV are attained when the resistance is infinite for MFC1, MFC2, and MFC3, respectively.

It can see that the voltage for MFC1 drops dramatically after one to 117.11 mV, which corresponds to a current density of 0.00366 A/cm^2. Following the swift voltage drop, it can see a linear path that represents the all-region. Similarly, it see before a dramatic voltage drop for MFC2 and MFC3, corresponding to the maximum current densities of 0.0453 A/cm^2, and 0.00281 A/cm^2, respectively.

It's crucial to understand the components that influence cell voltage in order to better understand linear line in the polarization curve. First, because the fuel cell has an internal resistance that affects the power generation process, the recorded OCV at infinite resistance does not represent the maximum theoretical electric potential. There were able to identify linear all region by the same MFCs polarization curve to the one discovered in the experiment.

1. A significant potential drop occurs at high resistances.
2. A somewhat straight route indicating a reduction in electric potential following the quick drop.

3. At low value resistances, there is yet another dramatic and quick potential drop beyond the linear fall.

As a result, a more exact equation defining the voltage of a fuel cell at any resistance and current should be considered.

$$V=V_o-(\Sigma V_{an} + \Sigma V_{cat} + I + R\Omega) \quad (3)$$

Where: Van=anode over potential

Vcat cathode over potential

I= current generated by cell

RΩ=internal resistance of fuel cell

The electrodes over potentials cause some of the voltage losses, which change as the current changes. Activation losses, bacterial metabolism, and mass movement are three types of voltage losses that could cause the electrodes to over-potential(Khaloufi and Elasli, 2019).

1. Activation losses: In order to continue with the oxidation-reduction reactions, an energy barrier must be overcome, and energy in the form of heat is lost in the process. Furthermore, we observe further energy loss as a result of electron migration from the bacteria to the anode surface, either directly or indirectly(Khaloufi and Elasli, 2019).
2. Bacterial Metabolism: Represents energy losses as a result of the bacteria's demand for energy to carry out its metabolic activity, namely the generation of the proton gradient in its electron - transport chain(Khaloufi and Elasli, 2019).
3. Losses in mass transfer: There are two sub-issues that can result in energy losses. First, the mass transfer (also known as flux) of the reactant-representing substrate to the anode is frequently insufficient. Second, protons' migration from the anodic to the cathodic chambers is occasionally restricted, resulting in a buildup of H^+ and lowering the pH at the anode while increasing it at the cathode(Khaloufi and Elasli, 2019)

Lower the activation losses by using a variety of bacteria at the anode to limit energy losses due to electrodes over potentials. New bacteria can be added to the substrate to compensate for voltage losses; these bacteria may have a more efficient metabolism. Finally, we can use effective proton exchange membranes to facilitate protons' migration to the cathode while retaining a sufficient buffer capacity to avoid mass transfer losses (resistance to pH change)(Khaloufi and Elasli, 2019).

Discovered another sort of energy loss termed Ohmic Losses in addition to electrode over-potential losses. The internal resistance of the electrode material and the wire causes energy loss during the transmission of electrons from the electrode through wires at the point of contact of the electrodes and the conducting wire(Khaloufi and Elasli, 2019). The potential loss due to Ohmic losses can be computed using the equation below.

$$\Delta \boldsymbol{V} = \frac{\mathbf{\nabla W \times I}}{\boldsymbol{\delta}}$$

Where: ∇W: The distance between anode and cathode

I: Current normalized to anodic surface (A/cm^2)

δ: Represent the solution of conductivity (µS/cm)

As a result, if it can boost solution conductivity, it may be able to lower Ohmic losses as well. Furthermore, the shorter the distance between the electrodes, the lesser the Ohmic losses both chamber of MFC. Nevertheless, Ohmic losses are sometimes unavoidable; for example, because bacteria can only operate within specified boundaries and conductivity ranges, it is impossible to raise the solution conductivity at random. Furthermore, low-internal-resistance electrode materials can be costly.

4.4. Power Density Curve

MFCs operate in the same way as traditional electricity generators, generating a current and a certain cell potential. Because generators are designed to produce useful power, it is natural to try to optimize the fuel cell for power generation. To do this, we employed a variety of external resistors of varying values to determine the optimal current and potential for maximizing power.

Power described as following equation.

$$\mathbf{P=V\times I} \qquad (4)$$

Where; P: Power

V: Voltage

I: Current

The following alternate equation is used to express the power in this laboratory research $\mathrm{P} = \frac{\mathrm{v}^2}{\mathrm{Rex}}$ where R is the applied external resistance and V is the cell potential. Alternatively we can express power by $\mathrm{P} = \mathrm{I}^2 \times \mathrm{R}_{\mathrm{ext}}$.(5)

The electrodes utilized, as previously noted, do have the same surface area. Electrode were used an 8cm×4cm cathode and anode using aluminum electrodes, giving us a total surface area of 32 cm^2. In terms of surface area, it can consider both sides of the electrodes, but for computations, it only needs to consider one side. Steel electrodes are 8 cm long and 4 cm wide, with 32 cm^2 surface area. The anodic surface area is frequently used to standardize the unit of power. As a result, power density is expressed as:

$$\mathbf{P} = \frac{\mathbf{v}^2}{\mathbf{Aan} \times \mathbf{Rex}} \qquad (6)$$

Where Aan: The anode’s surface area

V: Electric Voltage

Rex: External Resistor

Normalizing power to the cathode surface area, if either is greater than the anode, is also important. The fuel cell volume, including the cathodic chamber volume, should be used to normalize power. This enables for a more precise study of the fuel cell's power output. However, in order to acquire correct results, it kept the reactor volume and substrate amount constant.

The goal of this part is to figure out how much power each MFC can produce, thus it has made “power density as a function of current density” graphs for each one. The power density curve for excel expression MFC1 (Urine).

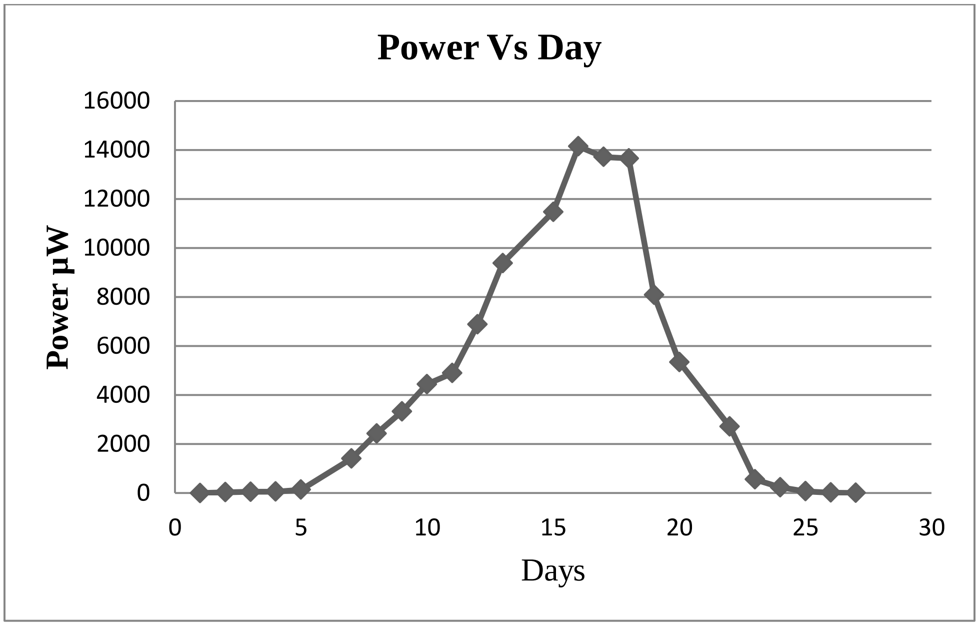


Figure 4.7 8 Power Vs day MFC1(Urine)

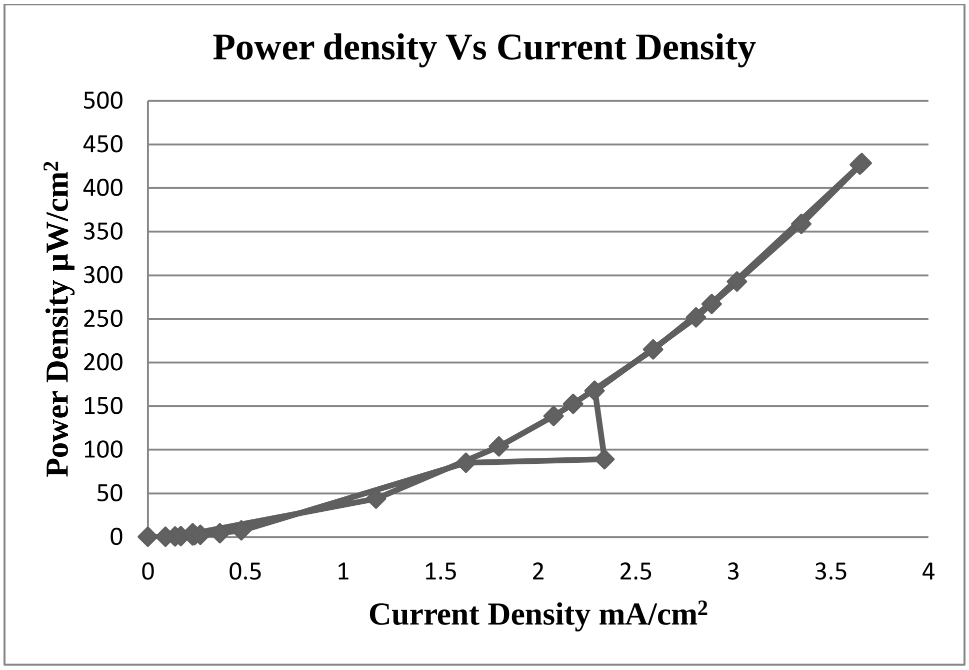


Figure 4.8 Power density curve for MFC1 (Urine)

Power density curve for MFC2 (Blackwater)

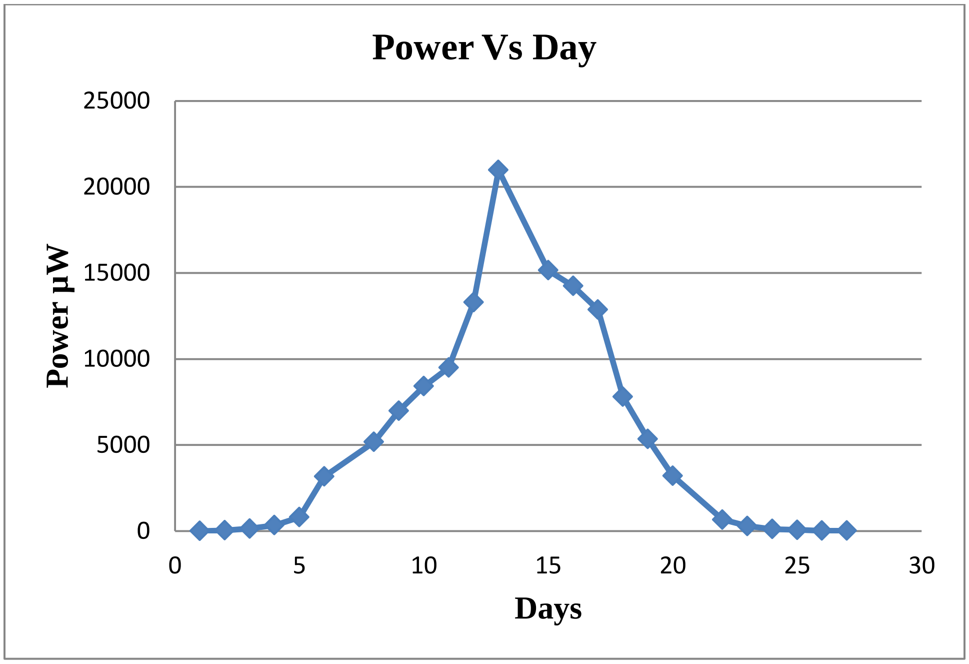


Figure 4.9 Power Vs day MFC2(Blackwater)

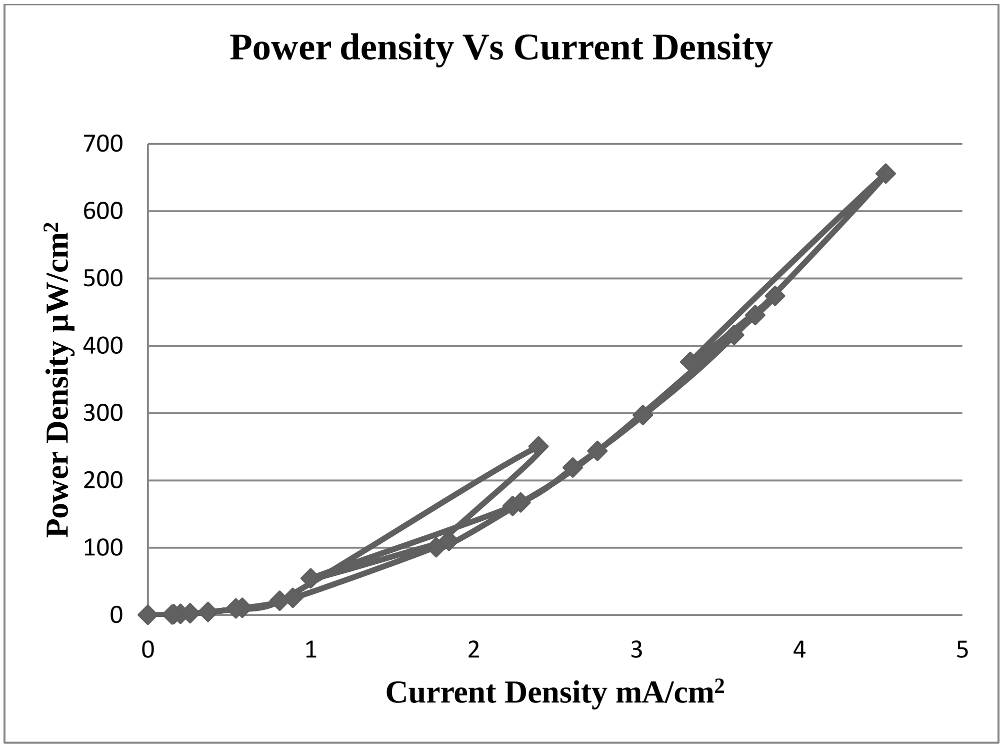


Figure 4.10 Power density curve for MFC2 (Blackwater)

The Power density curve for MFC3 (Graywater) excel graph interpretation.

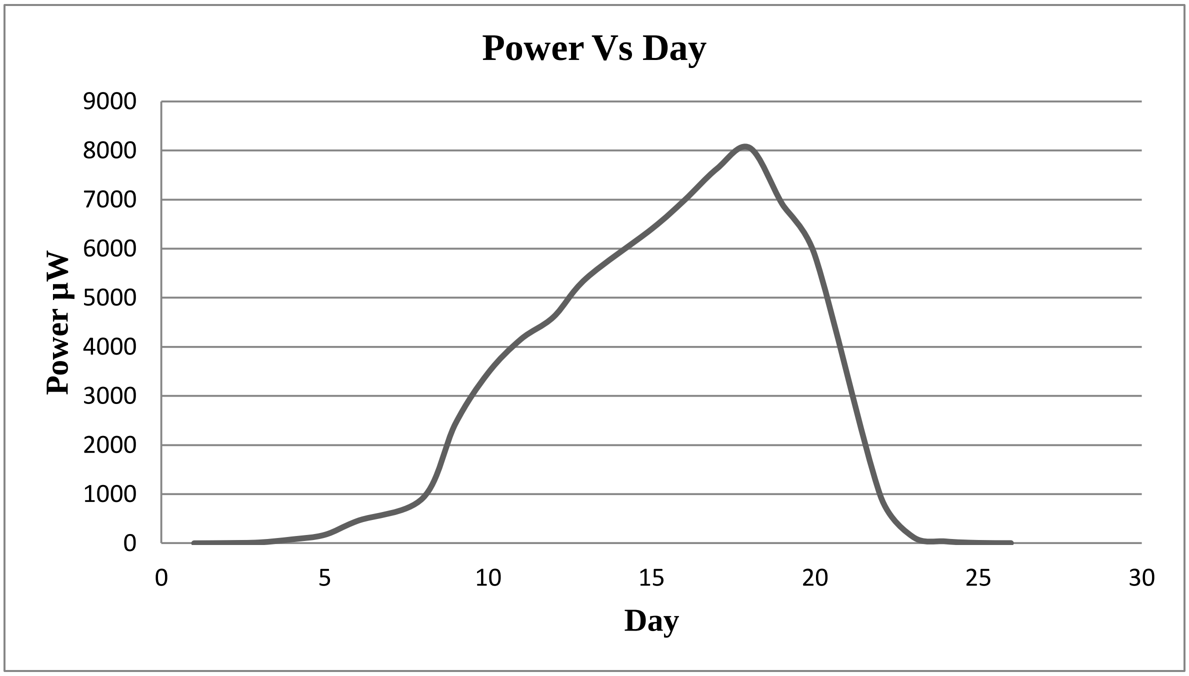


Figure 4.11 Power Vs day MFC3(Graywater)

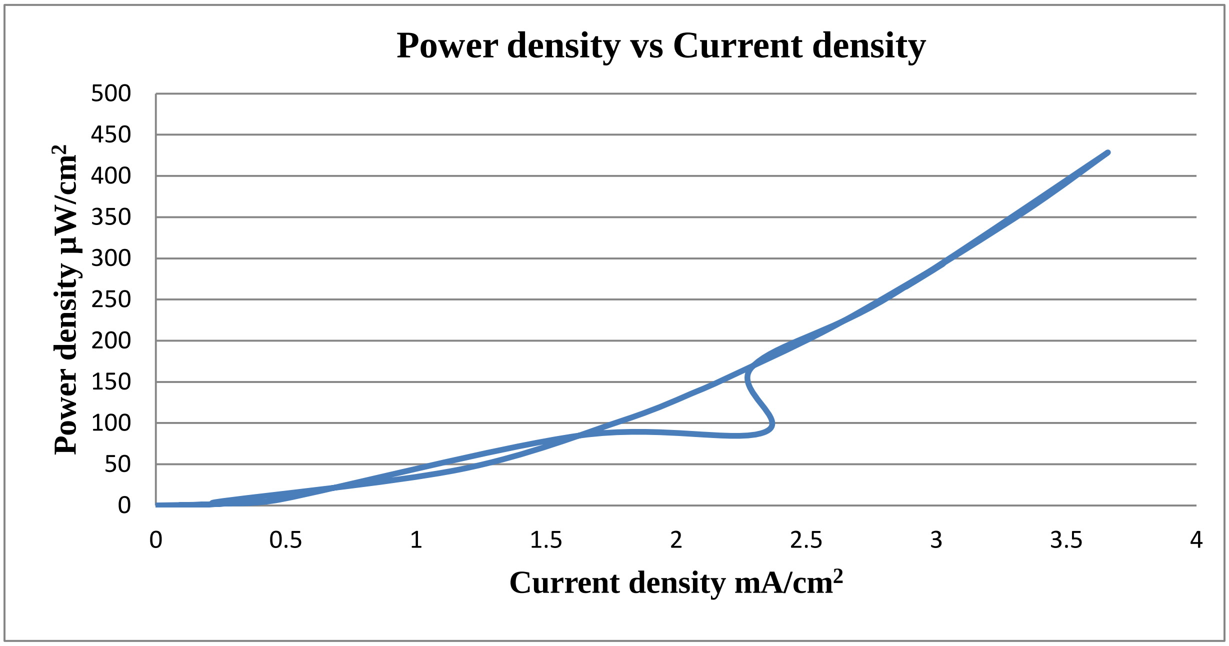


Figure 4.12 Power density curve for MFC3 (Graywater)

Power curves as a function of day usually take on a nonlinear-shaped shape, allowing us to establish the maximum power density as the plot's peak value. There were able to produce a similar but not perfect liner shaped curve after normalizing the modest amounts of power to the anodic surface area.

MFC 2 had the highest maximum power, with a value of 0.00655 W/cm^2 equating to 0.00453A/cm^2 of current. There were found the following values for the other fuel cells: 0.00442 W/cm^2, and 0.00251 W/cm^2, corresponding to current values of 0.00372 A/cm^2, and 0.00281 A/cm^2 are respectively value of MFC1 and MFC3.

Therefore, conclude that the best evaluated system in terms of maximum power generation is the one that stack MFC2 in series (Blackwater), followed by the MFC1 where it used aluminum electrode at the anode and aluminum at the cathode with used substrate mixed one. Then, MFC3 where it has steel at the anode and aluminum at the cathode with graywater substrate produced low power density. Because MFC1, MFC2, and MFC3 all operated under the same conditions with the only difference being the substrate source, it may conclude that blackwater outperformed them all in studied tests. Finally, because the electrodes in the graywater substrate are the different as in MFC 3, the lowest maximum power density value was observed.

4.5. Internal Resistance

Internal resistance is a significant element that can influence MFC performance. To put it another way, some MFCs can have the same reactor volume, and hence the same quantity of substrate, but produce different currents. The total maximum power is calculated in theory as

$P= \frac{\boldsymbol{OCV^2}}{\boldsymbol{R\ in+R\ ex}}$. The principles of electric circuits reveal that when Rint = Rext, it can record the maximum power; thus:

$$P_{\max} = \frac{\mathbf{OCV^2}}{\mathbf{4*R\ int}} \quad \textbf{(7)}$$

This equation can be used to calculate the MFC's internal resistance. In early experiments, a variety of external resistances were used to construct the polarization and power density curves. Each MFC's internal resistance is determined by recording the maximum power output and comparing it to the external resistance. That internal resistance is what we're up against. The internal resistances of the study MFCs (1-2-3) are 1kΩ, 1kΩ, and 1kΩ, respectively, as indicated in the early figures. The MFC3 is the best single MFC among the other single MFCs since study goal is to reduce internal resistance in the fuel cell. This result is consistent with MFC2's highest maximal power among single MFCs. Furthermore, the high internal resistance of MFC 3 can be justified by the high internal resistance of MFC 1, which is connected to MFC 2.

4.6. Columbic efficiency

The columbic efficiency is an important parameter to consider while evaluating the MFC's performance. Goal this research is to harvest as many electrons as possible from the anode's biodegradation of organic materials. The bacteria and the system as a whole are more efficient when the columbic efficiency is high.

Described by this equation $$\mathrm{Ce}=\frac{\text{electron recovered}}{\text{total electron in biomass}} \quad (8)$$

The term "electrons" refers to the charge of an electron in coulombs.

For MFCs’ the columbic efficiency defined as following equation used to described.

$$\mathrm{Ce}=\frac{\mathbf{8\int I \times dt}}{\mathbf{F\times Van\times \Delta COD}} \qquad (9)$$

Where F: Faradays constant

Van: Substrate volume in anode chamber

COD: proportional to substrate concentration 8 is constant value

It is critical to know the COD before estimating the columbic efficiency of the fuel cells. It kept Micrbial fuel cells running for 24 hours and then recorded the current, voltage, and other parameters. As a result, the COD change must also be documented after 24 hours. However, because the working period was insufficient to record a significant value, the average COD in urine, blackwater, and graywater was sought.

Table 4.8 Average COD value of substrates

No	Type of substrate	Average COD value
1	MFC1(urine)	65.83%
2	MFC2(black water)	87.94%
3	MFC(Graywater)	80.22%

Table 4.9 Columbic efficiencies of experimented value of each MFC

No	Type of MFC	Columbic efficiency (Ce)
1	MFC1(urine)	40%
2	MFC2(black water)	58%
3	MFC(Graywater)	32%

The instantaneous current value after 24-hour operation duration was used to calculate the columbic efficiency of the examined MFCs. It didn't utilize any external resistance because it didn’t want to restrict electron flow. According to the results of the experiment, the MFC2 has the maximum columbic efficiency because blackwater is the major substrate and both chambers are aluminum.

4.7. Electrode influence in Microbial Fuel Cell

Two different metals felt electrode pretreatments were tried to see how they affected emerging microbial populations. As a control, MFC’1 was fitted with untreated electrodes its surface area was 5×7cm steel electrode output of voltage was very little it generated 15mV but more effective in aluminum electrode surface area MFC1 it produce 117.1mV with 32cm^2 surface area while the other MFC’s were fitted with steel (MFC’2) its size 5×7 voltage generated was 55mV but MFC2 with it aluminum electrode surface area 32cm^2 generated the highest voltage it was 148.13 mV and aluminum (MFC’3) electrodes produce little bite voltage surface area 35 cm^2 but 32cm^2 surface area MFC3 with steel electrode produce 89.73mV. The kinetics of the electrode reactions within the fuel cell determine the performance of MFCs, and the performance of the electrodes is greatly impacted by the materials used to make them(Mustakeem, 2015).To enhance the productivity of MFC, a variety of materials have been investigated.

For MFCs to operate well in terms of bacterial attachment, electron transfer, and electrochemical efficiency, the electrode material must be chosen carefully. Various electrode factors, including as biocompatibility, active surface area, high conductivity, and the nature of the electrode surface, all influence the performance of an MFC.

This Study was discovering materials for MFC; the desire for cheaper electrode materials is creating chance MFC technology from being implemented outside of the lab. Material for the electrodes Metals such as aluminum and steel can boost power generation, and their widespread use would result in lower cost of materials(Bhargavi, Venu and Renganathan, 2018). Because it is a hub for critical bioelectrochemical reactions and a mediator of electron transport from exoelectrogens to electrode, an MFC's efficiency is largely determined by its anode performance. As a result, it's critical to concentrate on the anode materials and design. Surface area, chemical resistivity, lifespan, and electrical conductivity are all anodic factors that have a substantial impact on MFC performance. This study used two types of electrode in anode and cathode to improve MFC performance and lower costs. It has been determined that reactor design has a substantial impact on MFC performance. In terms of power generation and longevity, aluminum electrodes outperform steel electrodes. In general power production electrodes depend on type of substrates so that MFC2 produced high yield of voltage.

Table 4.10 Electrode influence factor in MFC

No	Electrode influence factor	MFC1 (Urine)	MFC2 (Blackwater)	MFC3 (Graywater)	Remark
1	Surface area of electrode in cm^2	32	32	25	Surface area increase in rising voltage production
2	Shape of container chamber	Cylindrical	Cylindrical	Cylindrical	Cylindrical shape better than rectangular
3	Type of electrode made material	Aluminum	Aluminum	Stainless steel	Aluminum preferable for its good conductance
4	Corrosion resistance	Excellent	Excellent	Good	Aluminum is good corrosion resistance
5	Type of substrate	Urine	Blackwater	Graywater	Blackwater was good potential produced voltage due to different mineral content

4.8. All Over Experimental Result

The main goal of this research was to generate electricity energy from human waste. study were able to demonstrate that stacking a particular number of microbial fuel cells in series using different electrodes in the two compartments of the fuel cell is the ultimate way to produce a minimum useable power by testing several types of electrodes and stacking two separate MFCs together in series.This result suggests that MFCs can be used as generators in the real world.

Table 4.11 All over experimental result

No	Types	MFC1	MFC2	MFC3
1	Substrate	Urine	Blackwater	Graywater
2	Anode material	aluminum	aluminum	steel
3	Cathode material	aluminum	aluminum	aluminum
4	Anode surface area(cm^2)	32	32	32
5	Cathode surface area(cm^2)	32	32	18
6	Voltage maximum output(mV)	118.93	144.84	89.76
7	Maximum current density(mA/cm^2)	3.66	4.53	2.81
7	Maximum power density($\mu W/cm^2$)	13712.4	20978	8056.86
8	External resistance	1kΩ	1kΩ	1kΩ
9	Columbic efficiency	40%	58%	32%

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

Based on laboratory data provided a strong indication of MCF viability, providing hope for a future generation free of green gas effect energy sources. As a result, the purpose of this study was to see if more research on MFCs for usage in underdeveloped nations in general and distant areas in particular should be done, rather than to recommend MFCs as a solution to Ethiopia's electrical problems. The study's objectives were investigated a laboratory-based experimental work. The capacity of microbial fuel cells and the type of substrate employed were evaluated using experimental research designs. The quantity of electric current produced by wastewater during treatment was measured using an experimental approach. The cross-sectional methodology was used to inspect the capability of each three type of substrates power generation capability during the research design. Maximum Voltage output or OCV (open circuit voltage) values of 118.93 mV, 144.84 mV, and 89.76 mV are attained when the resistance is infinite for MFC1, MFC2, and MFC3, respectively. The maximum voltage production achieved in blackwater substrate it generated 144.84mV and the smallest generated from graywater it was 89.76mV.

The MFC that employed graywater as a substrate produced the least amount of electricity of the three, but it was the most stable. COD reduction was highest in Blackwater waste, at roughly 87.94%, compared to 65.83 % and 80.22 % for urine and graywater waste, respectively and BOD_5 removal of substrate urine, blackwater and graywater are 67.79%, 91.35% and 28.89% respectively value in the BOD reduction also blackwater substrate attained the highest reduction.

This Study was discovering materials for MFC; the desire for cheaper electrode materials is creating chance MFC technology from being implemented outside of the lab. Material for the electrodes Metals such as aluminum and steel can boost power generation, and their widespread use would result in lower cost of materials. The power productions of electrodes depend on type of substrates and surface area of electrode so that MFC2 produced high yield of voltage. Generally use the microbial fuel cell the best option to solve problem of energy shortage for rural community.

5.2. Recommendations

Based on laboratory analytical result the study was recommending the following important point to be considered and applied.

- This study scope was restricted to the relatively modest Wolkite University complex in Ethiopia. Understanding what individual organisms are degrading organic matter requires identifying the microbial community structure in the anode and cathode from the lab-based pilot system will be needed.
- More research will be done to see if pathogens may be eliminated from the MFC digestion and to producing composite from remaining sludge after digestion of waste in the microbial fuel cell.
- MFCs may be employed as an alternative substrate used for effective wastewater digestion in MFC. However, in order to improve the feasibility of producing electric current from wastewater, better suited material should be sought for and tested.
- It is also suggested that this research be carried out by another Jimma University researcher. Research should be conducted on a regular basis to ensure that the method is feasible. All of the findings in this study show that more research on MFCs should be done by the researcher in order to learn more about how MFCs are seen and if they are fully functional.
- Microbial fuel cell is used for multiple purposes for treatment of wastewater and production of fertilizer so that more research need on MFC.
- Finally, in order to address the influence on the MFC treatment viability, attention will be focused on the user interface problems in Ethiopia for the MFC. MFC is considered for efficient generation of energy from constantly growing wastewater oxidation, according to the conclusions of this research study.

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APPENDICES

Appendix: a

Laboratory procedure

a) Total and fecal coliform (TC and FC).

1. Red alcohol (C2H5OH) is utilized to hold any equipment in this procedure, because bacteria can migrate from hand to equipment.
2. Sterilize all of the equipment (forceps, measuring cylinder, pipette, membrane filtration petri-dish, and membrane filtration apparatus) in the Autoclave sterilizer by steam at 120^0C for 15 minutes to kill any bacteria that may be present, including the H_2O to be used.
3. Dilute the sample with sterilized H_2O dilution factor of 100,000x (0.1ml sample with 9999.9ml of sterilized cooled H_2O).
4. To create the food for bacteria, combine the powder form of membrane filtering media with the required volume of sterilized H_2O (72.9g =1000ml ratio).
5. Filter paper and a filter pad should be ready.
6. Fill the membrane filtering equipment with step 3 and place the filter paper inside. Then, to filter it down, open the vacuum pump.
7. Fill step 4 with a pipette after inserting the filter pad into the membrane filtering petri-dish.
8. Take the filter paper from step 6 and place it in the petri-dish with step 7.
9. Count the spore generated at 37^0C (for 24hr-48hr) for TC and 44.5^0C (for 24hr) for FC, and put the colonies/100ml in the fourth chapter.

   Col/100ml=Nọ of count × dilution factor(D.f=10^4 for calculation).

Here below are laboratory picture during done determination of total and fecal coliform.

A) Petri-dish

B) WW Sample

C) Pipetting

D) Agar Media Red Alcohol

E) Pour on Petri-dish

C) Incubation

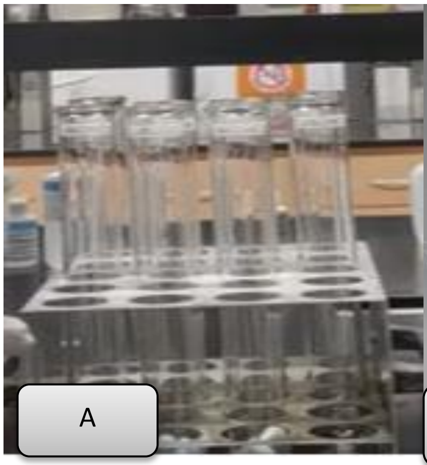
D) Digital Counting device

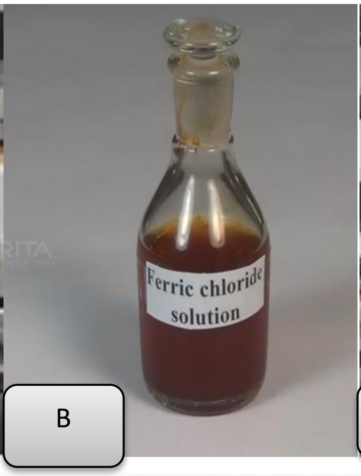
E) Counting

Figure 5 During lab experiment of total and fecal coliform WKU biology department laboratory.

b).Total Nitrogen

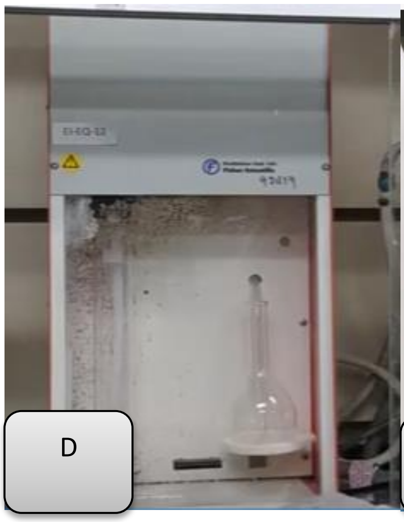
1. Fill the empty LCK 338 (kit) with 0.2 mL sample, 2.3 mL total nitrogen reagent A, and 1 piece total nitrogen reagent B, and digest for 1 hour at 100^{o}C in the Hath Lange Lt 200 digester**.**
2. Add 1 Microcap from reagent C after digestion is complete and shake the kit until only the plastic component of the Microcap remains.
3. Add 0.5ml of step 2 above to another LCK 338 contain chemical produced by the producer.
4. Add 0.2ml total nitrogen reagent D to step 3 above and shake vigorously for a brief time, then set aside for 15 minutes to cool.
5. Finally, using the spectrophotometer DR 5000, read TN automatically by inputting LCK 338 from step 4.


A

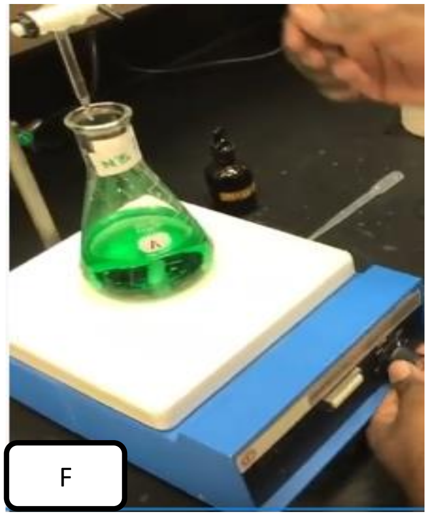


B


C


D


E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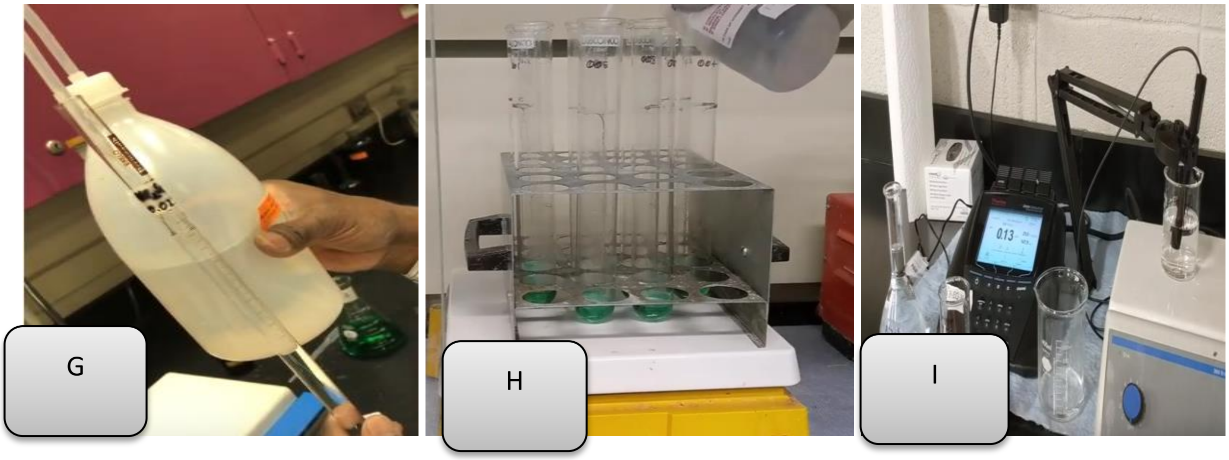

Figure 6 The Nitrogen Test Chemistry the Lab WKU

C).Total phosphorous (TP)

1. Carefully remove the foil from the Dosicap Zip that has been fastened on.
2. Remove the Dosicap Zip.
3. 0.4 mL sample pipette.
4. The Dosicap Zip is screwed back in place, with fluting at the top.
5. Firmly shake
6. For 60 minutes, heat at 100°C in the thermostat.
7. Pipette 0.5ml reagent B (LCK 350 B) into the cooled cuvette and immediately shut the reagent B after use.
8. Attach a grey Dosicap C (LCK 350 C) to the cuvette with a screwdriver.
9. Invert the image a few times. After 10 minutes, invert the cuvette a few times more, carefully cleans the outside of the cuvette, and use a spectrophotometer model DR 500 to determine the total phosphorus available.

Figure 7 Phosphorus test in the Spectrometer at WKU

d) Chemical Oxygen Demand (COD)

1. Invert the substrate a few times to bring it into suspension.
2. Pipette a 2.0 mL sample with care.
3. Close the cuvette and clean the outside thoroughly.
4. For 15 minutes, set the thermostat to HT 200 S and use the regular program HT.
5. After the lock on the HT 200 S opens, carefully remove the heated cuvette and invert twice.
6. Allow the HT 200 S to cool to room temperature in the thermostat.
7. Before evaluating the substrate, it must be totally settled. Clean the cuvettes's outside and test it with a spectrophotometer.

Figure 8 During COD experiment

E) Total potassium

1. Using an evaporator, concentrate a 100-ml sample's potassium content until only around 5 ml remains. Fill a 25-ml centrifuge tube halfway with deionized distilled H_2O to make up to 10 ml of concentrated sample.
2. Because the reaction is time and temperature dependent, both should be kept fairly consistent for all samples and standards in a series of tests: 15 minutes and 5 degrees Celsius.
3. 1 mL of 1 mol L-1 nitric acid and 5 mL of trisodium cobalt nitrite solution, combined at room temperature. Allow for 2 hours of resting time.
4. Spin for 10 minutes in a centrifuge. Pour the liquid off carefully and wash the precipitate with 15 mL of 0.01 mol L-1 nitric acid. To ensure contact between the precipitate and the wash solution, stir using a tiny glass stirring rod.
5. Centrifuge for another ten minutes. Pour out the liquid and combine it with 10.00 mL potassium dichromate solution and 5 mL concentrated sulphuric acid in a mixing bowl.
6. Allow to cool to room temperature before serving. With deionized distilled H_2O, make up to 100 mL. Filter the turbid solution into a Nessler tube and make up to 100 mL.
7. Standardization is the process of developing a set of guidelines. Pipette portions of the standard potassium solution of 1, 2, 3, 4, 5, 6, and 7 ml into a series of 25-ml centrifuge tubes, and make up to 10 ml with deionized distilled H_2O. To obtain color standards containing 1.00 to 7.00 mg K, treat all tubes as indicated for the sample in steps 3 to 6 above.

8. The absorbance of the sample is measured, and the content of potassium is determined using microprocessor flame photometer and a calibration curve with absorbance plotted against mg K.

Figure 9 During conducted of the experiment potassium in the Microprocessor Flame photometer chemistry lab WKU.

F).Total solid (TS) and Volatile solid (VS)

1. In a furnace, heat a clean evaporating dish to 550°C for 1 hour for VS.
2. Preheat oven to 103 to 105 degrees Celsius for 1 hour.
3. Place dish in desiccator till needed.
4. Weigh everything right before you use it.
5. Pipet a pre-weighed dish with a measured volume of well-mixed material.
6. Dry in a drying oven until completely dry.
7. If necessary, after evaporation, add additional sample pieces to the same plate.
8. Place the dish in the desiccator to cool and balance the temperature, then weigh it.
9. In a muffle furnace, ignite the residue obtained by procedure 2540B.
10. Cool completely in a desiccator in a dry environment.

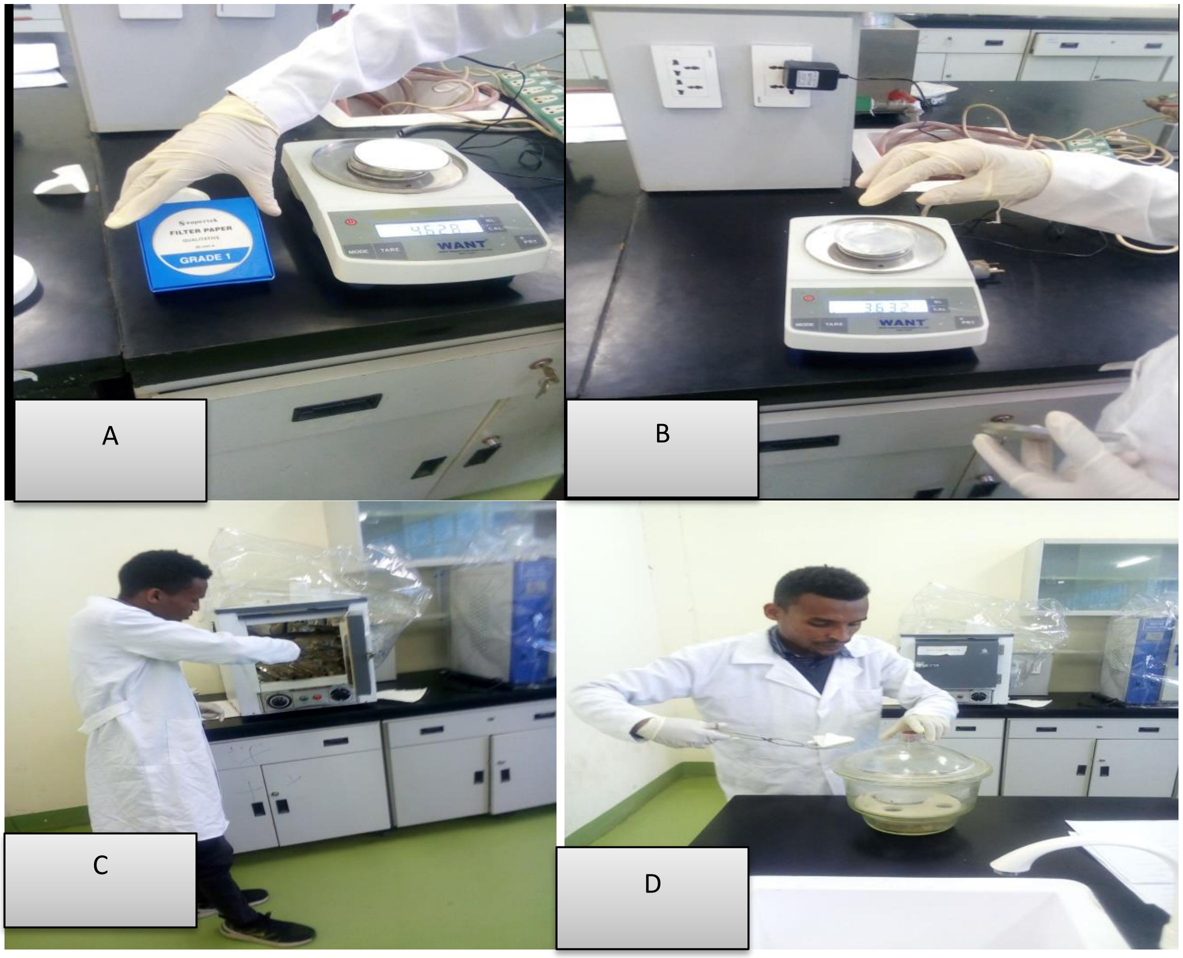

Figure 10 The analyses of Total Solid and Volatile Solid

G) Biochemical oxygen demand

1. Take precise measurements of the sample using proper overflow and, if necessary, add a nitrification inhibitor (ATH)
2. Place the magnetic stirring rod in place.
3. Fill the seal gasket with 3-4 drops of KOH solution and place it in the bottle's neck.
4. Attach the BOD sensors to the sample vial with a screwdriver.
5. Insert the bottle into the bottle rack.
6. Begin taking measurements.
7. Incubate the sample for 5 days at 20^0C according to the BOD_5 instructions.
8. Use a digital biological incubator to measure after 5 days.

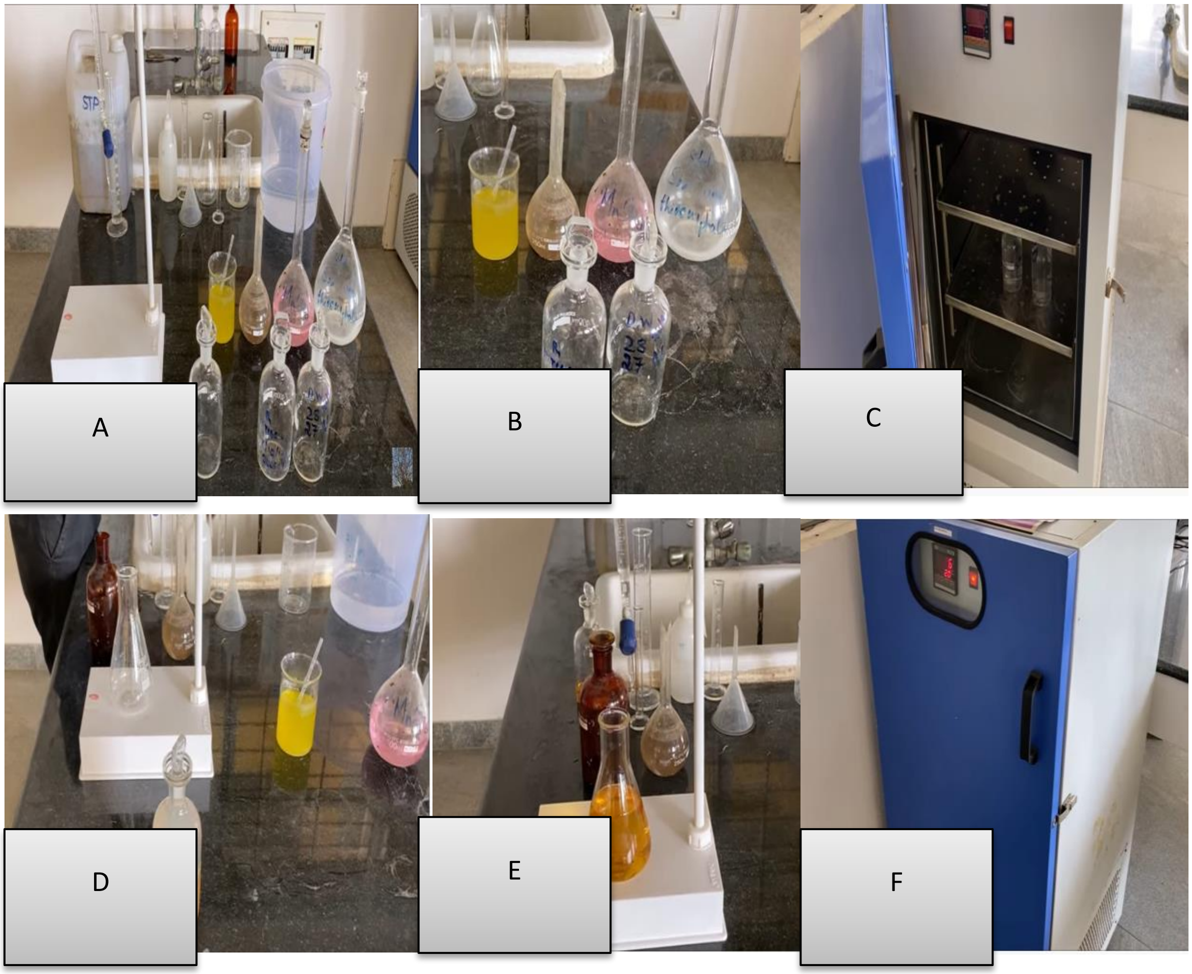

Figure 11 The Biochemical Oxygen Demand experiment in the food lab WKU.

H).Ammonia

1. Titrate ammonia in distillate using standard
2. 0.02NOnly samples that have gone through preliminary distillation should be used for this procedure Solution of sulphuric acid and boric acid indicator

Appendix .b

	Urine (MFC1)						
	Date	Conductivity µS/cm	pH	COD mg/L	Turbidity NTU	TS mg/L	VS mg/L
1	1/4/2021	19065	6.3	400	37	385	208
2	2/4/2021	20235	6.1	400	35	382	206
3	3/4/2021	21712	6.7	400	30	382	204
4	4/4/2021	19502	6.6	400	29	380	204
5	5/4/2021	17836	6.6	400	29	378	203
6	6/4/2021	19518	6.2	400	30	378	200
7	7/4/2021	-	-	-		-	-
8	8/4/2021	18564	6.9	400	29	371	167
9	9/4/2021	20091	6.4	400	29	371	153
10	10/4/2021	16782	6.7	400	30	371	150
11	11/4/2021	19054	6.6	400	29	371	120
12	12/4/2021	18734	6.8	400	27	365	115
13	13/4/2021	19345	6.4	400	26	362	111
14	14/4/2021	-	-	-	-	-	-
15	15/4/2021	241519	6.7	400	25	251	98
16	16/4/2021	28954	6.6	400	25	242	92
17	17/4/2021	12452	6.8	400	25	232	79
18	18/4/2021	11112	6.4	400	25	215	72
19	19/4/2021	9051	6.7	400	25	213	70
20	20/4/2021	10213	7.1	400	23	167	69
21	21/4/2021	-	-	-		-	-
22	22/4/2021	6745	6.9	400	22	132	58
23	23/4/2021	5642	6.4	400	22	86	54
24	24/4/2021	3422	6.7	400	22	73	46
25	25/4/2021	3892	6.6	400	22	56	34
26	26/4/2021	2967	6.8	400	22	46	31

Appendix .c

	Blackwater (MFC2)						
No	Date	Conductivity µS/cm	pH	COD mg/L	Turbidity NTU	TS mg/L	VS mg/L
1	1/4/2021	27894	6.7	900	49	3982	1231
2	2/4/2021	25643	7.1	854	45	3923	1201
3	3/4/2021	23421	6.8	823	44	3802	1200
4	4/4/2021	32851	6.6	764	40	3788	1165
5	5/4/2021	23465	8.6	765	40	3643	1102
6	6/4/2021	34214	8.6	734	39	3398	1067
7	7/4/2021	-	-	-	-	-	-
8	8/4/2021	31204	6.6	713	36	2967	1143
9	9/4/2021	29549	6.9	684	34	2912	1006
10	10/4/2021	30235	6.6	645	28	2728	974
11	11/4/2021	31246	7.6	600	30	2467	970
12	12/4/2021	38045	6.9	549	27	2215	974
13	13/4/2021	39342	8.6	538	27	2000	963
14	14/4/2021	-	-	-	-	-	-
15	15/4/2021	35634	6.9	560	25	1764	956
16	16/4/2021	27595	7.9	453	22	1510	954
17	17/4/2021	12654	6.9	395	23	1500	956
18	18/4/2021	10987	6.9	334	21	1500	945
19	19/4/2021	15643	8.6	342	22	1494	929
20	20/4/2021	12432	7.2	400	19	1489	918
21	21/4/2021	-	-	-		-	-
22	22/4/2021	5432	6.8	299	16	1454	894
23	23/4/2021	4321	6.8	231	18	1420	890
24	24/4/2021	5436	7.1	186	15	1321	886
25	25/4/2021	3408	6.8	167	12	1284	883
26	26/4/2021	3219	6.9	120	13	1256	881

Appendix .d

	Graywater (MFC3)						
No	Date	Conductivity µS/cm	pH	COD mg/L	Turbidity NTU	TS mg/L	VS mg/L
1	1/4/2021	12271	8.0	900	29	56	37
2	2/4/2021	14980	7.8	896	27	56	35
3	3/4/2021	12869	7.9	888	25	55	35
4	4/4/2021	13000	7.7	856	26	56	33
5	5/4/2021	14500	7.7	860	25	54	33
6	6/4/2021	12789	7.6	870	24	54	33
7	7/4/2021	-	-	-	-	-	-
8	8/4/2021	13067	8.2	790	24	53	33
9	9/4/2021	14002	7.0	781	25	51	32
10	10/4/2021	12543	7.6	764	24	48	33
11	11/4/2021	12954	7.6	737	24	48	32
12	12/4/2021	13623	7.5	720	22	46	32
13	13/4/2021	14342	7.6	702	22	47	31
14	14/4/2021	-	-	-	-	-	-
15	15/4/2021	15657	7.4	653	21	46	29
16	16/4/2021	16254	7.6	627	22	43	29
17	17/4/2021	17512	7.7	543	21	42	27
18	18/4/2021	16512	7.4	500	21	42	28
19	19/4/2021	17120	7.7	430	20	41	26
20	20/4/2021	16514	7.3	421	18	41	28
21	21/4/2021	-	-	-	-	-	-
22	22/4/2021	5515	7.3	410	17	39	25
23	23/4/2021	5000	7.3	400	18	39	24
24	24/4/2021	4890	7.5	400	16	37	23
25	25/4/2021	4678	7.3	396	16	38	22
26	26/4/2021	4518	7.2	378	14	38	23

Formula used determine electrochemical analysis

Ohm law $\mathbf{V=I\times R}$

Where V=Voltage

I=Current

R=Resistance

Power described as following equation.

$$\mathbf{P=V\times I}$$

Where; P: Power

V: Voltage

I: Current

For MFCs' the columbic efficiency defined as following equation used to described.

$$Ce=\frac{\mathbf{8\int I\times dt}}{\mathbf{F\times Van\times \Delta COD}}$$

Where F: Faradays constant

Van: Substrate volume in anode chamber

COD: proportional to substrate concentration 8 is constant value

Appendix. e

Electrochemical analysis MFC1 (Urine Substrate)

Nọ	Date	Voltage (mV)	Current (mA)	Current density (mA/cm^2)	Power (µW) 1000	Power density (µW/cm^2)
1	1/4/2021	0.00	0.00	0.00	0.00	0.00
2	2/4/2021	5.41	5.41	0.17	29.268	0.92
3	3/4/2021	7.32	7.32	0.23	53.58	1.68
4	4/4/2021	7.64	7.64	0.24	58.37	1.83
5	5/4/2021	11.69	11.69	0.37	136.66	4.27
6	6/4/2021	-	-	-	-	-
7	7/4/2021	37.45	37.45	1.17	1402.50	43.81
8	8/4/2021	49.31	49.31	1.53	2431.48	75.44
9	9/4/2021	57.64	57.64	1.80	3322.37	103.75
10	10/4/2021	66.63	66.63	2.08	4439.56	138.59
11	11/4/2021	69.95	69.95	2.18	4893.00	152.49
12	12/4/2021	82.93	82.93	2.59	6877.38	214.79
13	13/4/2021	96.85	96.85	3.02	9379.92	292.49
14	14/4/2021	-	-	-	-	-
15	15/4/2021	107.11	107.11	3.35	11472.55	358.82
16	16/4/2021	118.93	118.93	3.72	14144.34	442.92
17	17/4/2021	117.10	117.10	3.66	13712.41	428.59
18	18/4/2021	116.84	116.84	3.65	13651.59	426.47
19	19/4/2021	89.90	89.90	2.81	8082.01	251.62
20	20/4/2021	73.12	73.12	2.29	5346.53	167.44
21	21/4/2021	-	-	-	-	-
22	22/4/2021	52.09	52.09	1.63	2713.37	84.91
23	23/4/2021	23.14	23.14	0.72	553.46	16.66
24	24/4/2021	15.32	15.32	0.48	234.70	7.35
25	25/4/2021	8.52	8.52	0.27	72.59	2.30
26	26/4/2021	4.38	4.38	0.14	19.18	0.61
27	27/4/2021	2.92	2.92	0.09	8.52	0.26

Appendix. f

Electrochemical analysis MFC2 (Blackwater Substrate)

Nọ	Date	Voltage (mV)	Current (mA)	Current density (mA/cm^2)	Power (µW) 1000	Power density (µW/cm^2)
1	1/4/2021	0.00	0.00	0.00	0.00	0.00
2	2/4/2021	6.34	6.34	0.20	40.20	1.26
3	3/4/2021	11.91	11.91	0.37	141.85	4.43
4	4/4/2021	18.45	18.45	0.58	340.40	10.63
5	5/4/2021	28.34	28.34	0.89	803.16	25.10
6	6/4/2021	59.32	59.32	1.85	3171.94	109.96
7	7/4/2021	-	-	-	-	-
8	8/4/2021	71.98	71.98	2.24	5181.12	161.91
9	9/4/2021	83.65	83.65	2.61	6997.32	218.67
10	10/4/2021	91.76	91.76	2.87	8419.90	263.12
11	11/4/2021	97.43	97.43	3.04	9492.60	296.64
12	12/4/2021	115.31	115.31	3.60	13296.40	415.51
13	13/4/2021	144.84	144.84	4.53	20978.63	655.58
14	14/4/2021	-	-	-	-	-
15	15/4/2021	123.10	123.10	3.85	15153.61	473.55
16	16/4/2021	119.33	119.33	3.73	14239.65	444.99
17	17/4/2021	113.41	113.41	3.54	12861.83	401.93
18	18/4/2021	88.32	88.32	2.76	7800.42	243.76
19	19/4/2021	73.12	73.12	2.29	5346.53	167.08
20	20/4/2021	56.7	56.7	1.77	3214.89	100.47
21	21/4/2021	-	-	-	-	-
22	22/4/2021	25.87	25.87	0.81	669.26	20.91
23	23/4/2021	17.23	17.23	0.54	296.87	9.28
24	24/4/2021	10.93	10.93	0.34	119.46	3.73
25	25/4/2021	8.31	8.31	0.26	69.06	2.16
26	26/4/2021	5.21	5.21	0.16	27.14	0.85
27	27/4/2021	4.63	4.63	0.15	21.44	0.67

Appendix.g

Electrochemical analysis MFC3 (Graywater Substrate)

Nọ	Date	Voltage (mV)	Current (mA)	Current density (mA/cm^2)	Power µW 1000	Power density (µW/cm^2)
1	1/4/2021	0.00	0.00	0.00	0.00	0.00
2	2/4/2021	2.12	2.12	0.07	4.50	0.14
3	3/4/2021	4.09	4.09	0.13	16.73	0.52
4	4/4/2021	8.90	8.90	0.28	79.21	2.47
5	5/4/2021	13.11	13.11	0.41	171.87	5.37
6	6/4/2021	21.32	21.32	0.67	454.54	14.21
7	7/4/2021	-	-	-	-	-
8	8/4/2021	30.48	30.48	0.95	919.30	28.73
9	9/4/2021	49.34	49.34	1.54	2438.44	76.08
10	10/4/2021	58.90	58.90	1.84	3469.21	108.41
11	11/4/2021	64.45	64.45	2.01	4153.80	129.54
12	12/4/2021	67.89	67.89	2.12	4609.05	143.93
13	13/4/2021	73.45	73.45	2.30	5394.90	168.59
14	14/4/2021	-	-	-	-	-
15	15/4/2021	79.99	79.99	2.50	6398.4	199.95
16	16/4/2021	83.56	83.56	2.61	6982.27	218.20
17	17/4/2021	87.34	87.34	2.73	7628.28	238.38
18	18/4/2021	89.76	89.76	2.81	8056.86	251.77
19	19/4/2021	83.12	83.12	2.60	6908.93	215.90
20	20/4/2021	76.56	76.56	2.40	5861.43	183.16
21	21/4/2021	-	-	-	-	-
22	22/4/2021	31.23	31.23	0.96	975.31	30.48
23	23/4/2021	11.42	11.42	0.36	130.41	4.08
24	24/4/2021	6.13	6.13	0.19	37.58	1.17
25	25/4/2021	2.78	2.78	0.9	7.73	0.24
26	26/4/2021	1.56	1.56	0.05	2.43	0.08