BIOREMEDIATION OF BENZENE AND ITS DERIVATIVES PRODUCING RENEWABLE ENERGY THROUGH BENTHIC MICROBIAL FUEL CELLS

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by

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DECLARATION BY AUTHOR

This dissertation is composed of my original work and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my Ph.D research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. In addition, the present work (around 100%) was already published in various journals by us (Mohammad Faisal Umar and main supervisor Assoc. Prof. Dr. Mohd Rafatullah. The list of publications was given at the end of the thesis.

Regard

Mohammad Faisal Umar　　　　　Assoc. Prof. Dr. Mohd Rafatullah

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(MOHAMMAD FAISAL UMAR)

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LIST OF SYMBOLS

e-	Electron
H^+	Proton
CO_2	Carbon dioxide
H_2O	Water
mW/m^2	Milli watt per meter square
Cm	centimeter
Ω	Ohm
%	Percentage
A	Ampere
V	Volt
mV	Milli volt
mA	Milli ampere
mA/m^2	Milli ampere per meter square
J	Current density
A	Area
m^2	Meter square
kΩ	Kilo ohm
F/g	Faraday per gram
Hz	Hertz
mHz	Milli hertz
Nm	Nanometer
Ppm	Parts per million
λ_{max}	Maximum wavelength

LIST OF ABBREVIATIONS

Ag/AgCl	Silver chloride
BES	Bioelectrical electrical system
B.E%	Bioremediation efficiency
BMFC	Benthic microbial fuel cell
BTX	Benzene, Toluene and Xylene
BOD	Biological oxygen demand
CNT	Carbon nano tube
COD	Chemical oxygen demand
Cp	Specific capacitance
CV	Cyclic voltammetry
DCMFCs	Double chamber microbial fuel cells
EIS	Electro impedence spectroscopy
ETC	Electron transport chain
FADH	Flavin adenine dinucleotide
MDC	Microbial desalination cell
MES	Microbial electrolysis cell
MFC	Microbial fuel cell
MSC	Microbial saline-wastewater electrolysis cell
MnO_2	Manganese oxide
MQ	Menaquinone
NADH	Nicotinamide adenine dinucleotide
ORR	Oxygen reduction reaction
PANI	Polyaniline
PCR	Polymerase chain reaction
P.D	Power density
PPy	Poly-pyrrole
Rct	Charge transfer resistance
Rs	Solution resistance
SBMFC	Single chamber benthic microbial fuel cell
SCMFC	Single chamber microbial fuel cell
SEM	Scanning electron microscopy

SMFC	Sediment microbial fuel cell
UQ	Ubiquinone
W	Warburg
ZrO_2/CB	Zirconium oxide/Carbon Black

BIOREMEDIASI BENZENA DAN DERIVATIFNYA MENGHASILKAN TENAGA DIPERBAHARUI MELALUI SEL BAHAN BAKAR MIKROB BENTIK

ABSTRAK

Aktiviti antropogenik sebahagian besarnya bertanggungjawab terhadap sejumlah besar bahan pencemar seperti hidrokarbon aromatik polisiklik, sianida, fenol, derivatif logam, sulfida dan bahan kimia lain dalam air sisa melalui industri petrokimia dan kimia sumber. Lebihan benzena, toluena dan xilena (BTX) boleh menyebabkan ketoksikan teruk kepada organisma hidup dalam air sisa. Beberapa kaedah telah digunakan seperti penapisan, pemendapan, pengozonan dan lain-lain. BMFC ialah teknologi baru muncul untuk pengeluaran serentak tenaga boleh diperbaharui dan bioremediasi BTX daripada air sisa dengan menggunakan substrat tebu. Ia berbeza daripada sel bahan api mikrob kerana ia tidak mempunyai membran. Benzena dan toluena telah dioksidakan kepada asid benzoik perantaraan, manakala xilena ditukar kepada asid 2-metil benzoik kemudian ditukar sepenuhnya kepada karbon dioksida. Potensi maksimum dikira menggunakan multimeter, manakala ketumpatan arus dan ketumpatan kuasa melalui lengkung polarisasi BMFC. Spektroskopi boleh dilihat UV dan voltammetri kitaran digunakan untuk menentukan kecekapan bioremediasi dan kapasitans spesifik, masing-masing. Spektroskopi impedans elektrokimia menentukan rintangan dalaman keseluruhan, dan analisis mikrobiologi mendedahkan bakteria yang ada. Voltan tertinggi reaktor BMFC semasa bioremediasi benzena, toluena, dan xilena masing-masing dijana pada 430 mV, 530 mV dan, 410 mV, apabila rintangan luaran ialah 1 kΩ. BMFC yang dibangunkan terbukti berkesan dan telah mengkaji kecekapan bioremediasi benzena, toluena dan xilena adalah 82.3 %, 98.22 % dan 87.88 % melalui spektroskopi boleh dilihat UV

adalah lebih baik daripada keputusan lain. Ketumpatan kuasa benzena, xilena, dan toluena dijana melalui reaktor kira-kira 24.2 mW/m^2, 63 mW/m^2 dan 34.7 mW/m^2, manakala ketumpatan arus benzena, toluena, dan xilena dicapai 95mA/m^2, 170.94 mA/m^2, dan 120 mA/m^2 masing-masing. Daripada lengkung voltammetri kitaran, kapasitans khusus untuk benzena ialah 0.00017 F/g selepas 30 hari, manakala kapasitans khusus untuk toluena dan xilena ialah 0.141 F/g dan 0.124 F/g pada hari ke-40 dan ke-41 semasa operasi BMFC. Imej SEM menunjukkan morfologi komuniti mikrob dalam bentuk biofilem, yang tidak teratur diedarkan pada permukaan elektrod. Keputusan pyrosequencing 16S rDNA mengenal pasti *Pseudomonas* sp. dan *Bacillus* sp terlibat dalam bioremediasi benzena, *Staphylococcus* sp dan *Pseudomonas* sp yang dikaitkan dengan toluena, manakala *Staphylococcus edaphicus*. dan *Staphylococcus sparophiticus* terlibat dalam bioremediasi xilena. Ini adalah laporan pertama sisa tebu sebagai substrat dalam BMFC untuk bioremediate benzena, toluena, dan xilena bersama-sama dengan penjanaan kuasa. Keputusan ini menunjukkan bahawa BMFC boleh digunakan untuk penjanaan kuasa dan bioremediasi benzena, toluena dan xilena.

BIOREMEDIATION OF BENZENE AND ITS DERIVATIVES PRODUCING RENEWABLE ENERGY THROUGH BENTHIC MICROBIAL FUEL CELLS

ABSTRACT

Anthropogenic activities are largely responsible for the vast amounts of pollutants such as polycyclic aromatic hydrocarbons, cyanides, phenols, metal derivatives, sulphides, and other chemicals in wastewater through the source petrochemical and chemical industries. The excess benzene, toluene and xylene (BTX) can cause severe toxicity to living organisms in wastewater. Severral methods have been utilized like filtration, sedimentation, ozonation etc. BMFC is an emerging technology for the simultaneous production of renewable energy and bioremediation of BTX from wastewater by using sugar cane substrate. It is a different from microbial fuel cells because they lack a membrane. Benzene and toluene were oxidized into intermediate benzoic acid, while xylene was converted into 2-methyl benzoic acid then completely converted into carbon dioxide. The maximum potential was calculated using a multimetre, while current density and power density through the polarization curve of BMFC. UV–visible spectroscopy and cyclic voltammetry were used to determine bioremediation efficiency and specific capacitance, respectively. Electrochemical impedance spectroscopy determined the overall internal resistance, and microbiological analysis revealed the bacteria present. The highest voltage of BMFC reactor during bioremediation of benzene, toluene, and xylene was generated at 430 mV, 530 mV and, 410 mV respectively, when was external resistance was 1 kΩ. The developed BMFC proved to be effective and examined bioremediation efficiencies of benzene, toluene and xylene were 82.3 %, 98.22 % and 87.88

%through UV–visible spectroscopy was better to other results. The power density of benzene, xylene, and toluene were generated through a reactor of about 24.2 mW/m^2, 63 mW/m^2 and 34.7 mW/m^2, while the current density of benzene, toluene, and xylene were achieved 95mA/m^2, 170.94 mA/m^2, and 120 mA/m^2 respectively. From cyclic voltammetry curve, the specific capacitance for benzene was 0.00017 F/g after 30 days, while specific capacitance for toluene and xylene were 0.141 F/g and 0.124 F/g at 40th and 41th day during BMFC operation. The SEM images showed morphology of microbial community in the form of biofilm, which are irregular manner distributed over the electrode's surface. The 16S rDNA pyrosequencing results identified *Pseudomonas* sp. and *Bacillus* sp were involved in bioremediation of benzene, *Staphylococcus* sp and *Pseudomonas* sp associated with toluene, while *Staphylococcus edaphicus*. and *Staphylococcus sparophiticus* involved in bioremediation of xylene. This is the first report of sugar cane waste as a substrate in a BMFC to bioremediate benzene, toluene, and xylene along with power generation. These results showed that the BMFC could be used for power generation and benzene, toluene and xylene bioremediation.

CHAPTER 1

INTRODUCTION

1.1 Research background

There are several sources to contaminate water bodies from the chemical and oil filtration industry containing inorganic pollutants (sulphide, ammonia) and organic pollutants (benzene, toluene, ethylbenzene and xylene) (Afferden et al., 2011; Olmos et al., 2004). However, groundwater is continuously contaminated by BTX (benzene, toluene, and xylene) due to leakage of BTX from many industries such as petrochemical, oil refining, and chemical industry. These pollutants have a severe and harmful issue to the environment and health hazards (Gibson, 1968). On the 9th of March 2019, the river in “Kim Kim” Pasir Gudang, Johor, Malaysia, was poisoned due to the disposal of numerous unregulated chemicals. Many people were affected by this chemical waste due to its dangerous fumes. Several chemicals such as acrylonitrile, methane, furan, indole, oxadiazole, pyrroles, triazoles and BTX were interacting with water which was source of water pollution followed by air pollution (Lee Goi, 2020). Many pollutants are very harmful to human health and the environment, as its adverse health effects include chronic toxicity formation, cancer, and mutagenesis (Saha et al., 2018). BTX are the most toxic of all the BTX compounds due to its carcinogenic nature. They are usually found in groundwater samples and this poses severe risks to human health as cause headache, weakness and aberration of respiratory, nervous, immune, hematological, hepatic, renal, cardiovascular, and reproductive systems (Liu et al., 2018). Various bioremediation techniques are used to treat contaminated groundwater which produces hazardous organic compounds. These techniques include coagulation, flocculation, sedimentation, filtration, infection, fluoridation membrane bioreactor, membrane filtration, precipitation, adsorption, and electrochemical treatment technologies (Du et al., 2017; Khodaei et al., 2017). Bioremediation is an economical and environmentally friendly approach that involves biological remediation of polluted groundwater by various microorganisms that decompose pollutants into non-poisonous or less toxic compounds (Al-Mailem et al., 2017). BTX biodegradation are effective because it converts pollutants into environmentally friendly end products like biomass, CO_2, and water. However, biodegradation and oxidation methods have shown greater performance than others, which has been a drawback to their adoption (Montagnolli et al., 2017). Furthermore, aerobic bioremediation requires expensive mechanical aeration to supply dissolved oxygen to groundwater samples. Aeration also causes air pollution through volatile organic carcinogenic compounds from groundwater (Liu, et al., 2018).

MFC has been gaining prominence as an emerging technology, depends on microbial metabolism for energy production. Unlike other wastewater treatment methods, the microbial fuel cell (MFC) generates electricity with pollutants degradation (Gude, 2016; Santoro, Arbizzani, et al., 2017). MFC is an electrochemical technique for biological treatment that employs electrodes and biofilms to serve as biocatalysts, allowing contaminants to be biodegraded. These pollutants store houses of chemical energy that are converted into electrical energy upon their biodegradation (Logan & Regan, 2006; Manman Zhang et al., 2019). Electrons could be transferred either through short range electron transfer, long range electron transfer and direct electron transfer. Electrons could be transferred to electrode through the direct electron transport mode via biofilms which is quick mechanism in MFCs. The biofilm developed over electrode surface by microbes, which will help to transfer of electrons from microbe to anode which finally increased biodegradation rate of organic pollutants. It has been demonstrated that the MFC can effectively promote BTX biodegradation in wastewater (Li et al., 2013). Daghio et al., (2018) reported that bioelectrochemical treatment positively impacted xylene and toluene degradation.

MFC is an electrochemical device that converts chemical energy into electrical energy via microbial degradation of toxic organic compounds. In recent years, the microbial fuel cell has been considered an alternative source for bioremediation of a vast range of toxic organic pollutants and one of the beauties is that to generate the electrical energy by microbes from chemical energy. A benthic MFC (BMFC) is an MFC type that produces power from a potential developed between an anode immersed in sediment (anoxic environment) and cathode enclosed in overlying water (oxic environment) (Abbas & Rafatullah, 2021; Sajana et al., 2013). The fundamental theory of BMFC is similar to that of the microbial fuel cell (MFC), in which exoelectrogen microbes releasing electrons (e-) and hydrogen ions (H+) at the anode in anoxic condition decompose the organic matter. The electrons migrate to the cathode through an external circuit where oxygen is reduced and interacts with hydrogen ions (passed to the cathode through the membrane) and converts to water. In BMFC, as the anode is deployed under the sediment and the cathode at the benthic zone above the sediment or water interface, membranes in these cells are not needed (Pushkar et al., 2018). Few studies have been reported on the construction of MFCs to remove organic pollutants like benzene and simultaneous electricity production (Chang et al., 2017; Wei et al., 2015). Imran et al., (2019) reported only 63.81 mW/m^2 power density using a coating of cerium at anode and platinum coating at the cathode in the designed BMFC. Recently, the benthic microbial fuel cell or sediment microbial fuel cells introduced electrical energy depending on a potential difference between nonaerated bio-sediment and aerated water. Reimers et al., (2001) first discovered the general prototype of BMFC utilized the platinum mesh and carbon cloths act an anode and cathode. In BMFC, the anode is embedded in bio-sediment without oxygen, while cathode with oxygen in contact with water generates renewable energy. The electron and protons are delivered from anode to cathode through an external circuit and flow through bio-simulation to cathode area fused with oxygen to form water, finally bioremediation of organic pollutants and generating energy. Previous researchers have used various materials to construct the anode and cathode, but it is costly and cannot last for many days. It is also toxic for the growth of microbes, which can hinder the production of electricity. This is why a graphite electrode has been recommended because its unique feature, such as non-toxicity, has long durability.

1.2 Problem statements

Anthropogenic VOCs, generally referred to as aromatic hydrocarbons, are the most frequently measured by previous studies. Aromatic VOC, particularly benzene, toluene, ethylbenzene and xylenes (BTEX) partitions is of great concern as it may cause a detrimental impact on human health (Latif et al., 2019). On the 9th of March 2019, the river in Kim, Kim, Pasir Gudang, Johor, Malaysia, was poisoned due to the disposal of numerous aromatic pollutants (Lee Goi, 2020). This component highly injurious to human health as its causes many chronic diseases like cancer and tumors (Saha et al., 2018). There was large quantity of BTX in water which is a critical challenge to solve with green and feasible approach. Authors were reported for the treatment of toxic organic (benzene and its derivative) by different physical and chemical methods. These methods are effective, but require a high amount of energy and their limited application (Adelaja et al., 2017). Usually, the accumulation of reductive substance and the lack of accessible electron acceptors were the main limitations for the bioremediation by substrate underwater in anaerobic conditions. The MFCs is the most emerging and promising approach but it has some challenges which need to address. Benthic microbial fuel cell (BMFC) is a special type of microbial fuel cell working on the seafloor. Benthic microbial fuel cells (BMFC) are the potential sources for energy generation in which the chemical energy stored in the bonds between organic and non-organic substrate are turned into electricity using microorganisms as the catalysts (Tavakolian et al., 2020). One of the challenges is lie mainly on the bioremediation efficiency of benzene, toluene and xylene through BMFC with the help of organic substrate. Additionally, the transportation of electrons from bacteria cell to anode electrode surface was an essential step for renewable energy. In the last decade, several efforts were done to improve the bio-generation of electron transportation between bacteria cell and electrodes which ultimately affect the efficiency of the BMFC. A wide variety of organic substrate have been used for BMFC have already been studied such as natural organic substrate and synthetic substrate (Aldrovandi et al., 2009; Hassan et al., 2014; Jafary et al., 2013; Mäkinen et al., 2013). However, natural organic substrate (rice straw, rice paddy, wheat straw,) and synthetic substrate (lactate, cellulose, glucose) failed to bioremediation of pollutants and not enough power energy (Kouzuma et al., 2013; Niessen et al., 2004; Rezaei et al., 2009). The above-mentioned organic substrates presented the drawback: difficult to break down into simple molecule due to polysaccharide sugar (Shibuya & Iwasaki, 1978), they did not bioremediate of any type of pollutants. One another break through, a wide variety of materials to build the anode electrodes for MFCs have already been studied metal-based anodes and carbon based anodes (Liu et al., 2020). Metals utilized for anodes, such as silver, nickel, copper and stainless steel. Metal based materials presented the following drawbacks: low power density, poor chemical stability, small surface area, large pore size, poor mechanical stability, non-biocompatibility, corrosion, un-known time period of microbial community, toxic for microbes. Furthermore, stainless-steel surface is a problem to stick of microbes due to the smooth surface, while carbon cloth is easy to developed bacteria over the electrode and create a biofilm. Therefore, the electron flows easily through an external circuit from the anode to the cathode by biofilm and produces more power density. The above metals are not appropriate to design for anode of BMFC (Imran et al., 2019; Prakash,et al., 2018). On the other hand, carbon-based materials specially graphite rods have an important role in the BMFC anodes because they are relatively economical in comparison to metal, and they are easy to handle and also have mechanical strength, inertness, commercial availability and they have a fixed surface area and non-reactive (Mashkour et al., 2017; Zhao et al., 2019).

The improved anode electrode and natural substrate which contained the disaccharide can address the bioremediation performance issue in BMFCs such as slow redox reaction to convert the toxic organic pollutant into nontoxic state. With the help of natural substrate and biocompatible anode electrode of BMFC bring major breakthrough in improving the bioremediation performance and renewable energy of BMFCs. The natural organic substrate and biocompatible anode electrode may enhance the bacterial biocompatibility and boost the redox reaction which may lead to the high organic pollutants bioremediation performance and renewable energy.

1.3 Research questions

- Which BMFC is better for removal of Aromatic organic pollutant (Benzene, Toluene, and Xylene)?
- Why graphite rod employed at both anode and cathode electrodes in BMFC?

- What is the mode of bio-production of electron pathway inside membrane of microbes?
- Which microbes are mostly responsible for BMFC operation and generation of high-power density?

1.4 Objectives of the research

The main aim of this proposed work is the bioremediation of benzene and its derivatives producing renewable energy through BMFC. The studies were carried out with the following specific objectives:

- To determine bioremediation efficiency of toxic benzene, toluene, and xylene through BMFC at different time period.
- To optimize the maximum power density, current density and internal resistance of BMFC with respect to external load at different time period.
- To determine the pathway and mechanism of electrons inside the cytoplasm as well as inner membrane of microbes through CV of BMFC.
- To identify the microbial community related to benthic microbial fuel cell performance.

1.5 Scope of the research

Last work reported about MFC, bioremediate of pollutants and produced energy, but by using synthetic substrate or natural substrate to boost of microbes. Some authors have been used natural substrates for example rice husk, and wheat straw (Kouzuma et al., 2013; Niessen et al., 2004; Rezaei et al., 2009). They are effective but have some drawbacks presented following: not broken into simple molecules due to polysaccharide sugar, they have not produced simultaneously bioremediation of pollutants and power density. Furthermore, they did not determine the electron pathway and did not isolate the microbes.

In this study, synchronization was performed by BMFC which is rapid biological treatment due to availability of electron donor and electron acceptors. A lab scale BMFC was operated to determine their optimized performance in term of bioremediation efficiency of BTX in single solution., not in binary mixture of BTX, and power generation by using sugarcane waste substrate its easily break down into simple molecules due to disaccharide sugar to provide the energy of microbes. The experiment was conducted in the single or double chamber to use for BMFC system. It's showed the bio-generation pathway of electron inside membrane of microbes by CV. The electro-microbiology and morphology of electrode biofilm were also covered in this study.

The reduction of the oxygen molecule by the cathode is the main limitation of the BMFC system. The reduction of oxygen at the cathode has recently been identified as a significant limiting factor in this study, despite the fact that various electrodes have been commonly used for the cathodic reaction. Even this study was conducted on a laboratory scale rather than a field scale or real solution.

1.6 Thesis organization

The organisation of this thesis is divided into 5 major chapters. Chapter 1 discusses the introduction of this research work that includes research background on BTX pollutants in water, treatment methods and BMFCs. This chapter covers the problem statement and solutions, research questions, objective and scope of the research.

Chapter 2 summarizes the literature review of sources of wastewater pollution, giving an overall idea of BMFCs and different power densities have found from exoelectrogens and organic substrate. This is followed by mechanism of bioremediation of BTX organic pollutants, bio-generation pathway of electron and discussed the operating factors. This literature review also discusses the applications of BMFC.

Chapter 3 presents the methodology part, with the description of chemicals and apparatus used for this research. The configuration of double chamber BMFC and single chamber multi anode BMFC both are without membrane and embedded with sugarcane substrate, power density calculated by ohms law while specific capacitance and internal resistance measurement by cyclic voltammetry and electro-impedence spectroscopy. The BTX bioremediation efficiency was analysed using UV–Vis spectrophotometer. This chapter also discussed the morphology and microbial characterisation of microbes.

Chapter 4 reports the results and detailed discussion of BMFC. The outcomes include bioelectricity production, power and current density, bioremediation efficiency of BTX. This chapter also include optimisation of specific capacitance and internal resistance by cyclic voltammetry and electrochemical impedence spectroscopy. Finally, the morphology and microbial characterisation of identified microbes are analysed.

Chapter 5 describes overall conclusions and future recommendations for research work.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In different regions of the world, wastewater rises daily, which is a significant source of pollution in shallow and groundwater (Asano & Cotruvo, 2004). The human immune system is directly affected by water in different ways such as water pollution, degradation, and ecology. Overall, many types of pollutants exist in wastewater, such as organic matter (phenol, penta-chlorophenol, nitrobenzene, pyrene, phenanthrene, anthracene) and inorganic matter, which includes nitrogen, phosphorus, ammonia, iron chlorides, nitrate, nitrite and involves heavy metals (Tengrui et al., 2007). Wastewaters are mostly accumulated in domestic areas from different regions such as laundry wastewater, kitchen utensil wastewater, petrochemical industries, and processing plant oil, containing organic compounds and incredibly aromatic hydrocarbons such as benzene, toluene, and xylene (BTX) (Norton-Brandão et al., 2013). Petroleum aromatic hydrocarbons are considered potential water pollutants due to their adverse effects on human beings, including carcinogenic and mutagenic effects (Adelaja et al., 2017; Lamichhane et al., 2016). Petrochemical wastewaters are the aggregate composition and the most invasive are hydrocarbons and aromatic hydrocarbons (BTX) (Lamichhane et al., 2016). The sewage composition from refinery wastewater includes lubricant and petroleum compounds, which comprise three hydrocarbons such as naphthalene (cyclohexane (C_6H_{12}) and dimethyl cyclopentane (C_7H_{14})), paraffin (methane (CH_4), ethane (C_2H_6) and propane (C_3H_8), and aromatic compounds (BTX). They are all present in wastewater from petrochemical factories which cause well-known lethal effects. BTX compounds may promote unfavourable health effects in the nervous and respiratory systems. If the desired concentration of BTX can be kept at a minimum level, it will be impossible for the compounds of BTX to have an unfavourable effect on the nervous and respiratory systems of human beings (Amini et al., 2017). Amongst the compounds of BTX, benzene is the most hazardous and is extremely can-cerous in humans, according to the World Health Organization (WHO 1996) and the International Agency for Research on Cancer (IARC 2012). According to the WHO, a concentration of 1.0 $\mu g/m^3$ of benzene would cause up to six leukaemia cases in a population of 1 million people (Kuranchie et al., 2019). In recent years, several techniques have been utilized for the investigation of wastewater before irrigation, such as lagoon ponds, constructed wetlands, and conventional wastewater treatment plants, which involved (coagulation, flocculation, sedimentation, filtration, disinfection, fluoridation, storage and distribution), membrane bioreactor, membrane filtration, precipitation, coagulation–flocculation, adsorption, membrane filtration, and electrochemical treatment technologies. Despite the significance value these techniques provide, the space demands/requirements and huge capital requirements are existing problems dampening their adoption (Abbas et al., 2018; Norton-Brandão et al., 2013). Recently, microbial fuel cells (MFCs) have been considered a substitute source for the bioremediation of vast ranges of BTX, which is non-expensive, and one of the beauties of this method is that microbes can generate the electrical energy from chemical energy (Kugarajah & Dharmalingam, 2021). The electrical energy produced by any variation of benthic microbial fuel cell (BMFC) depends on the potential difference between non-aerated bio-sediment and aerated water (Girguis et al., 2010). However, there is a variance between MFCs and BMFCs; in MFCs, synthetic substrate and the non-synthetic substrate are used as fuel for removing toxic substances with renewable energy production; on the other hand, BMFCs utilize natural organic waste substrate as fuel. Actually, natural energy is costly due to existing sustainable energy produced by different energy sources such as solar, wind and hydro-energy, since they rely on the climatic and environmental factors of a particular place. The conclusions that many environmental microorganisms can establish direct electrochemical communication with a solid electrode have led to microbial fuel cell technology (Abbas & Rafatullah, 2021; Liu et al., 2015; Yadav et al., 2020). The BMFC has some advantages over the conventional treatment methods because they have maintainable power generation, they are also progressing at an appropriate fast rate, and a developing scheme illustrates their practical possibility. The prototype of double chamber BMFCs consists of an anode in the non-aerated benthic sugarcane waste and a cathode in the aerated groundwater, which completes the connection for both electrodes of BMFCs from the external circuit.

There are different industries around us such as petrochemical, chemical industries, and textile industries. These industries are very useful and sometimes harmful because they released toxic pollutants mostly aromatic pollutants, and water becomes polluted. Among aromatic pollutants, BTX is the most toxic in the water. This component causes carcinogenic diseases like cancer and tumors. Some conventional methods were used for the bioremediation of BTX. These methods are effective but required a large amount of energy and limited application. In previous works, most MFCs consist of an anode and cathode separated by a pro-ton exchange membrane, allowing proton transfer from the anode to the cathode to bioremediation water. Previous researchers have used various materials in the construction of the anode and cathode, but it is costly and cannot last for many days. It is also toxic for the growth of microbes, which can hinder the production of electricity. This is why a graphite electrode has been recommended because its unique feature, such as non-toxicity and has long durability. BMFCs may provide an additional advantage that membranes lack, and are biocompatible due to the use of natural organic substrates to generate electricity, another aim of BMFCs is the bioremediation of BTX pollutants from wastewater.

2.2 Sources of BTX

2.2.1 Related industries producing BTX-containing wastewater

It is undeniable that the progressive development in the petroleum industry and consumption of its products across the world in the last century produce enormous amounts of toxic chemicals into the environment. Among varies petroleum pollutants of interest, Benzene (B), Toluene (T), and Xylene (X: of ortho, meta, and para-positions), termed as BTX, mono-aromatics has found to be the most dangerous environmental problem (Navarro Amador et al., 2018). The major source of BTX released in air and wastewater from different industries which included petrochemical fuel derivatives such as petrol or gasoline, automobile emissions, and other related industries such as glue factories or paint, municipal waste, traffic, plastics, agriculture effluents, and solvent extraction (Afshari et al., 2018; Hoseini et al., 2019; Hossini Asl et al., 2020). This component may be continuously released due to petrol and diesel fuel leakage from cracked and old underground gasoline storage tanks in urban areas. BTX is the ability of water to move deep through an urban area that contaminates water (Radwan et al., 2019; Tiburtius et al., 2005; Torabian et al., 2010). The world health organization (WHO) was announced that the maximum permissible value of BTX in drinking water are B= 0.01 ppm, T =0.7ppm, and X= 0.5 ppm (Hossini Asl et al., 2020). BTX compounds are volatile and easily vaporize at temperatures above 20 °C. Therefore, fugitive emissions from fuel storage facilities and transportation activities are also important sources of BTEX. A number of studies have reported high ambient concentrations of BTX during fuel tank filling (Moolla et al., 2015; Tsangari et al., 2017), from oil and gas operations (Jiang et al., 2017) and during solvent usage and spills from accidents (Latif et al., 2019; Tsangari et al., 2017). BTX in Southeast Asian countries also can be emitted from other sources such as petrol stations, residential cooking, and open burning of solid waste and agricultural residues (Phuc & Kim Oanh, 2018). Moreover, BTX can be classified as industrial wastewater and hazardous air pollutants among the organic compounds (Chang et al., 2001).

2.2.2 Impact of BTX

Volatile organic compounds when released into the atmosphere, not only affect the human health but also cause various environmental complications such as ozone layer depletion, deterioration of crops and vegetation (Masih et al., 2018) as well as formation of secondary organic aerosol and ground level ozone. Exposure to volatile organic compounds can be deleterious to human health, causing headache, nausea, weakness, lack of concentration, loss of appetite and fatigue (Bernstein et al., 2008). Aromatic VOC, particularly (BTX) partitions is of great concern as it may cause a detrimental impact on human health (Atkinson & Arey, 2003). The nature and extent of these health risk effects depend on the concentration levels of the species and the duration of their exposure to pose adverse health effects (Garg & Gupta, 2019).

It is known that BTEX compounds are harmful to human health; long-term exposure may lead to respiratory and cardiovascular illnesses and also affects the function and development of the immune, metabolic and reproductive systems (El-Hashemy & Ali, 2018; Latif et al., 2019; Pouresmaeili et al., 2018). Ran et al., (2018) studied various diseases such as respiratory and cardiovascular disease, particularly, congestive heart failure and chronic obstructive pulmonary disease, they all may be caused by atmospheric BTX. The health risk assessment of BTX (carcinogenic and non-carcinogenic) suggested by the United States Environmental Protection Agency (USEPA) is usually used to estimate the nature and likelihood of adverse health impacts of each monitored BTX species (Dehghani et al., 2018; El-Hashemy & Ali, 2018; Hu et al., 2018). Benzene exposure has been linked with occurrence of a number of blood diseases such as aplastic anemia and a variety of cancers including the acute myeloid leukemia (Buczynska et al., 2009; Duarte-Davidson, 2001). According to W.H.O., benzene concentration of 1.7 mgm^{-3} is likely to cause leukemia to 1 in 100,000 individuals (World Health Organisation, 2010). Toluene is a known teratogen and causes fatal abnormalities (Donald et al., 1991). Xylene acts as a skin sensitizer and can cause dryness, rupture and blistering of skin. At higher concentrations, toluene and xylene can cause weakening of nervous system, kidneys and liver (Masih et al., 2018).

2.3 Treatment of BTX-containing wastewater

2.3.1 Conventional methods

The development of appropriate technologies for the bioremediation of wastewater with BTX is urgently required due to the detrimental effects of BTX that have already been described. When compared to the use of activated carbon adsorption systems, conventional procedures like flocculation, sedimentation, and filtering seem impracticable for the treatment of BTX (Carvalho et al., 2012). As activated carbon has a high porosity and substantial internal surface area, it is regarded as one of the effective solutions for the removal of BTX (Alalm et al., 2015). In particular, modified poly(butyl methacrylate) resin showed a significant ability for the adsorption of BTX in its various forms (Yang et al., 2016). Author used a cationic surfactant prepared from ostrich bones as a bio-adsorbent of petrochemicals. However, the application cannot be implemented on a large scale because of the high cost of activated carbon regeneration (Gar Alalm et al., 2018). It is possible that the nanocomposite materials' increased water dispersibility could have an impact on the probability of secondary contamination. In addition, the recovery of the nanocomposite from the treated wastewater might result in material degradation if conventional methods such as flocculation, sedimentation, and filtering are using (Zhao et al., 2018). When applied to a large-scale real water purification process, the use of traditional separation technologies may result in an increase in both the time and expense, which is incompatible with the implementation of sustainable chemistry (Wei et al., 2022). To address these issues, microbial fuel cell is an ecofriendly method that produces no secondary pollution, especially when a range of pollutants are destroyed efficiently.

2.3.2 Advanced treatment

In Advanced treatment, it has been shown that some advanced oxidation process may remove BTX from water and wastewater with a reasonable amount of effectiveness (Al-Sabahi et al., 2017; Rakoczy et al., 2013; Xue et al., 2018; You et al., 2020; Zhang et al., 2018). Advanced oxidation process are preferred technologies for the rapid degradation of bioresistant organic contaminants such as pharmaceuticals, pesticides, phenolic compounds and BTX (Gar Alalm et al., 2015). The dominant classification of advanced oxidation processes includes UV–hydrogen peroxide processes, Fenton and photoFenton, ozone-based processes, photocatalysis and sonolysis. These processes are effective and give better results but have some drawbacks inefficient for heavily polluted streams, slow reaction rate, low solubility in water/drawbacks in aqueous reactions, improper catalyst selection and parameter versatility (Cardoso et al., 2021; Liu et al., 2021; Pandis et al., 2022; Vieira et al., 2021; Wang et al., 2022). A microbial fuel cell can produce simultaneously bioenergy and treatment pollutants from the wastewater used by substrates. It is a fast reaction and their substrate is the proper catalyst. A microbial fuel cell was successfully applied for the treatment of benzene and ammonium co-contaminated groundwater (Wei et al., 2015). Zhang et al., (2018) found the bioremediation efficiency for toluene at 88% and the power energy 18.3 mW/m^2 for toluene by successfully applying the MFC. It has emerged as a promising solution to the growing awareness of the benefits of solar-powered pre-and post-treatment for wastewater and produced energy.

2.4 Bioremediation of BTX

2.4.1 Bioremediation mechanism of BTX

Compared to a microbial fuel cell, an anode inserted into the soil and a cathode in the overlying water comprise a benthic microbial fuel cell. In BMFCs, the redox potential difference between the sediment and marine water is responsible for electrons' movement. The sediment and water edge play an essential role; they work on the microbial fuel cell proton exchange membrane. There are two possible factors in the treatment of BTX pollutant in wastewater by BMFCs. Research has shown that microbial communities are influenced by (i) direct extracellular electron transfer ability and (ii) the use of pollutants as a carbon source (Jia et al., 2019). Direct extracellular electron transfer involves exoelectrogens from microbial communities forming biofilms over the electrodes. The anodic biofilm contains electroactive microbes, which are key in the bioremediation process of BTX. Biofilm formation accelerates BTX bioremediation as it encourages electron activation and the breakdown of BTX as a carbon source. These electrons, once activated, migrate to the anode and renewable energy is produced. The diversity of the microbial community, which has both biodegradative and electroactive microbes, makes the mechanism of the bio-electroremediation of BTX unique. These microbes catalyse the anode region, causing the activation of electrons from BTX (Kaur et al., 2019). These activated electrons move through an external circuit from the anode to the cathode region. The electroactive microbes improve direct extracellular electron efficiency. The biodegradative microbes are responsible for the initial ring cleavage of BTX, while the electroactive microbes are essential in the bioremediation process of BTX. Firstly, benzoic acid is formed under the mediation of carboxylase in a process called carboxylation (Yang et al., 2020). Then, benzene and toluene are converted into benzoic acid, but xylene is converted into 3-methylbenzoic acid. Benzene is more difficult to convert to benzoic acid through carboxylation when compared to toluene and xylene. This is because benzene may first be converted into thylated or hydroxylated forms before it becomes benzoic acid. Then, the cleavage reaction converts benzoic acid into smaller acid molecules, CO_2, and electrons, as shown in Figure 2.1.

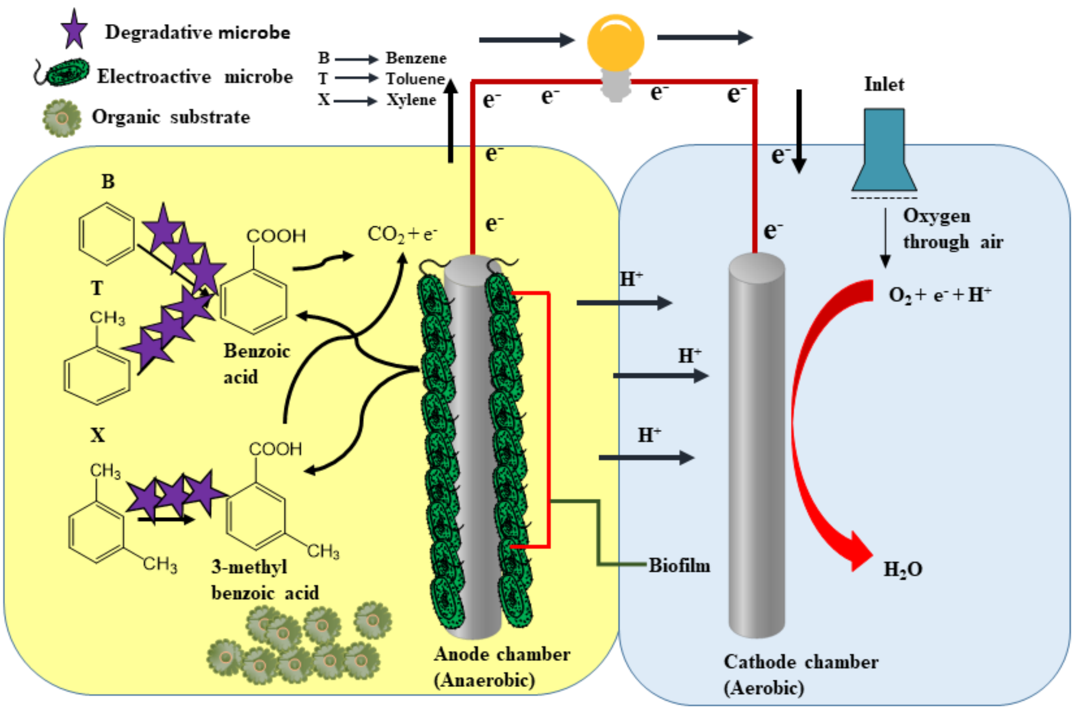

Figure 2.1: Schematic illustration of general mechanism of benzene, toluene, and xylene (BTX) by BMFCs. The bioactivity degradative microbes are pollutants degrading microbes which produced the pollutant intermediates, while electroactive microbes attach to the anode and form biofilm. This biofilm attacks the intermediate to generate CO_2 and electrons.

The MFC technology has been previously researched and reported to have a functional potential to bio-remediate benzene at the anode. Zhang et al., (2010) practically stated that the bioremediation of benzene was carried out by a graphite electrode and an electron acceptor from polluted marine-sediment, demonstrating the potential of electrode-based systems for the degradation of aromatic hydrocarbons in anoxic environments. During the concurrent generation of power in 1000 mg of glucose, the author reported that a packaging-type MFC was used, and 600 mg of benzene was degraded entirely within 24 h. It was also reported that the degradation of benzene and the production of electricity occurred concurrently with potassium ferricyanide acting as an electron acceptor. The aromatic ring of benzene was possibly triggered in the analysis and cleaved by mono and/or dioxygenases, suggesting aerobic or micro aerobic conditions due to rapid reaction kinetics; benzene can be degraded effectively under oxygen-restricted conditions (Abbas,et.al., 2017).

In another study, benzene was introduced to the system, and the current production was reported. Thirty electrons were said to be theoretically released over the entire degradation of benzene. The current production depends heavily on the efficiency with which electrons are transported to the anodes as benzene is oxidised (Wei et al., 2015). Benzene oxidation is the primary reaction at the anode and the electrons' release is effectively delivered at the anode, as demonstrated by the anodic and cathodic reaction of MFCs. The degradation efficiency and power density of BTX pollutants have been evaluated by electrochemical reactions such as the anode and cathode reactions (Wei et al., 2015). This study's scope is the bioremediation of BTX pollutants coupled with electricity production using BMFCs (Abbas, et.al., 2017).

The author reported together BTX biodegradation studied was used by MFC (Zhang et al., 2020). In this study the enhancement of biodegradable of BTX caused by biocathodes while double chamber MFC was used to calculate the removal efficiency and power generation. The individual toluene and ethylbenzene removal efficiencies were 94.8% and 86.3%, while xylene removal efficiency was only 71.6%. This was biodegradation efficiency of xylene was lowest due to under oxygen limited condition and has different structure from xylene and toluene. Moreover, toluene, ethylene benzene, and blend (toluene and ethyl benzene) were produced maximum power densities of 10.1, 5.8, and 9.9 mW/m^2, respectively, corresponding with the open circuit voltages of 0.47 V, 0.49 V, and 0.54 V while xylene lowest power density was produced by MFC 2.3 mW/m^2 but the value of xylene power density continuously increased 6.1, 4.0, or 3.2 mW/m^2. Some species were responsible to produced power density such as *Hydrogenophaga sp.*, *Chryseobacterium sp.*, *Geobacter sp. Pseudomonas sp. Alicycliphilus sp.* and *Rhodococcus sp.* Another study, used exoelectrogens *Shewanella oneidensis* MR-1 in the MFC from external side to biodegradation of xylene. The o-xylene biodegradation efficiency was increased from 54% to 73% at a concentration of 300 g/m^3, and the maximum power density 52.1 to 92.5 mW/m^3 was after adding the *Shewanella oneidensis* MR-1. Therefore, the power density was 1.3 times higher than without adding *Shewanella oneidensis* MR-1. Other species involved *Chryseobacterium sp., Hydrogenophaga sp.*, and *Sedimentibacter sp.* to degrade the xylene.

2.4.2 Effective BTX-degrading bacteria

The ability of BTX degradation of certain microorganisms is known since 1908, when Stormer observed the capacity of the bacteria *Bacillus hexabovorum* to grow aerobically in a medium containing toluene and xylene. The ability of natural microorganisms in the soil in BTEX degradation was first demonstrated by Gray and Thornton in 1928 (Mazzeo et al., 2010) . The degradation of BTEX compounds has been extensively studied by many researchers (Mathur et al., 2007; Shim et al., 2002; You et al., 2018) in order to identify the substrates degradation. The main reasons of the interaction effects (synergistic and/or antagonistic) during the BTX components degradation can be attributed to the competitive inhibition (Bielefeldt & Stensel, 1999), toxicity and formation of toxic by-products (Mazzeo et al., 2010), and enzyme induction (Deeb et al., 2001). Dou et al., (2008) studied the interactions in the anaerobic degradation of BTEX compounds. The authors observed that low concentrations of m-xylene increase the degradation of benzene, but at high concentrations of m-xylene the same was not observed. The toluene addition stimulates the degradation of o-xylene, while ethylbenzene addition inhibited the degradation of o-xylene. When the concentration of BTEX mixtures was higher than 150 mg/L, the degradation of benzene, o-xylene, m-xylene, and p-xylene was inhibited.

Alvarez & Vogel, (1991) measured the degradation for individually, binary and ternary mixtures of benzene, toluene, and para-xylene by two pure cultures of bacteria (*Pseudomonas* sp. CFS-215 and *Arthrobacter* sp. HCB), and the mixture of the cultures. The authors observed cases of non-interaction, competitive inhibition, and co-metabolism. The toluene degradation was faster when individually compared with binary and ternary mixtures, suggesting the same metabolic way and thus competitive inhibition. On the other hand, Arthrobacter was unable to degrade toluene and para-xylene, individually and in mixtures. The degradation of these compounds occurred only in the presence of benzene, suggesting that co-metabolism was responsible for the beneficial effect.

Otenio et al., (2005) evaluated the degradation of BTEX compounds by *Pseudomonas putida* CCMI 852 and reported that the culture was able to metabolize toluene and p-xylene, but not benzene. The toluene was degraded at a rate twice higher than p-xylene. Jo et al., (2008) evaluated the synergistic and antagonistic effects during biodegradation of the BTEX compounds using a mixed culture of microorganisms, and showed that by increasing the xylene concentration was possible to increase the BTEX removal. Some species were responsible to produced power density such as *Hydrogenophaga* sp., *Chryseobacterium* sp., Geobacter sp. *Pseudomonas* sp. *Alicycliphilus sp.* and *Rhodococcus* sp. Another study, You et al., (2020), used exoelectrogens *Shewanella oneidensis* MR-1 in the MFC from external side to biodegradation of xylene. The o-xylene biodegradation efficiency was increased from 54% to 73% at a concentration of 300 g/m3, and the maximum power density 52.1 to 92.5 mW/m^3 was after adding the *Shewanella oneidensis* MR-1. Therefore, the power density was 1.3 times higher than without adding *Shewanella oneidensis* MR-1. Other species involved *Chryseobacterium* sp., *Hydrogenophaga* sp., and *Sedimentibacte*r sp. to degrade the xylene.

2.4.3 Renewable sources of energy

Resources that can be repeatedly replaced in nature and do not run out with time are known as renewable resources and energy produced from these resources is known as renewable energy or clean energy. Solar, wind, tidal, hydro and geothermal energy have been successfully employed to harvest renewable energy around the world. Recently, bioenergy has garnered significant attention as a potential renewable energy source. Bioenergy is the energy derived from biomass which ranges from food waste and plants to wastewater (Rittmann, 2008). In recent years, direct derivation of renewable energy from the waste has become a potential option and bio electrochemical systems have played a significant role in extraction of usable energy from waste. Different bio-electrochemical systems have been developed over the years with microbial fuel cell (MFC) being the most explored technology. Figure 2.2

presents the flow chart of the various techniques available for generating renewable energy.

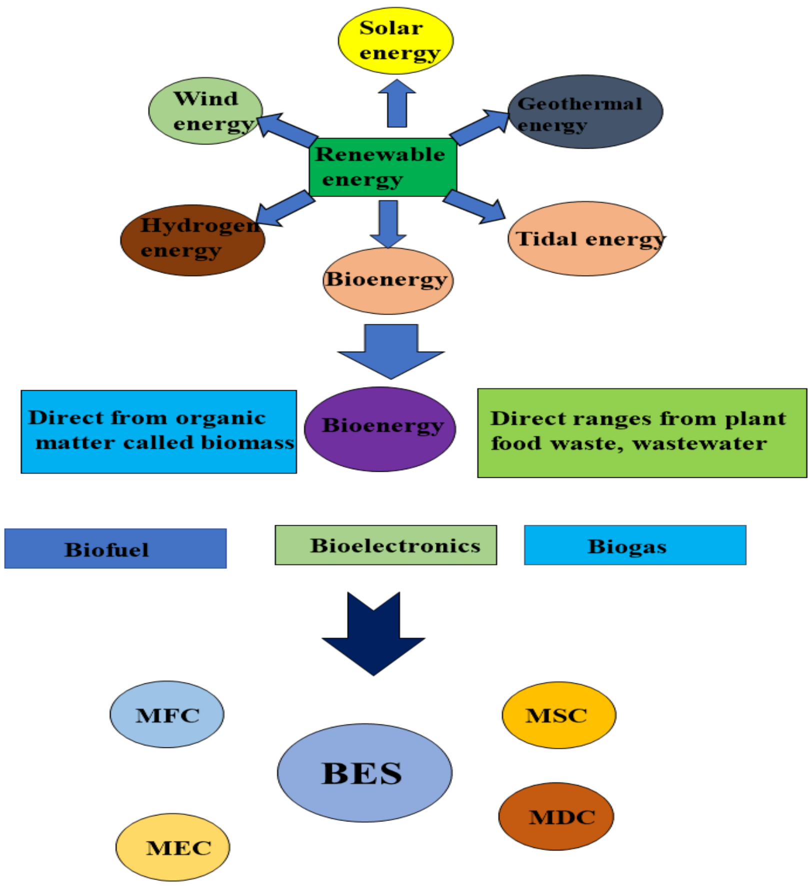

Figure 2.2: Flow diagram showing different sources of renewable energy with special reference to bioenergy.

The need and interest in finding the sustainable alternatives to the non-renewable sources of energy which are cost effective and performance efficient has brought the focus of the scientists across the world to MFC technology. MFC is a technology capable of harvesting energy from organic and inorganic chemical wastes in the form of electricity. The technology has garnered significant attention because of its capability of harvesting energy from waste with almost zero energy input

(Kumar et al., 2018). MFC can be defined as the technology that utilises active microbial population to catalyse the oxidation of organic and inorganic waste to harvest energy in the form of bioelectricity. It acts as a connecting link between microbial metabolism and electrochemistry of the system (Kumar et al., 2018). The first idea of harvesting electric energy with the aid of microorganisms dates back to the early 20th century when Potter and co-workers in 1911 suggested that living cultures like *E.coli* and *Sacchromyces* can assist in the production of electricity (Potter, 1911). However, the work did not gain much attention until the 1931 work by Cohen, who connected MFCs in series producing voltage larger than 35 V. Further, NASA in 1960s researched the applications of MFCs in space missions (Santoro,et al., 2017). Later, in 1980s, Allen and Bennetto discovered that the performance of MFC can be greatly improved by using mediators to support electron conduction from microbes to the electrodes (Chaturvedi & Verma, 2016). However, the instability and toxicity of the mediators offered obstructions in their practical application. A major breakthrough came when Kim et al., (2017) showed that the electricity conduction did not require mediators and some microbial communities can transfer electrons to the electrodes directly using microbial metabolites. Bruce Logan and team are considered the firsts to develop a laboratory-scale MFC (Ghasemi et al., 2013; Rinaldi et al., 2008).

The basic principle of MFC is based on the redox reaction taking place within the system where the oxidation taking place at the anode generates electrons and protons lowering the redox potential at the anode. The electrons produced travel through the external circuit while the protons migrate across the membrane to the cathode where they are accepted by the terminal electron acceptor at higher redox potential. The flow of electron across the circuit as a result of developed potential