SIMULTANEOUS WASTEWATER TREATMENT AND BIOELECTRICITY GENERATION WITH CONSTRUCTED WETLAND INCORPORATING MICROBIAL FUEL CELL

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A project report submitted in partial fulfilment of the requirements for the award of the degree of Bachelor of Civil Engineering (Environmental) with Honours

Faculty of Engineering and Green Technology
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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

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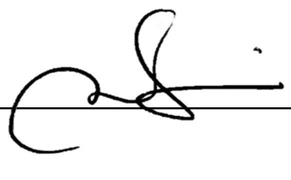
Signature : 

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Specially dedicated to
my beloved parents

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ABSTRACT

Constructed wetland (CW) and microbial fuel cell (MFC) complement each other well due to the similar configuration in both systems. Therefore, incorporation of CW and MFC had emerged in years ago, which is known as CW-MFC. In contrast to the standalone systems, the integrated system had a higher wastewater treatment (WWT) efficiency with simultaneous bioelectricity generation. In this context, the open circuit and closed circuit CW-MFCs were developed to compare the effectiveness of CW-MFC and CW in this study. The performance of CW-MFC varied with the configuration and operating condition that affect the complex mechanisms in the system. This study aims to optimise the CW-MFC system for optimum removal efficiency of chemical oxygen demand (COD) and power generation. A few aspects that were considered in this study include the electrode material, the hydraulic retention time (HRT), and the external resistance. The respective effect was analysed. Waste materials, namely copper wire and iron strip were employed in this study. The CW-MFC with the former electrode material had a higher treatment efficiency. The effect of HRT was then investigated by varying from 1 d to 7 d. Subsequently, the optimal external resistance was determined among the resistors of 110 Ω, 560 Ω, and 1000 Ω. The findings show that the copper CW-MFC achieved the most outstanding performance on day 7 when it was externally connected to the smallest resistance. By applying the optimum operating condition, the system removed an average of 85.1 % COD from the municipal wastewater and generate a power density of 40.3 mW/m^2. The total cost of this study was RM 200.80. This highlights that utilisation of waste material as the electrode form a cost-effective

and more sustainable CW-MFC system for treating the wastewater and producing the bioelectricity concurrently.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS / ABBREVIATIONS

I	current density, mA/m^2
R_{ex}	external resistance, Ω
C_o	initial concentration of COD, mg/L
C_e	equilibrium concentration of COD, mg/L
P	power density, mW/m^2
T	temperature, °C
V	voltage, mV
AC	activated carbon
AS	activated sludge
B	biochar
BOD	biochemical oxygen demand
BOD_5	five-day biochemical oxygen demand, mg/L
$CaCl_2 \cdot 2H_2O$	calcium chloride dihydrate
CFB	carbon fibre brush
CFF	carbon fibre felt
COD	chemical oxygen demand, mg/L
CH_3COONa	sodium acetate
C_6H_5COONa	sodium benzoate
CW	constructed wetland
CW-MFC	constructed wetland integrated microbial fuel cell
DO	dissolved oxygen
DET	direct electron transfer
EAB	electroactive bacteria, electrochemically active bacteria
EPS	extracellular polymeric
FESEM	field emission scanning electron microscopy
FN	foamed nickel

GAC	granular activated carbon
GG	graphite granule
GF	graphite felt
GP	graphite plate
HRT	hydraulic retention time, day
IET	indirect electron transfer
IWK	Indah Water Konsortium
KH_2PO_4	potassium dihydrogen phosphate
MFC	microbial fuel cell
$MgCl_2 \cdot 6H_2O$	magnesium chloride hexahydrate
NaCl	sodium chloride
NH_4NO_3	ammonium nitrate
ORP	oxidation-reduction potential
PEM	proton exchange membrane
PVC	polyvinyl chloride
Pt	platinum
SSM	stainless steel mesh
TC	titanium cylinder
TM	titanium mesh
TSS	total suspended solids
UASB	up-flow anaerobic sludge blanket
UF	ultrafiltration
USEPA	United States Environmental Protection Agency
WWT	wastewater treatment
WWTP	wastewater treatment plant

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background

Economic development exerts a great impact on the environment. The best evidence is the pollution level before and after the coronavirus pandemic. During the pandemic, the lockdown in Malaysia that restricts the human activities had reduced the pollution levels. Particularly, several rivers were clearer than before the lockdown, including Kim Kim River, Gombak River, and Klang River (Lee Goi, 2020). Wastewater is the untreated or poorly treated effluent that is discharged into the water bodies. It may be present in the form of runoff, rainwater, infiltration, or outflow. The sources of water pollutants govern the types of wastewaters, notably domestic or municipal, agricultural, and industrial wastewaters (Shah and Rodriguez-Couto, 2021a). The concentration of organic contents-- fats, oils, and greases, biological oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids (TSS) correlates to the three classes of wastewater strength, from low to high (specifically from commercial sector) (Amador and Loomis, 2020).

Municipal wastewater is effluent discharged from various sources, especially from households (Dehghani, Karri and Roy, 2021). The domestic wastewater includes greywater and black water from households, commercial buildings, and institutions. Greywater from laundry, kitchen, and bathroom has a great amount of chemicals, fibres, and oils. On the contrary, black water is discharged from toilet use and consists of various organic materials such as faeces, bacteria, hormones, and ammonia (Shah and Rodriguez-Couto, 2021b; Kumar and Kumar, 2022). The amount and composition of the wastewater vary with climate, socio-economic aspects, technology, and so forth. Typically, urine produces primarily the nutrients load in domestic wastewater; effluent from kitchen contains organic matter in majority with a portion of chemicals; different types of chemicals can be found in the effluent discharged from laundry and bathroom (Chen et al., 2020). Stormwater runoff mixes with municipal wastewater through combined sewer system. Otherwise, it is excluded from municipal wastewater (Pettygrove, 2018).

To ensure water safety, wastewater treatment (WWT) is an essential precaution that removes the contaminants from the effluents before being discharged into the nearby water bodies (Jerold et al., 2021). In the past, various physical, chemical, and biological approaches were developed and have been implemented. Each approach has its own advantages and drawbacks by considering the aspects of economical, efficiency, feasibility, and environmental (Crini and Lichtfouse, 2019). For instance, some of the chemical treatments demand a large volume of chemical agents and generate toxic secondary products (Muthu, 2021). Despite that, none of the approaches is capable to solely treat the wastewater due to the complex nature of wastewater. To achieve the preferred water quality, combination of approaches is a common practice which is economical (Crini and Lichtfouse, 2019). Regardless, the practice of WWT in developing countries remains deficient due to poor economy, infrastructure, and technical and institutional capacity (Chaubey, 2021).

Considering the increasing environmental crisis, sustainability plays an important role in the era of globalization. A sustainable design involves various aspects: economical, environmental friendliness, technical feasibility, and social acceptance. Constructed wetland (CW) and microbial fuel cell (MFC) are renowned sustainable technologies that emerged recently, integrating the environmental aspects. CW is a man-made system which encompasses biological, chemical, and physical processes, akin to a natural wetland (Kumar and Kumar, 2022). The main role of CW is to eliminate the pollutants from the effluent such as municipal wastewater. In Malaysia, CW has been designed and built for treating the stormwater runoff (Sim et al., 2008). CW is an economical wastewater treatment system operated at low energy input. The essential elements such as wetland plants, soils, and relevant microbial assembly are naturally available at the local. Apart from environmental protection via water remediation, CW also promote economic growth and social equity through low energy input and use of natural materials (Stefanakis, 2018; Dasgupta et al., 2019).

Similarly, MFC, a microbial electrochemical technology, also offers environmental protection in addition to recovery of energy, water, and invaluable metal (Gude, 2018). MFC produces renewable energy through the conversion of chemical energy in the organic substances present in the biodegradable organic-rich wastewater, supporting by the microbes (Kundu and Dutta, 2018; Khan et al., 2021). In other words, MFC can remove the pollutants in wastewater and produce renewable energy simultaneously. However, the treatment efficiency of MFC is low (Nawaz et al., 2022).

1.2 Problem Statements

About 80% of global municipal wastewater (including industrial wastewater) discharged into the environment is untreated (Programme, 2021). Untreated or inadequate treated water sources have detrimental effects on human and the environment. In municipal wastewater, there are millions of pathogens, including bacteria, protozoa, viruses, and helminths, present in sewerage (Dehghani, Karri and Roy, 2021); that cause fatal water-borne diseases: typhoid, cholera, and diarrhoea, especially in developing countries (Mishra et al., 2020; Shah and Rodriguez-Couto, 2021b). According to Mohd Uzir Mahidin (2020), the number of water-borne diseases and food increased by 11.8% from 15,346 in 2015 to 17,157 in 2019. Exceedance of nutrients (specifically phosphorus and nitrogen) in natural water can lead to eutrophication and cause impairment of water quality. Algal bloom releases various toxins that threaten aquatic ecosystem and human health (Yan and Guo, 2017). Moreover, organic content of municipal wastewater, made up of 40-60% proteins (amino acids), 25-50% carbohydrates (sugars, starches, and cellulose), and the remaining which are lipid (fats, oils, and grease), are the food of microbes. With the additional supply of organic matter, dissolved oxygen in natural water gradually depleted, initiating anaerobic conditions. As a result, aquatic organisms are dying. Suspended solids that deposit as a sludge or form a scum layer on water surface are inert materials (rags, silt, and paper). Apart from that, endocrine-disrupting compounds principally from hospital may trigger feminization of fish and indirectly affect human health (Gupta and Bux, 2019; Riffat and Husnain, 2022).

The pollutant degradation rate of CW is relatively low due to the prominent anaerobic regions. This has limited the number of electron acceptors viable for oxidation of organic substrate. As a result, the requirement of large land area (3-10 m^2 per person) is necessary. On the contrary, the power generation of MFC is considerably low (Srivastava et al., 2019). The electrode material such as platinum is expensive as well (Gude, 2018). Hence, integration of CW with MFC, which forms CW-MFC system, may be an alternative solution to those problems owing to their similarity; that is oxidation and reduction processes occurred in the anaerobic and aerobic regions, respectively (Sengar, Chaudhary and Bhadauriya, 2022). Such system can improve the performance of CW and increase the power generation of MFC, creating synergistic effect. Nonetheless, the interactions between the biotic and abiotic components of CW-MFC influence the effectiveness of the system. Optimisation of the factors affecting CW-MFC is thereby crucial.

Apart from wastewater treatment, solid waste management is also significant aspect that influences the quality of living environment. In Malaysia, about 1.17 kg of municipal solid waste was generated daily per capita, contributing to a total waste of 30 thousand tonnes each day. The amount of solid waste inclines with the growth of population and the economic development in addition to the higher living standards. However, most of the waste generated was disposed at the landfill. In other words, the practices of reuse and recycling are poorly implemented in the country. At this rate, all the landfills will reach their maximum capacity and being closed for the waste disposal (Malaysian Investment Development Authority, 2021; International Trade Administration, 2022; Nuradzimmah and Nor Ain, 2023). At present, the study of CW-MFC with the utilisation of waste material is rare.

In line with the problems above, this study incorporates waste material as the electrode pair in CW-MFC to further enhance the values of cost-effectiveness and sustainability of the system and promote wate recovery. The performance of this novel system is thus studied in terms of COD removal and power generation. Furthermore, the effects of several parameters on the performance of CW-MFC incorporated with the waste material are investigated, including electrode material, hydraulic retention time (HRT), and external resistance. Optimisation of the system's parameters is conducted to maximize its performance.

1.3 Aims and Objectives

The objectives of the thesis are shown as following:

i) To incorporate waste material as the electrodes in the CW-MFC for municipal wastewater treatment and electricity generation concurrently.
ii) To study the effects of electrode material, HRT, and external resistance on the performance of CW-MFC in respect of COD removal and power generation.
iii) To optimize the COD removal and power generation of CW-MFC through the parameters such as electrode material, HRT, and external resistance.

1.4 Scope of Study

In this study, a CW-MFC using the alternative electrode materials will be developed to remediate the municipal wastewater and generate the electricity simultaneously. Prior to the operation, characterization of microbes present in the inoculum sample and development of biofilm will be carried out. Subsequently, a preliminary assessment of the circuit connection will be conducted before the parameter studies or optimisation procedure of CW-MFC. The performance of CW-MFC system will be assessed in terms of COD removal and power generation based on various parameters so called CW-MFC configurations such as electrode material, HRT, and external resistance. The ranges of the parameters for this research will be selected based on previous works done by the researchers. At the end of the experiment, the peak performance of CW-MFC will be determined through the investigation of the optimum operating operational conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 Characteristics of Municipal Wastewater

Municipal wastewater denotes effluent that is collected and conveyed through the sewers to a wastewater treatment plant (WWTP). Majority of the wastewater discharged from households and partially from institutions. It is also known as sewerage. If cracks occur on sewer pipe, groundwater will infiltrate into the wastewater (Chen et al., 2020). This implies that the composition of municipal wastewater is rather complex as shown in Table 2.1. It also reveals that the concentration of wastewater is dependent on the pollutant and the water contents. For instance, high water consumption or stormwater dilutes the wastewater, producing wastewater with low concentration. Domestic wastewater comprises greywater originated from kitchen, laundry, and bathing and black water from toilet use.

Table 2.1: General composition of raw municipal wastewater with partial industrial wastewater (in g/m^3) (Tyagi et al., 2021)

Parameter	Concentration Range (mg/L)
Chemical Oxygen Demand (COD)	200 – 1200
Suspended COD	300 – 720
Biochemical Oxygen Demand (BOD)	230 – 560
Total Nitrogen	30 – 100
Ammonia Nitrogen	20 – 75
Total Phosphorus	6 – 25

Total Suspended Solids (TSS)	250 – 600
Volatile Suspended Solids (VSS)	200 – 480

Physical parameters include solids, turbidity, colour, odour as well as temperature. Water temperature affects solubility of oxygen, microbial activity, and processes like condensation and evaporation. Total solids in wastewater made up of 40% to 65% of insoluble TSS and the remaining are soluble solids. It is found that 60% of TSS are settleable, fixed solids while the rest are volatile (majority are organic) at a temperature of 600℃. Qualitative parameter of colour reveals age of wastewater. A typical raw wastewater appears in grey. Wastewater collected in the septic has black appearance (Theodore and Dupont, 2019). Extensive bacterial decomposition under anaerobic environment usually occurs in a dark grey or black wastewater. Similarly, development of sulphides such as ferrous sulphide also darkens the wastewater (Kyzas, 2015). Decomposition occurred in wastewater contributes to odour. Fresh wastewater generates a musty odour whereas odours of sewage and rotten egg are identical (Das, 2020).

In municipal wastewater, there are about 70% of organic and 30% of inorganic contents that are correlated to the chemical characterization (Spellman, 2020). Alkalinity is significant to buffer wastewater in biological treatment as it demonstrates the capability of wastewater to neutralize acids. The typical pH range of municipal wastewater is 6 to 9 (Shah, 2020). Monitoring of dissolved oxygen (DO) is vital for proper respiration. Extreme pH and DO can affect the water quality and harm the organisms (Malik and Marathe, 2021). For BOD, five days is essential to quantify biodegradable matter in effluent. On the contrary, measurement of oxidizable substance is termed as COD (Spellman, 2021). Excessive phosphorus accelerates algae growth and depletes DO, threatening aquatic organisms. Similar consequences arise for excessive nitrogen too (Lichtfouse, Muthu and Khadir, 2021). Other chemical characteristic is heavy metal which includes nickel, copper, chromium, cadmium, lead, and mercury (Dehghani, Karri and Roy, 2021).

Biological characteristics of municipal wastewater mainly focus on the microorganisms. The common microorganisms in the wastewater are bacteria, viruses, protozoa, algae, and helminths. This characteristic may benefit certain biological WWTs (Arni and Elwaheidi, 2020). They tend to break down the complex compounds contained in the wastewater, aiding by the enzymes. Among the wide variety of microorganisms, only a few kinds are dangerous as they can cause diseases. The classification of microbes into aerobic, anaerobic, and facultative depends on their respiration (Malik and Marathe, 2021). Therefore, the municipal wastewater should be treated at the WWTP before it is discharged into the environment.

2.2 Conventional Treatment of Municipal Wastewater

The conventional treatment of municipal wastewater at a WWTP is split into three stages: primary treatment, secondary treatment, and tertiary treatment. Initially, solids are being removed, followed by suspended and dissolved organic matters. Subsequently, the third stage ensures thorough removal of organic and inorganic substances (Das and Dash, 2021). For an effective and efficient removal of contaminants, a conventional wastewater treatment normally combines the physical, chemical, and biological methods. The nature and desired water quality are the primary factors affecting the type of WWT implemented.

2.2.1 Physical Treatment

In a WWTP, physical WWT would be implemented in the preliminary and primary stages. It is a significant process that prevents the failure of the equipment used for the following treatments. This treatment typically eradicates the solids and particulate matters such as detritus from the wastewater through physical process without modifying its chemical structure (Kapoor and Shah, 2022; Pirzadeh, 2022).

The well-known methods include sedimentation, floatation, filtration, stripping, ion exchange, adsorption, and so forth.

Sedimentation process is usually implemented at the primary stage of conventional WWT to remove the suspended solids. In a sedimentation tank where flow is very slow, most of the solids will settle at the bottom due to the effects of gravity. The deposited solids or sludge will be eradicated from the tank. In the meantime, the scum on the water surface will be skimmed off. This process is called floatation, which is often integrated with sedimentation process. Through these processes, approximately 80 % – 90 % suspended solids, 40 % BOD, and 55 % faecal coliforms may be removed from the wastewater (Theodore and Theodore, 2021).

In the recent years, application of advanced physical treatment such as membrane filtration technology in WWTP has been surging. Bonelli et al. (2020) claimed that ultrafiltration (UF) is an alternative technology of some secondary wastewater treatment such as coagulation, flocculation, and sedimentation. This process utilises the difference in pressure or concentration to separate the suspended solids and big particles by using a semipermeable membrane with a pore size of 0.005 – 0.100 μm. Through UF, effective removal of colloidal silica, enzymes, gelatins, iron, manganese, proteins, total organic carbon, salts, bacteria, viruses, and chlorine-resistant pathogens can be achieved (Verlicchi, 2020). Therefore, physical treatment of wastewater is important to ensure the performance of the equipment used in the subsequent treatment.

2.2.2 Biological Treatment

Application of biological WWT is extensive due to its flexibility and low costs. The capital and operating costs required are respectively 5 – 20 and 3 – 10 times lower than the chemical treatment. However, high retention time of wastewater is necessary (Bonelli et al., 2020). Biological wastewater treatment comprises a biological reactor where degradation of organic matter by microbes occurs and a secondary clarifier for settling of microbes. The microbes in this treatment are recyclable. It is important to distinguish between aerobic and anaerobic treatment. Both aerobes and anaerobes are active in a warm environment (Rodriguez-Couto, Shah and Biswas, 2021).

Aerobic wastewater treatment degrades the organic pollutants and inorganic nutrients like nitrogen and phosphorus in the presence of oxygen. This process produces the by-products so-called carbon dioxide and biomass. Air blower and compressor are mechanical aeration devices for supplying oxygen continuously. Various significant technologies under aerobic treatment are activated sludge (AS) method, fixed film bioreactor, moving bed biofilm reactor, oxidation pond, rotating biological contractor, root zone technology, and trickling filter (Chaubey, 2021). Rodriguez-Couto, Shah and Biswas (2021) claimed that this treatment is more suitable for low strength wastewater because of high generation of sludge and operating, although the rapid decomposition prevents regeneration of microbes. Hence, people are shifting to the use of anaerobic treatment.

In contrast to aerobic biological treatment, no requirement of oxygen in anaerobic biological treatment, leading to generation of methane or biogas (Chaubey, 2021). Ahamad, Siddiqui and Singh (2021) affirmed that this treatment can also treat high strength municipal wastewater at a high loading rate (about 10 to 20 times of AS method). In addition, the costs and production of sludge are lower compared to the aerobic treatment. The relevant technologies are anaerobic baffled reactor, anaerobic fluidized bed reactor, up-flow anaerobic sludge blanket (UASB) reactor and expanded granular sludge bed. For instance, UASB reactor may achieve 80.0 % removal of COD in maximum according to Gandhi and Shah (2021). Although biological treatment is less efficient than chemical treatment, it is less likely to generate the harmful by-products.

2.2.3 Chemical Treatment

The primary, secondary, tertiary stages of municipal wastewater treatment often involve the chemical treatment (Crini and Lichtfouse, 2019). This approach requires the addition of a specific chemical which depends on the process implemented. Common chemical methods are precipitation, chemical oxidation, and formation of an insoluble gas followed by stripping.

Guo et al. (2021) stated that chemical precipitation is favourable for treating wastewater containing high phosphorus concentration. In this method, addition of proper precipitant precipitates the phosphorus in the wastewater. The recovered phosphorus may be transformed into fertilizers. Caustic soda (or sodium hydroxide), soda ash (sodium carbonate), limes (hydrated lime and pebble lime), or magnesium hydroxide, sodium sulphides or sodium hydrosulphide may be used in chemical precipitation (Ahamad, Siddiqui and Singh, 2021).

For chemical oxidation, oxidizing agent is required to treat the wastewater. This process converts the organic and inorganic pollutants existed in the wastewater to less harmful products such as carbon dioxide and water (Karri, Gobinath and Dehghani, 2021). Potential oxidants are chlorine or sodium hypochlorite, hydrogen peroxide, ozone, and permanganate, whereas reductants to be considered are sodium bisulphite and metabisulphite (Ahamad, Siddiqui and Singh, 2021).

Disinfection is a process that uses chemicals to destroy specific pathogens such as bacteria and viruses in the wastewater. Gandhi and Shah (2021) opined that chlorination method is a way to disinfect municipal wastewater by adding bleaching powder or purging chlorine gas. Chlorine is a robust oxidizing agent and disinfectant though it is very corrosive and toxic in small amount. However, chlorination is only applicable for wastewater to be treated for human use because of chlorine residual, which is harmful to aquatic organisms but reduces contamination throughout distribution (Spellman, 2020).

2.3 Emerging and Sustainable Constructed Wetland Incorporating Microbial Fuel Cell

2.3.1 Constructed Wetland (CW)

Constructed wetland has been the most prevailing choice of decentralized WWT. CW is an engineered system integrated natural processes. Figure 2.1 outlines the types of CW according to plants and flow patterns. Deng, Liu and Wang (2020) emphasised on the key elements of biological process in CW system. Firstly, soils will adsorb soluble pollutants partly from the wastewater. Secondly, plants remove the nutrients, refractory organics, and heavy metals from wastewater via uptake; toxic excretions from the roots may destroy pathogens as well. Microbes eradicate biodegradable contaminants such as colloidal solids, BOD_5, nitrogen, and refractory organics. Additionally, CW also comprises physical and chemical processes (Bahadir and Haarstrick, 2022). Biological removal processes involved in CW are listed in Table 2.2. This synergy process thereby enhances the efficiency of WWT in short.

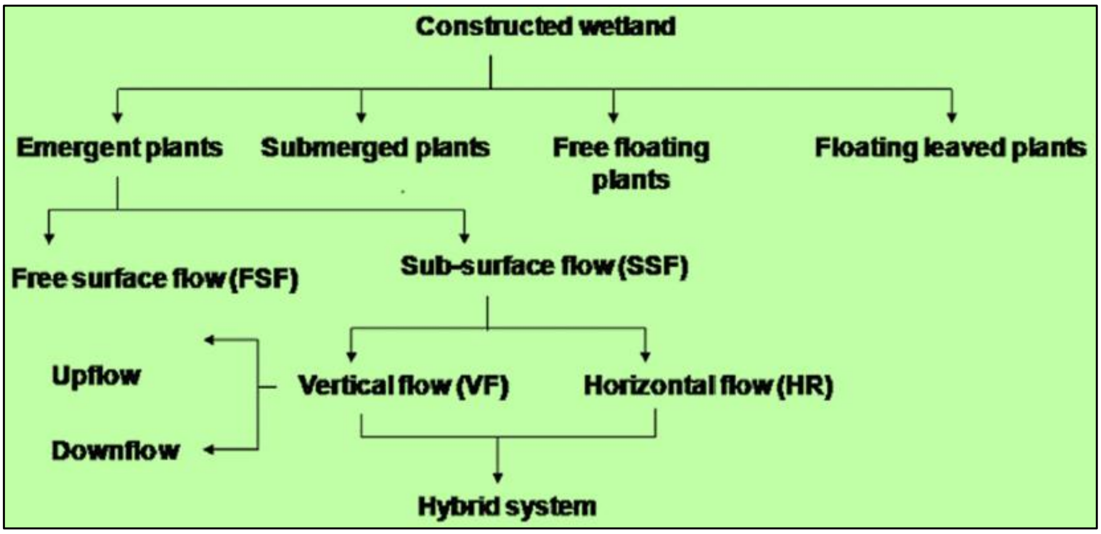

Figure 2.1: Classification of CWs based on macrophytes and flow regime (Srivastava et al., 2019)

Table 2.2: Biological mechanism of pollutants removal in constructed wetlands (Kumar, Saxena and Shah, 2020)

Parameters	Mechanism
Suspended solids	Biodegradation
BOD	Biodegradation
COD	Biodegradation, Phytodegradation, Phytovolatilization, Plant uptake
Nitrogenous	Biodenitrification, Nitrification, Plant uptake
Phosphorus	Microbial uptake, Plant uptake
Metals	Plant uptake
Pathogens	Natural death, Exposure to natural toxins bacteriophage attacks

Despite the benefits, constructed wetlands also have several downsides. Verma (2021) affirmed that construction of CW demands a large land area opposed to conventional wastewater treatment methods. This requirement has been a challenge in countries with high population density, for example, India. For economical purpose, CW must be constructed near the source of wastewater. However, this is normally unfeasible due to the land availability near the source. Issues like odour and groundwater contamination also may occur under certain circumstances. In case of subsurface flow CW, there is a necessity for mosquito control (Chakraborty, 2021).

2.3.2 Microbial Fuel Cell (MFC)

Since years ago, application of emerging technology called MFC has been surging globally in the field of WWT. A typical MFC is illustrated in Figure 2.2. The reason behind this context is the dual advantage offered by the technology, that is simultaneous bioelectricity generation and WWT. Besides, the costs required for construction and operation are relatively low (Kundu and Dutta, 2018).

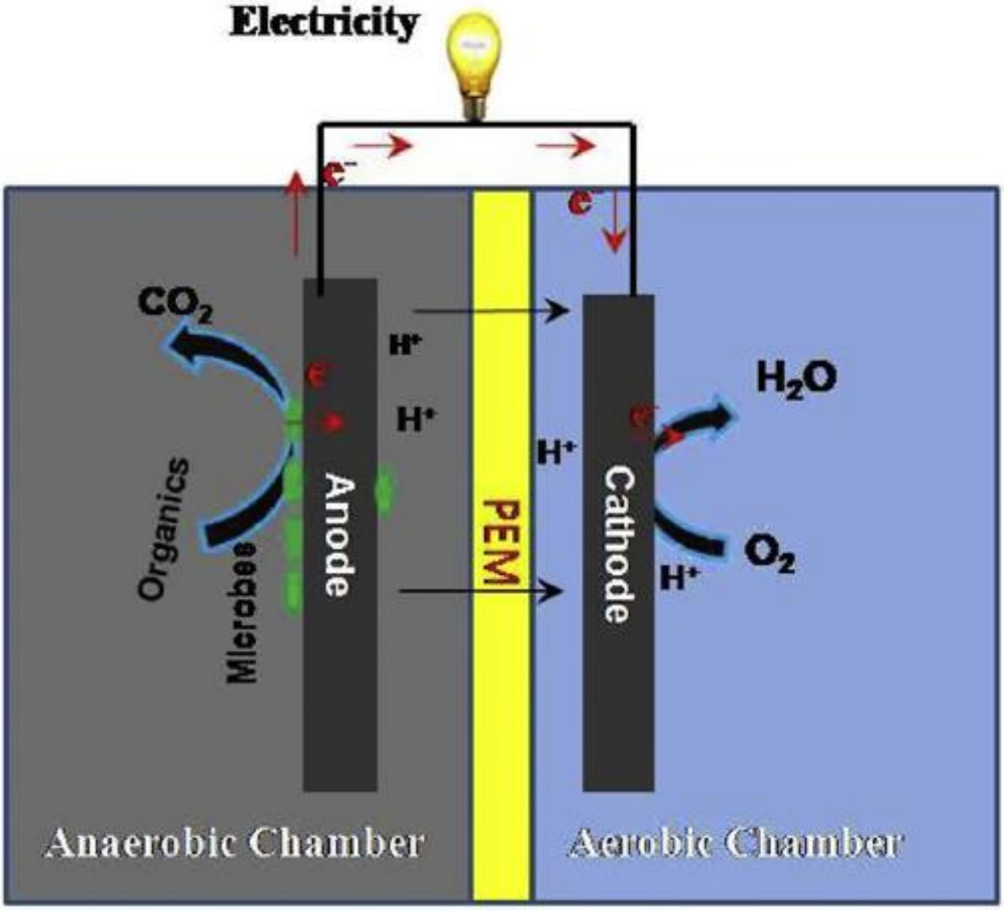

Figure 2.2: A typical setup of MFC (Srivastava et al., 2019)

A general MFC comprises two chambers: anodic and cathodic, facilitating aerobic and anaerobic conditions respectively; separated by a proton exchange membrane (PEM). Separator prevents oxygen but cations to diffuse in between the dual chamber. In anaerobic chamber, oxidation process occurs. The EAB will consume the fuel, specifically organic and inorganic materials, in the wastewater; thereby producing electrons and protons. The EAB may be found in sediments of ocean and freshwater, aerobic and anaerobic sludges, manures, or wastewater (Abbassi et al., 2020). Examples of EAB involved in MFC are *Geobacter suldurreducens*, *S. oneidensis*, and *Shewanella putrefaciens* (Gurunathan, Sahadevan and Zakaria, 2021).

The electrons generated via oxidation by EAB is then donated to the anode through either direct or indirect mechanisms. Direct physical contact between EAB and electron acceptors enables direct electron transfer (DET), either through a medium called conductive nanowires or outer-membrane redox protein with cytochrome cascade. Otherwise, indirect electron transfer (IET) occurs. In this case, electron mediator generated from bacterial metabolism or provided artificially will assist the extracellular electron transfer. Figure 2.3 depicts the mechanisms of DET and IET (Krishnaraj and Sani, 2019; Srivastava et al., 2019).

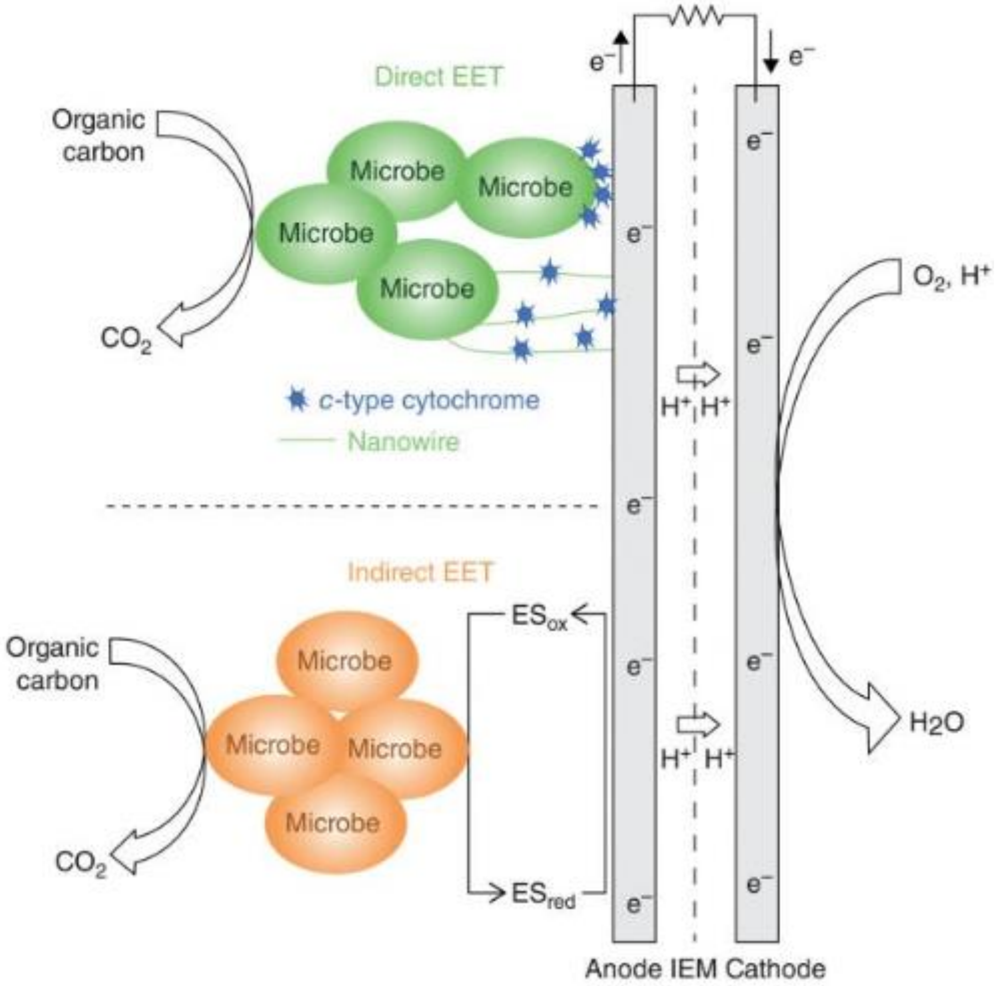

Figure 2.3: Direct and indirect electron transfer to the electrode by electroactive bacteria (Krishnaraj and Sani, 2019)

Presence of electron acceptor with greater oxidation-reduction potential (ORP) or redox potential than anode in the anaerobic chamber should be eliminated. Additionally, sufficient redox gradient between anode and cathode ensures electron transfer through the electrical circuit. Electrons will be attracted to the anode and subsequently transfer to the cathode through an external circuit (or wire). Electron acceptors at cathode with higher ORP, namely oxygen will receive electrons generated at anode. The flow of electron generates electric current. Meanwhile, protons will enter aerobic sector via PEM. Eq. (2.1) and Eq. (2.2) below represents the respective reaction at anode and cathode (Gupta and Nguyen, 2022).

Oxidation reaction: $$C_nH_{2n}O_n \rightarrow CO_2 + nH^+ + ne^- \quad (2.1)$$

Reduction reaction: $$O_2 + nH^+ + ne^- \rightarrow H_2O \quad (2.2)$$

However, the power density, power stability, energy conversion efficiencies of MFC are low and limited. In this context, various incorporations of MFC with other WWT methods have been investigated, notably plant-MFC, sediment or benthic microbial fuel cell. Plant-MFC applies the principle of rhizodeposition. There were expectations that power output could be enhanced by the natural occurring microbes in the rhizosphere. In contrast to plant-MFC, sediment-MFC involves conversion of biomass to electricity. In this system, excretion of oxygen from the roots of the plants will increase the efficiency of cathode.

2.3.3 Integration of Constructed Wetland and Microbial Fuel Cell (CW-MFC)

In general, integration of CW and MFC enables simultaneous WWT and generates bioelectricity with better performances than the individual systems. The synergy effects include enhanced removal of pollutants from wastewater and higher power density. Both CW and MFC are feasible in treating synthetic and real wastewaters. In addition, both aerobic and anaerobic conditions are vital for the biological treatment processes in the respective systems (Srivastava et al., 2019). Thus, the naturally occurring redox gradient in CW is complement with MFC. There will be sustainable amount of electron acceptor and donor. CW also nurture the microbes for MFC (Kumar, Saxena and Shah, 2020).

The collaboration of the plants, microbes, and rhizodeposition in CW-MFC are the key driving force of its wastewater treatment and energy production. In CW-

MFC, microbes are responsible for the pollutant degradation. A variety of microbes can be found in different compartments of the integrated system such as EAB in the anaerobic zone and aerobic microbes in the aerobic zone. The plants supply the dissolved oxygen for the microbes to degrade the pollutants in the wastewater. Apart from the wastewater, the root of plants also provides carbon resources through rhizodeposition that enhance the microbial activity (Saz et al., 2018; Oodally, Gulamhussein and Randall, 2019).

Figure 2.4 depicts the combination of CW and MFC. The anode and cathode are installed at anaerobic and aerobic (air-water interface) regions respectively in the chamber. The electron acceptor or anode increases metabolism and growth of microbes, enhances oxidation of biodegradable pollutants, and consequently produces more electrons. Anode accepts the electrons donated by microbes which then transfer to the cathode through the external circuit and initiates the electric current. Subsequently, reduction of oxidants such as oxygen occurs at cathode (Abbassi et al., 2020). Subsurface flow has been implemented in CW-MFC, either vertical (downflow and upflow) or horizontal.

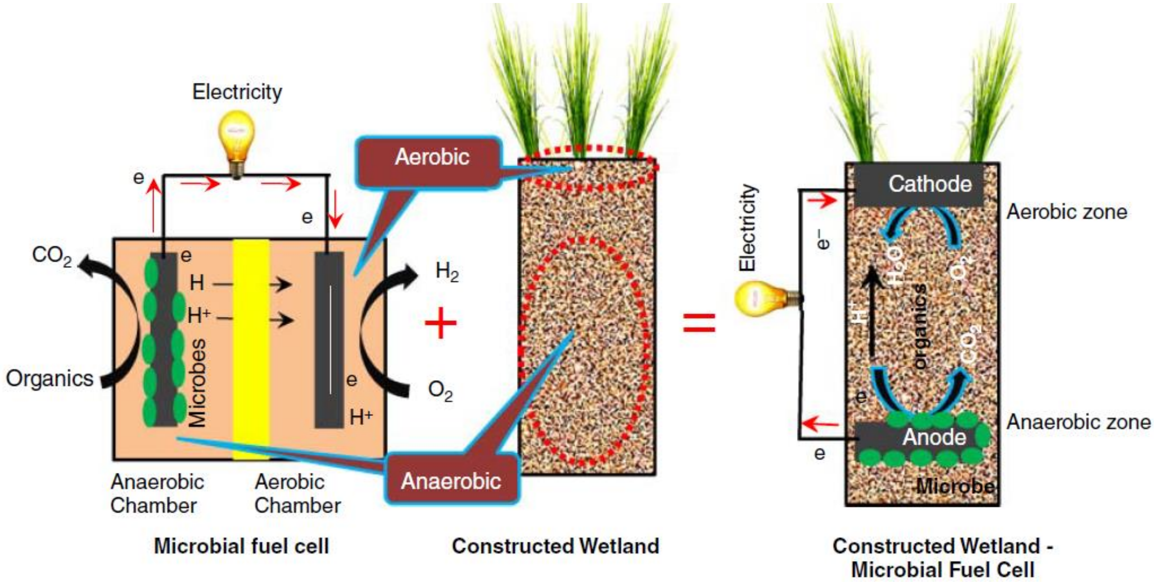

Figure 2.4: Incorporation of MFC and CW (Stefanakis, 2018)

Based on Table 2.3, the synthetic wastewater has been the focus of many studies. Meanwhile, the research on CW-MFC in treating real wastewater is limited, including swine and municipal wastewater. *Canna indica, Phragmites australis*, and *Ipomoea aquatica* (mostly in early studies) are renowned plant species used in CW-MFC. Those plants could be acquired easily and has remarkable features such as robustness and pollutant removal efficiency (Ebrahimi, Sivakumar and McLauchlan, 2021). Majority of the experiments were conducted in continuous mode whereby the wastewater was pumped vertically upward from the bottom of the camber. Energy is essential for pumping the wastewater. Furthermore, different electrode materials were proposed by the researchers which are mainly classified into carbon-based and metal-based electrodes. Composite electrode has been widely used in CW-MFC nowadays as it is more cost-effective. The expensive electrode materials such as platinum and stainless steel were incorporated too.

Notable indicators of CW-MFC performance are COD removal and bioelectricity generation. According to the studies, the COD removal efficiency of CW-MFC varied from 46.9 % to 86.3 % while the power density extended to 3741 mW/m^2. On the contrary, for real WWT, Oodally, Gulamhussein and Randall (2019) successfully achieved a maximum COD removal efficiency of 97.0 %, though the corresponding power density was relatively low. The performance variation reflected the significant impacts of design and operation conditions on the system. The factors influencing the performance of CW-MFC are categorized into physical (architecture, flow condition, hydraulic retention time (HRT), filtration media, and presence of separator), chemical (concerning wastewater properties), biological (plants and microbes), and electrochemical (associates to electrode and resistance) (Stefanakis, 2018; Ebrahimi, Sivakumar and McLauchlan, 2021). The selection of material and operational parameters for CW-MFC is thereby crucial.

Table 2.3: Recent studies of CW-MFC

Wastewater	Plant Species	Flow Regime	Anode/Cathode	HRT (day)	External Resistance (Ω)	COD Removal (%)	Power Density	References
Synthetic	*Phragmites australis*	Vertical	AC-TM/TM	3	1000	82.3	3741 mW/m^2	(Xu et al., 2018)
Synthetic	*Hydrilla verticillata*	Vertical	CFB	—	1000	64.0	12.4 mW/m^2	Shen et al. (2018)
Domestic	*Cyperus prolifer* *Phragmites australis*	Batch, vertical	GAC/Pt coated carbon	—	1000	97.0 94.0	229 mW/m^3 109 mW/m^3	(Oodally, Gulamhussein and Randall, 2019)
Domestic	*Canna indica*	Batch, vertical	GP	2	1000	70.2	86.5 mW/m^2	(Das et al., 2019)
Synthetic saline	*Phragmites australis*	Continuous, up-flow	TC-AS-B/TM	3	1000	68.2	16.4 mW/m^3	(Xu et al., 2019)
Swine	*Acorus calamus* *Canna indica* *Ipomoea aquatica*	Integrated vertical	SSM/CF	2	1000	88.1 80.2 84.7	181 mW/m^2 210 mW/m^2 248 mW/m^2	(Liu et al., 2020)
Synthetic	*Iris pseudacorus*	Vertical	GG-SSM	5 – 11	983	51.6	25.14	(Yang et al.,

							mW/m^2	2020)
	Phragmites australis					46.9	21.70 mW/m^2	
Synthetic (domestic)	*Canna indica*	Continuous, up-flow	GAC-SSM	3	1000	83.2 – 86.3	92.05 mW/m^3	(Wen et al., 2021)

Note: AC; activated carbon; AS: activated sludge; B: biochar; CFB: carbon fibre brush; GG: graphite granule; GF: graphite felt; GP: graphite plate; GAC: granular activated carbon; Pt: platinum; SSM; stainless steel mesh; TC: titanium cylinder; TM: titanium mesh.

2.4 Parameters of CW-MFC

A variety of parameters that are potential to influence performance of CW-MFC have been identified, including biological, chemical, and electrical parameters. The pollutant degradation and bioelectricity generation of CW-MFC depends greatly on the design parameters and operation condition.

2.4.1 Electrode Materials

To date, various modifications have been done to the electrode for enhancement of electrical conductivity and biocompatibility (Wang et al., 2020). The selection of electrode material should consider various aspects, including biocompatibility, corrosion resistance, cost, electrical conductivity, sustainability, and porosity in addition to the potential performance of CW-MFC. Metal and carbon are major electrode materials that have been employed in CW-MFC. Carbon-based electrode has greater specific surface area and biocompatibility than metal electrode that forms ideal attachment with EABs. Common carbon-based electrodes are graphite, carbon brush, carbon cloth, carbon felt, and carbon fibre (Ebrahimi, Sivakumar and McLauchlan, 2021; Huang et al., 2021).

Although carbon-based electrode has good conductivity, it tends to generate greater internal resistance than metal electrode. Metal electrodes are more competent than carbon-based electrodes in conducting the electricity; but they are mostly expensive and noxious to the microbial community (Gupta et al., 2021). For example, platinum which is potential in enhancing the reduction of oxygen in cathodic chamber. Stainless steel mesh becomes the metallic electrode that is extensively used in CW-MFC. Many studies have chosen carbon over metal as electrode material (Ebrahimi, Sivakumar and McLauchlan, 2021). In fact, there are no significant differences in the power generation when using metal or carbon-based electrodes (Guadarrama-Pérez et al., 2019). Later, researchers created composite electrode such as platinum-activated carbon electrode, with better conductivity, biochemical stability, and specific surface area (Huang et al., 2021).

Considering quality and cost, transformation of waste material to electrode may be an ideal approach for CW-MFC. Currently, there are only a few studies of electrode derived from waste material in MFC in lieu of CW-MFC. From previous research, conductive waste materials that were explored include natural waste, plastic, and industrial waste (Fonseca, Meng and Deng, 2015; Fang et al., 2018). More recently, electronic waste (e-waste) material was also utilised as a cost-effective current collector for supercapacitor, for instance, copper (Cu) from waste cable wires (Nagaraju, Sekhar and Yu, 2018). Such waste recycling for CW-MFC would benefit both the environment and economy. In the research of CW-MFC, it is considered as a novel approach as it can treats the real wastewater and produces the electricity at the meantime.

2.4.2 Hydraulic Retention Time (HRT)

HRT plays a vital role in the pollutant removal of CW-MFC. Wang et al. (2019a) found that HRT possess influences of over 45.0 % on TN removal and 50.0 % on removal of COD, TP, and NH_4^+-N. The longer the HRT, the longer the contact time between substrate and bacteria for adsorption and degradation of the pollutant. Likewise, at less HRT, heterotopic biofilm may form on cathode, hindering the mass transfer of electrons and protons (Shah, Rodriguez-Couto and Sengor, 2020).

On the contrary, long HRT may deteriorate the pollutants removal by intensifying the anaerobic condition that is unfavourable for the bacterial activities. For instance, Wang et al. (2019a) obtained a slight upward trend for removal of COD and nutrient when HRT exceeded 1.5 days. Ye et al. (2020) also studied the influence of HRT on COD removal and nutrients recovery by using a continuous flow mode CW-MFC. The HRT varying from 0.35 to 0.69 day produced insignificant improvement in COD removal above 92.0 %. However, the maximum power generation decreased; the lowest value of the maximum voltage outputs was 510 mV with HRT of 0.35 day.

2.4.3 External Resistance

The operational parameter, external resistance, has a significant operating parameter that influences the performance of CW-MFC. External resistance affects the availability of anode as electron acceptors and hinders the flow of electrons through the circuit. Variation of the resistance produces a unimodal distribution. With the increase in external resistance, the power density will raise continuously until the peak and then decreases. Due to greater anode potential at lower external resistance, bacteria can obtain more energy and consequently generate more electrons (Fang et al., 2018; Mohan et al., 2018).

By using domestic wastewater, Corbella and Puigagut (2018) tested a dual chamber CW-MFC with different external resistances under batch mode. The external resistance varied from 50 Ω to 1000 Ω. Among the external resistances, 220 Ω caused the highest COD removal. It was inferred that external resistance reduced the current output and organic removal rate. This shows that optimal external resistance is vital for achievement of the best performance of CW-MFC.

Among the parameters studied by Wang et al. (2019a), external resistance was the dominant factor of the bioenergy production by CW-MFC. Optimisation of external resistance contributed about 90.0 % of the total power generated. The optimal resistance that produced the maximum power density was 500 Ω. In contrast, maximum treatment efficiency of the wastewater was achieved at lower resistance which was 250 Ω. Based on Ohm’s law, current is inversely proportional to resistance (Das, 2017). The microbial activity reduced with the current affected when external resistance increased, leading to lower organic degradation. The rate of denitrification in anaerobic regime decreased due to less production of electron.

2.5 Summary of Literature Review

In this literature review, the characteristics of municipal wastewater were discussed beforehand in terms of physical, chemical, and biological. Various conventional treatment technologies for each category, namely physical, chemical, and biological, were then introduced with respect to their function and efficiency. Subsequently, emerging WWTs such as CW and MFC were presented, revolving around the structure, mechanism, pros, and cons. Also, the fundamental concept of CW incorporating MFC were explained, supporting with some case studies. In the following section, a few operating parameters with great influence on performance of CW-MFC in terms of WWT efficiency and bioenergy generation. Compared to synthetic wastewater, the studies on application of real wastewater in CW-MFC were indeed fewer. To the best of our knowledge, this study aims to study the performance of CW-MFC by using municipal wastewater. In addition, the novelty of this study will be the utilisation of waste material as the electrode pair.

CHAPTER 3

METHODOLOGY

In this chapter, the procedure and setup of the experiment were discussed. Figure 3.1 is a flowchart that summarizes the processes involved in this research project.

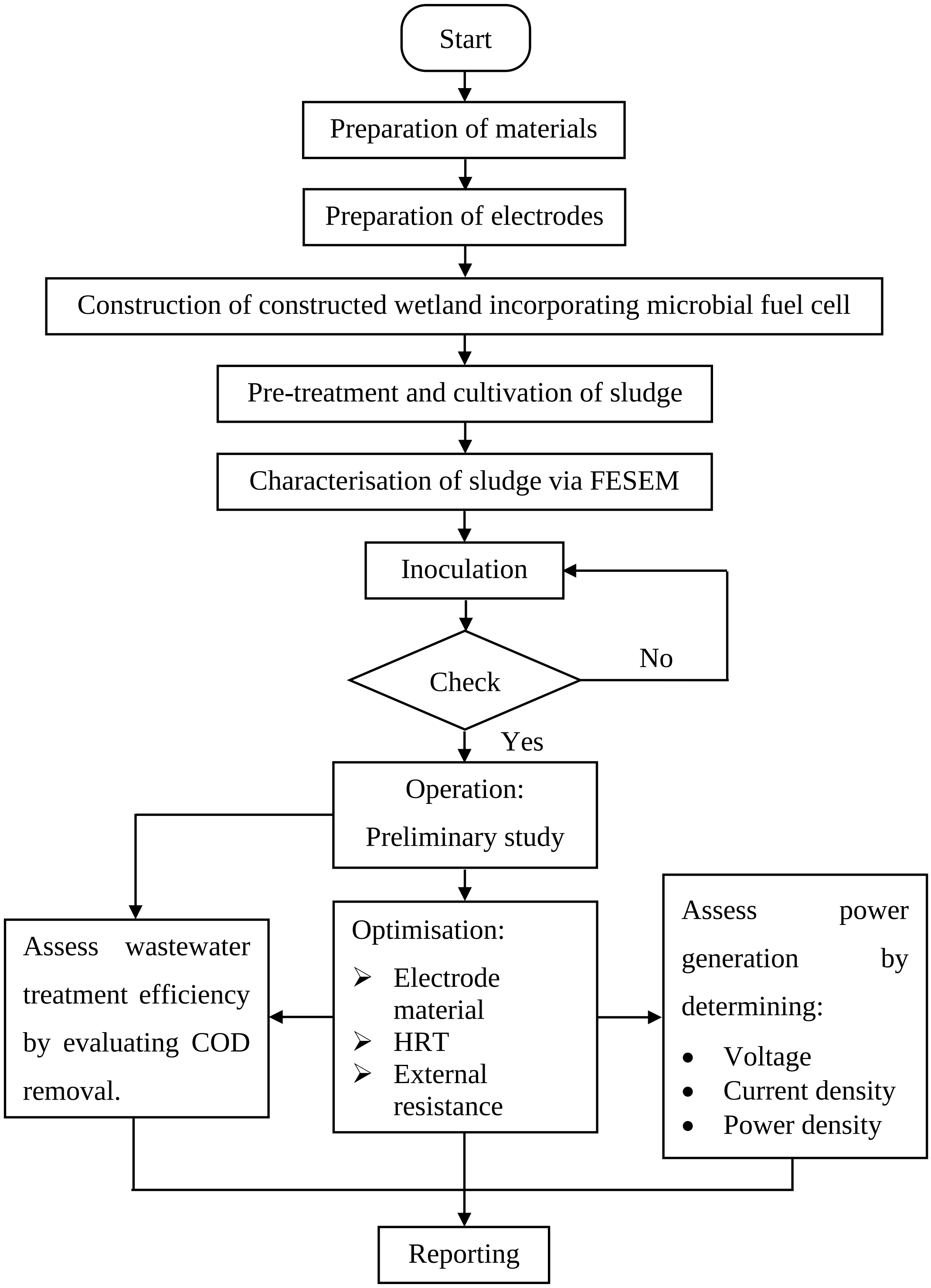

Figure 3.1: Flowchart of the experimental procedure

3.1 Chemicals and Materials

The materials that were required in this research project are listed in Table 3.1.

Table 3.1: List of chemicals and materials required

Chemical/Material	Purity (%)	Supplier	Function
Polyvinyl chloride (PVC) pipes	—	—	Used as a reactor.
Copper wire (waste material)	—	Green Bank	Used as anode and cathode.
Iron strip (waste material)	—	Engineering workshop	
Crocodile clip with wire	—	—	Used to connect the external circuit.
Resistor	—	Electronic engineering laboratory	Used to produce the external resistance.
Gravel	—	Acestoryaquatic	Used as filtration media.
Activated sludge (AS)	—	Indah Water Konsortium	Used as inoculum source.
Secondary effluent	—	(IWK) Sdn. Bhd.	Used to study the wastewater treatment efficiency of CW-MFC.
Chlorophytum comosum	—	The Green Pokok	Used as plant in CW.
Ethanol	70.0	Sigma-Aldrich	Used for electrode pretreatment.
Sodium benzoate (C_6H_5COONa)	> 99.0	Bio Basic Inc.	Used as carbon sources for

Sodium acetate (CH_3COONa)	99.0	Bendisen	inoculum.
Ammonium nitrate (NH_4NO_3)	99.9	Merck	Used to prepare the nutrient buffer
Calcium chloride dihydrate ($CaCl_2{\bullet}2H_2O$)	99.5	R&M Chemicals	required for inoculation.
Magnesium chloride hexahydrate ($MgCl_2{\bullet}6H_2O$)	99.5	R&M Chemicals	
Potassium dihydrogen phosphate (KH_2PO_4)	99.0	GENE Chemical	
Sodium chloride (NaCl)	99.0	EMSURE	
Deionized water	—	—	Used to prepare the blank.
Distilled water	—	—	Used for diluting and cleaning.
Low Range COD		HACH	Used for COD analysis.

3.2 Equipment

In this research, the equipment used were listed in Table 3.2.

Table 3.2: List of equipment.

Equipment	Manufacturer/ Model	Function
Multimeter	NJTY/ T-33	To measure voltage output.
Colorimeter	Hach (M) Sdn. Bhd./ DR890	For analysis of COD.
COD reactor	Hach (M) Sdn. Bhd./ DRB200	To heat up the COD vial.
FESEM	JEOL Ltd./ JSM-67011F	For morphological study.

3.3 Construction and Operation of CW-MFC System

In this study, laboratory scale CW-MFCs were developed to treat municipal wastewater and generate bioenergy simultaneously. The schematic diagram of the designed microcosm CW-MFC is illustrated in Figure 3.2. Four CW-MFCs were fabricated by using the identical PVC pipes with internal diameter of 11.0 cm and height of 60.0 cm. The outlet of the chamber was where the water tap attached to at the bottom of the pipe. Approximately 50.0 cm from the bottom of each pipe was filled with the lakeside gravel (3.00 mm – 5.00 mm) and planted with a *Chlorophytum comosum* with the soils being removed. The municipal wastewater was collected the sewerage treatment plant managed by IWK and would be discharged at the inlet at the top and collected from the outlet at the bottom. The systems were placed under the shade of the building to prevent direct exposure to sunlight intensity while stimulating the natural conditions.

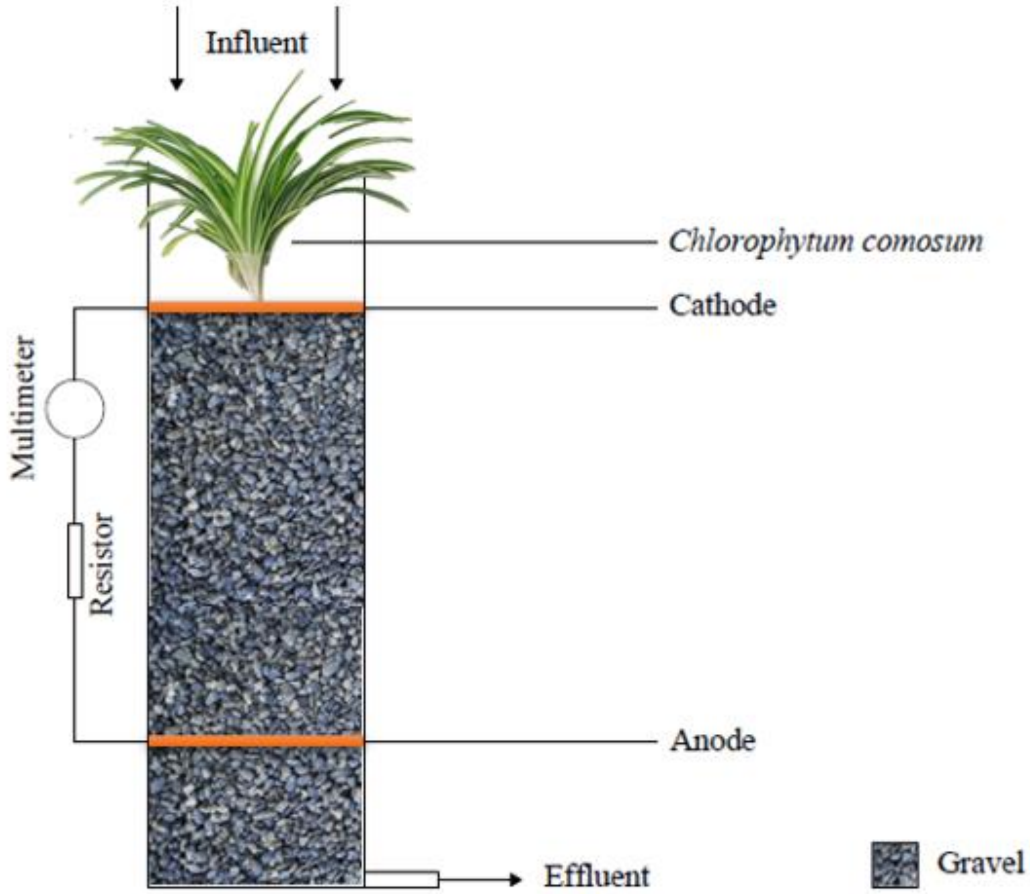

Figure 3.2: Schematic diagram of CW-MFC

Two types of electrode materials were prepared, namely iron and copper materials, which were collected from the engineering workshop and Green Bank respectively. The copper wire which was stripped from the electrical cable and iron strip was wound into spiral shapes as shown in Figure 3.3. For standardization purpose, both types of electrodes were fixed at 105 g. The CW-MFC system employed with the copper and iron electrode pairs would be termed as copper CW-MFC and iron CW-MFC, respectively. Anode was placed in the middle of the anodic regime, specifically 10.0 cm from the base; cathode was placed just below the surface of the gravels where abundant oxygen was available. The distance between the electrodes was about 40.0 cm. Before placement, the electrodes were pre-treated by soaking in 70.0 % ethanol to remove the impurities (Popovic et al., 2010; Costa et al., 2017).

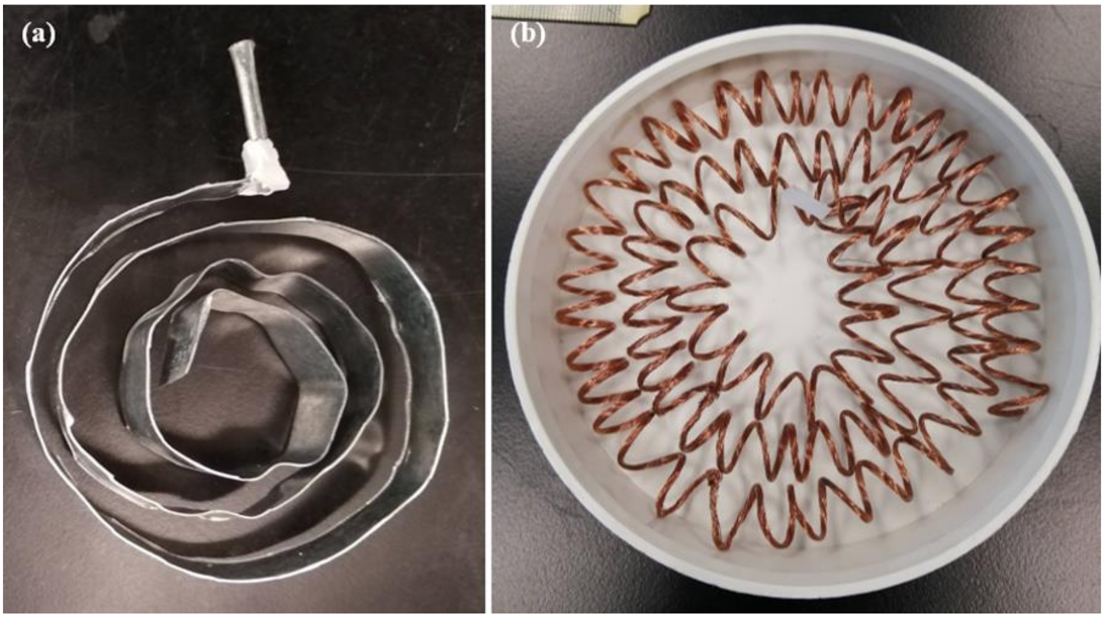

Figure 3.3: Electrodes made of (a) copper wire and (b) iron strip

Each microcosm was then inoculated with AS to create the biofilm. Prior to the start-up, the system was acclimatized for one month to promote the growth of microbes and stabilize the operation of the system (Oon et al., 2020; Yang et al., 2022). The systems were successfully started when the reproducible voltage and stable treatment efficiency were achieved (Yang et al., 2022). When the systems were stable, operation was conducted, without the energy input. In the preliminary study, the systems were operated in different circuit connection mode for comparing the performances between CW and CW-MFC. An open circuit CW-MFC acted as the control system in this research that simulated a CW system, whereby the anode and cathode were disconnected externally. The closed system, on the other hand, had it electrodes connected with the insulated copper wire through an electrical resistor, creating a closed circuit CW-MFC. To monitor the voltage output, a multimeter was connected to the external circuit of the system.

Table 3.3 shows the operational conditions for the parameter studies in this research. Initially, the experiment would be conducted to determine the most effective electrode material, followed by the HRT and external resistance. The effects of the varying parameters were investigated. Optimization of the CW-MFC system was done by implementing the best parameter in the subsequent study. Hence, at the final stage, the maximum attainable performance of the system was determined in terms of COD removal efficiency and power generation. Nevertheless, every CW-MFC system was operated in batch mode with an effective volume of 2.25 L.

Table 3.3: Operational parameters

Operational Parameters	Details		
Electrode material	copper		iron
HRT (d)	1	4	7
External resistance (Ω)	110	560	1000

3.4 Wastewater and Activated Sludge Collection

Fresh municipal wastewater was collected from IWK prior to secondary treatment. On the other hand, the AS was collected from the primary aeration tank. The collected samples would be stored at 4 ℃ for up to seven days. A black plastic bag was used to cover the wastewater sample in the bottle to minimize photodegradation (Scheer et al., 2020).

3.5 Inoculation

Activated sludge served as the inoculum for the CW-MFC. To obtain a mixed culture of aerobic and anaerobic microbes, anaerobic pre-treatment was done by storing the collected sludge in a sealed pipe as shown in Figure 3.4 (Gao et al., 2014). Nutrient medium was prepared which comprised C_6H_5COONa, CH_3COONa, NH_4NO_3, NaCl, $MgCl_2{\bullet}6H_2O$, $CaCl_2{\bullet}2H_2O$, and K_2HPO_4. Among the chemicals, sodium benzoate (C_6H_5COONa) and sodium acetate (CH_3COONa) were the predominant carbon sources that promoted the microbial growth (Oon et al., 2020). Refreshment of nutrient and the collection of the cultured sludge were carried out regularly.

Figure 3.4: Setup for anaerobic pre-treatment of AS

The filtration media and the electrodes were cleaned separately prior to the inoculation process. The enrichment and inoculation processes took about two months for completion in which the filtration media and electrodes were immersed in the cultivated sludge. The microorganisms would slowly acclimatize in the system.

3.6 Microbial Community Analysis

Field emission scanning electron microscopy (FESEM) was used to characterize the microbial community structure (Bhattacharya, Dev and Das, 2017). In more specific, FESEM determined the impacts of anaerobic pre-treatment and cultivation process on the inoculum—AS. The FESEM images depicted the structure and distribution of the microbes (Balasubramanian and Chowdhury, 2021).

In this study, the equipment of Model JEOL JSM-67011F was used. The samples of the sludges were collected and dried at 70 °C for two hours (Soonsorn et al., 2018). Prior to the FESEM analysis, the dried specimen was placed on the carbon tape and sputtered with a thin conductive layer of platinum. The variation of the microbial community was determined by comparing the surface morphologies of the fresh and pre-treated AS.

3.7 Analytical Methods

For water analysis, the sample was collected from the outflow of each system based on the HRT (Yakar et al., 2018). The physicochemical parameter, COD, was monitored to evaluate the treatment efficiency of the developed system. The assessments of the water quality parameters were based on the "Standard Methods for the Examination of the Water and Wastewater" (Baird et al., 2017) and standard methods approved by United States Environmental Protection Agency (USEPA).

To ensure the accuracy of the results, a sample size of two was obtained and analysed in this experiment. Thus, adequate sample volume was essential to assure a representative sample and enable replicate analysis besides waste minimisation. In this study, the average results would be calculated. In addition, the power generation of the system was monitored.

3.7.1 Chemical Oxygen Demand (COD)

COD test was performed based on Reactor Digestion Method (Method 8000) approved by USEPA. DO concentration in the water was measured by using a portable colorimeter (Model HACH DR890) in accordance with the standard operating procedure. The samples that contained solids were homogenized by shaking manually to obtain the representative samples. If the sample was not tested immediately, it would be preserved by adding the concentrated H_2SO_4 (approximately 2 mL/L) so that the pH was below 2. The preserved sample was then kept at 4 ℃ for 28 days in maximum.

The COD reactor (Model HACH DRB200) was preheated to 150 ℃ before operation. A clean pipet was used to add 2 mL of the sample to vial that was held at an angle of 45 º. Similarly, a blank sample was prepared by adding the deionised water. The cleaned vials were inverted gently several times and then put in the reactor. After heating for two hours, the vials were cooled down in the reactor to below 120 ℃, consuming approximately 20 minutes. While the vials were warm, each was inverted for a few times, followed by cooling to room temperature in a tube rack.

The colorimeter procedure was proceeded after the reactor digestion procedure. Prior to COD measurement, the spectrophotometer was calibrated by using the blank sample. The results were displayed in term of mg/L COD, indicating milligrams of oxygen that was consumed per litre of the sample under the analysing conditions. According to American Public Health Association, American Water Works Association and Water Environment Federation (2017), the reaction of oxidizable organic compounds reduced the dichromate ion (Cr^{6+}) to the green chromic ion (Cr^{3+}) during the digestion process. Both coloured ions possess different absorption level. The removal of COD by the CW-MFC was calculated using Eq. (3.1) where C_o and C_e are initial and equilibrium concentration of COD.

$$\text{Percentage removal (\%)} = \frac{(C_o - C_e)}{C_o} \times 100 \tag{3.1}$$

3.7.2 Power Measurement

Power generation of the system correlated to the voltage (*V*) drop in the developed CW-MFC. A digital handheld multimeter (Model NJTY T-33) was used to monitor the *V* output of the closed-circuit system with external resistance varying from 100 Ω to 1000 Ω. The current (*I)* and power (*P*) were calculated by using Eq. (3.3) and Eq. (3.4), respectively. To determine the current density (mA/m^2) and power density (mW/m^2), the calculated current and power were divided by the surface area of anode (Xu et al., 2018).

$$V = IR \tag{3.3}$$

$$P = VI \tag{3.4}$$

CHAPTER 4

RESULTS AND DISCUSSIONS

In this chapter, the experimental results of this research work were discussed. The first section analysed the characteristics of sludge applied in the CW-MFC systems. Subsequently, the preliminary study of the CW-MFC system was reviewed, specifically the treatment efficiencies of the CW-MFC and the control system. The following section assesses the factors affecting the operation of the systems, including electrode material, external resistance, and hydraulic retention time. The optimised was determined as well. Lastly, the costing analysis of this study was summarized.

4.1 Characterization of Sludge

The characteristics of AS employed in the CW-MFC systems were studied to elucidate the performances of the systems in terms of COD removal and power generation. Surface morphologies of the sludges were examined via FESEM analysis at the magnifications of 5000 × and 20 000 ×.

Figures 4.1 (a) and (b) show the morphology of the fresh AS. On the other hand, Figures 4.1 (c) and (d) present the morphology of the pre-treated AS, which was cultivated for about one month. Clusters of amorphous particles which were densely packed together, can be observed in the micrographs. The clusters might be identified as extracellular polymeric substances (EPS) matrix that provided protection to the embedded bacteria (Zeng et al., 2016). As shown in Figure 4.1 (a), the sludge contained cocci and rod-shaped microbes. The sizes of the particles varied between 0.2 μm and 3.5 μm in diameter, respectively. Weber et al. (2007) claimed that cocci tend to agglomerate, forming the clusters of cells. These outcomes were consistent with the research done by Zhao et al. (2010) and Zhi et al. (2019).

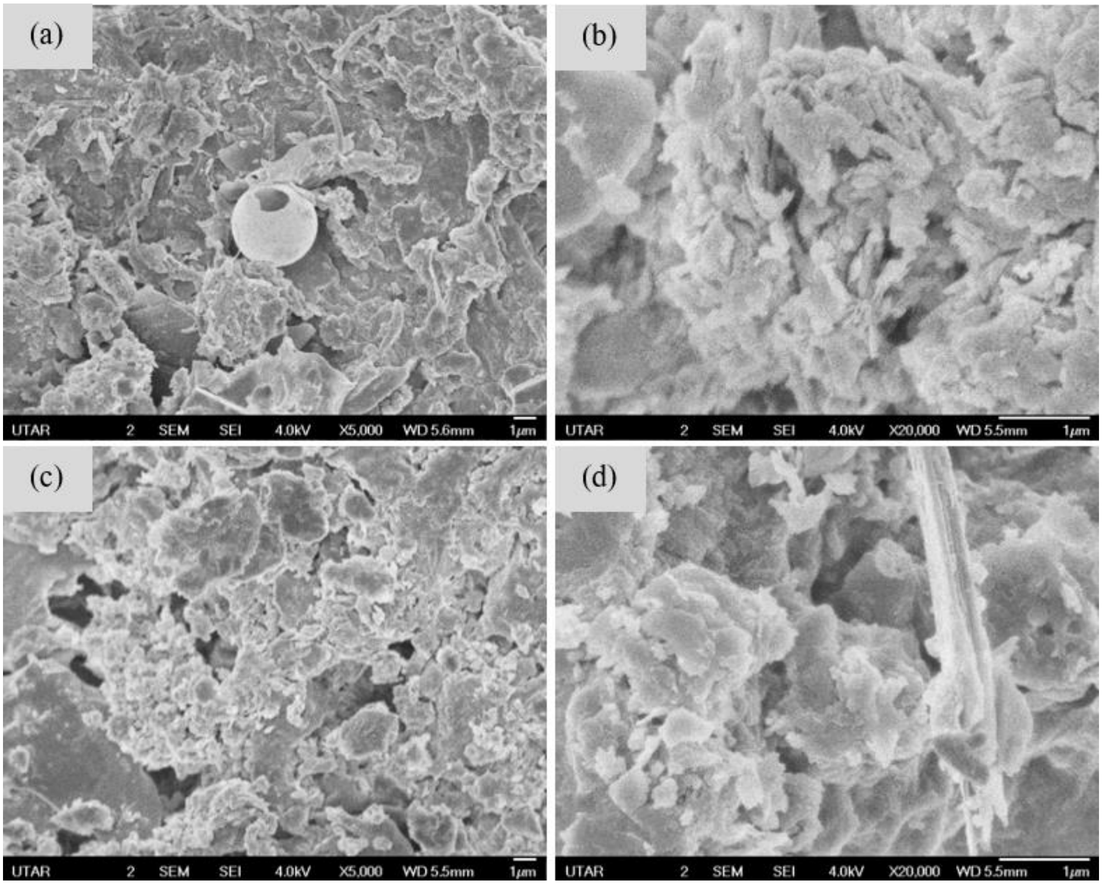

Figure 4.1: FESEM images of fresh AS (a and b) and pre-treated AS (c and d) at different magnifications of 5000 × and 20 000 ×

The sludge became more saturated after the anaerobic pre-treatment and cultivation as more clusters could be seemed in Figures 4.1 (c) and (d). Compared to the fresh sludge, the pre-treated sludge could have a more diverse microbial community, including the facultative bacteria and anaerobes (Cao et al., 2019). This finding emphasized the growth of EAB species in the sludge. Addition of the nutrients during the enrichment phase encouraged the growth of microbes. Therefore, the use of the mixed culture as inoculum drove the degradation of the complex pollutants in the wastewater. In addition, by using the inoculum with mixed microbial communities, a rapid increment of power density would occur in contrast to the pure culture (Gao et al., 2014; Singh and Mahapatra, 2019).

4.2 Preliminary Study of CW-MC

It had been discovered that the incorporation of MFC into CW could enhance the anaerobic oxidation of the pollutants (Srivastava et al., 2019). In light of this, investigation on the effectiveness of the CW-MFC and its control system was conducted. The closed system represented the CW-MFC whereas the open system acted as the closed system. The performances of both systems were compared by assessing the wastewater treatment efficiency, specifically the COD removal efficiency.

4.2.1 Removal of COD

The respective treatment efficiency of CW-MFC in closed circuit and open circuit (or control system) are shown in Figure 4.2. It was apparent from the figure that the performance of closed system was higher compared to the open system. The differences of the removal efficiencies between the open system and closed system were 8.20 % and 16.8 % for the iron electrodes and copper electrodes.

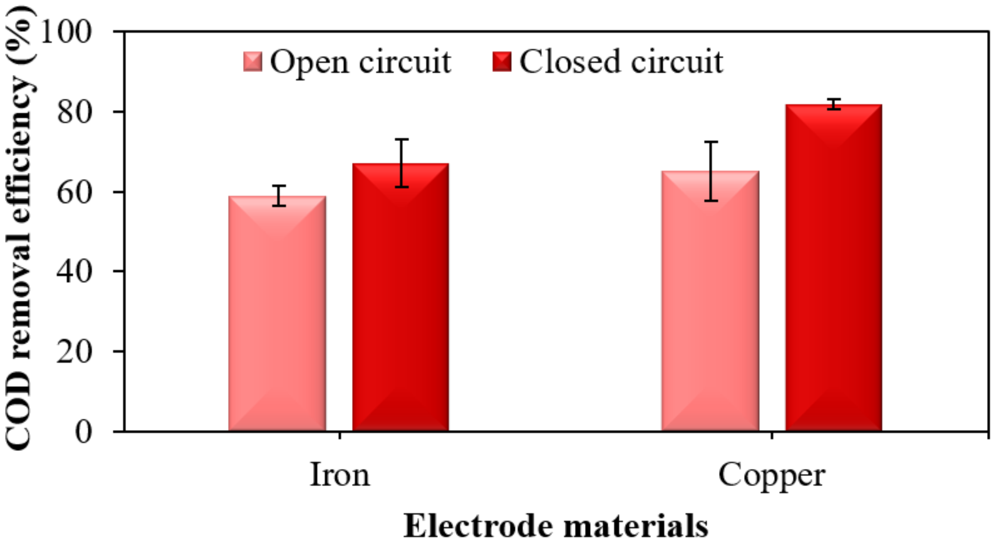

Figure 4.2: COD removal efficiency of copper CW-MFC and iron CW-MFC under closed circuit and open circuit connections

The better performance of the closed circuit CW-MFC was contributed by the electron transfer through the external circuit. The additional electron acceptor, anode, accelerated the anaerobic reaction (Srivastava, Yadav and Mishra, 2015). The increasing microbial electron transfer thereby promoted the growth of EAB and increased the consumption of organic matter (Noor, Sam and Kadir, 2022). On the contrary, in the open circuit CW-MFC, the electrons generated can only transfer through different media such as gravel and water. As a result, without the external circuit, only limited electrons were transferred from the anaerobic zone to aerobic zone in the open system (Srivastava, Yadav and Mishra, 2015; Srivastava et al., 2020). Therefore, a higher COD removal was reported for the closed system in lieu of the open system.

Similar investigations had been done previously. Research on the effect of the electron transfer from anaerobic zone to aerobic zone in the CW-MFC by using the synthetic textile wastewater, was studied by Fang et al. (2013). The treatment efficiency of the closed system was 12.7 % higher than the open system. A similar study was done by Srivastava, Yadav and Mishra (2015). They revealed the performance variation of the systems with different electrode materials that ranged from 12.0 % to 20.0 %. The differences of the removal rates between the open and closed systems in this study were consistent with other studies. Among the studies, the hybrid CW-MFCs developed by Srivastava et al. (2020) produced the slightest difference of 1.80 % between the open and closed systems. The variations highlight the significance of other factors such as HRT and electrode material which would be discussed in the following sections.

4.3 Effects of Various Operational Parameters

4.3.1 Effect of Electrode Material

The electrode material had a significant impact on the CW-MFC. In this study, two types of waste materials namely iron strip and copper wire, were chosen to create two pairs of electrodes: anode and cathode, for the respective system. It was apparent from Figure 4.3 that the copper CW-MFC outperformed the iron CW-MFC. With the respective initial COD of 35 mg/L and 37 mg/L, the corresponding COD removal efficiency of copper CW-MFC were 82.9 % and 81.1 %. On the other hand, the iron CW-MFC removed only 63.0 % and 71.4 % of the COD in the wastewater; with the initial COD concentrations of 46 mg/L and 56 mg/L. Based on the results obtained, it was evident that copper waste material was more capable than the iron waste material in performing as the electrode pair.

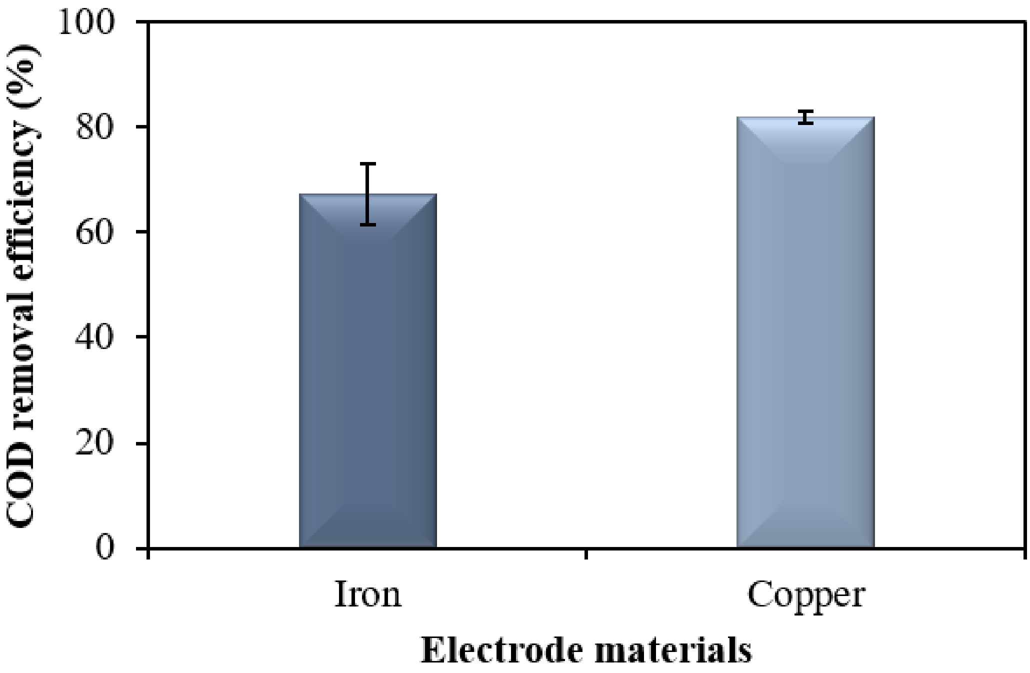

Figure 4.3: Effect of electrode material on the COD removal efficiency by close circuit CW-MFC

The performance variation of the CW-MFCs was mainly credited to the biocompatibility of the respective electrode material, specifically the ability of the material to host the microbes (Thomas, Balakrishnan and Sreekala, 2018). Most metals possessed the antimicrobial property whereby the growth of microorganisms was inhibited due to the toxicity of those metals, especially copper (Prager, 2018; Bhaskar et al., 2021; Kral, Aplin and Maier, 2021). However, since this research demonstrated the excellent performance by copper CW-MFC, this property might not apply to EAB. This statement was attested by Baudler et al. (2015) who observed the copper electrode in the MFC exhibited a great abundance of EAB, which could enhance the COD removal efficiency. On the contrary, the transition metals such as stainless steel performed poorer the noble metal. This could be associated to the poor removal efficiency of iron in this present study as iron was also a noble metal. This type of metal often generates an oxide layer that hindered the electron transfer from the microbes to the metal which served as the electron acceptor (Zhou, 2015).

Moreover, the copper electrode comprised multiple strands of copper wire, which might provide greater surface area than the solid plane iron. For instance, Wang et al. (2017) discovered that the electrodes: carbon fiber felt (CFF) and foamed nickel (FN) with larger surface areas were attached with greater population of EAB. The presence of abundant microbes would thereby accelerate the COD degradation in the copper CW-MFC (Rahimnejad, 2023). In this present study, thicker sludge was seen in the effluent collected from the iron CW-MFC compared to the copper CW-MFC after the inoculation process (Figure 4.4). This observation might indicate that the poor microbial attachment on the iron surface due to low biocompatibility or smaller surface area and was associated to the high removal efficiency of the copper CW-MFC. The copper CW-MFC was thereby chose to conduct the subsequent experiment for the maximum performance of CW-MFC.

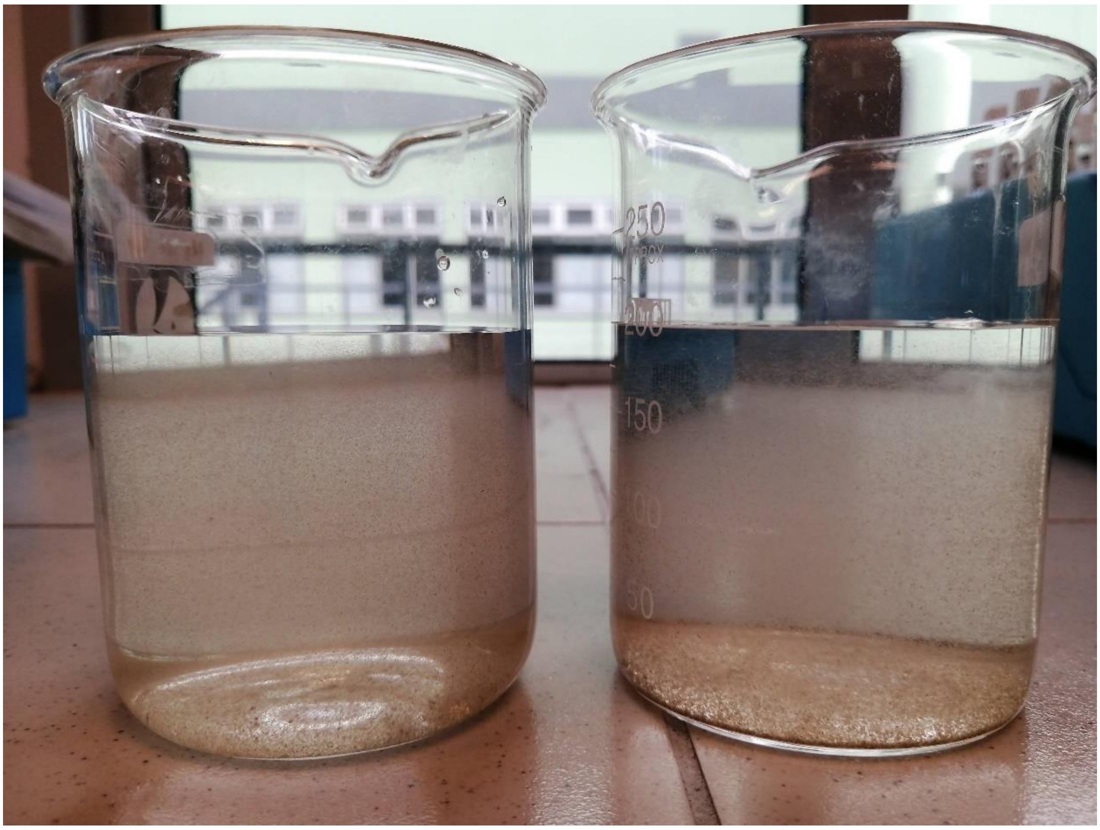

Figure 4.4: Denser sludge in the effluent collected from copper CW-MFC (left) compared to iron CW-MFC (right)

Apart from that, copper CW-MFC was more superior than iron CW-MFC in terms of power generation. According to Jaroszewski et al. (2018) and Tiwari et al. (2023), the electrical conductivity of copper and a pure iron were 58.0 MS/m and 10.0 MS/m, respectively. Hence, the higher conductivity of copper electrode increased the rate of electron transfer in the system, generating greater bioelectricity contrasted to the iron CW-MFC (Bolton and Higgins, 2020). Although no cost was incurred for both electrodes, copper was considered a better electrode material as it could be shaped easily and was relatively durable (Shah and Rodriguez-Couto, 2021a).

To date, there were limited findings on the performance of CW-MFC utilizing the metallic electrode, including the copper and iron waste materials. Previously, Wang et al. (2017) compared the treatment performances of several electrode materials, such as CFF, FN, SSM, and graphite rod. The COD removal efficiencies ranged from 35.7 % to 48.8 %, which were lower than using the copper and iron as the electrodes in this research. A comparable removal efficiency of above 80.0 % was achieved by using the granular activated charcoal or granular graphite as the electrodes in the CW-MFC (Srivastava, Yadav and Mishra, 2015). Nevertheless, in contrast to those electrode materials, the application of waste material in this study provided an exceptional benefit that was low cost.

4.3.2 Effect of HRT

The improvement on the wastewater treatment performance with the increasing HRT in the CW-MFC was attested with this study. In this study, the initial COD concentration of the wastewater was 35.0 mg/L. When the HRT changed from 1 d to 7 d, the concentration of the effluent reduced from 25 mg/L to 6 mg/L. An upward trend of the removal efficiency was thus extrapolated in Figure 4.5. A significant drop in the concentration was observed at HRT of 7 d. This revealed that the COD removal efficiency rose with the HRT. As a result, the peak efficiency yielded by the copper CW-MFC was 82.9 % for the HRT of 7 d, followed by 65.7 % for 4 d and 28.6 % for 1 d.

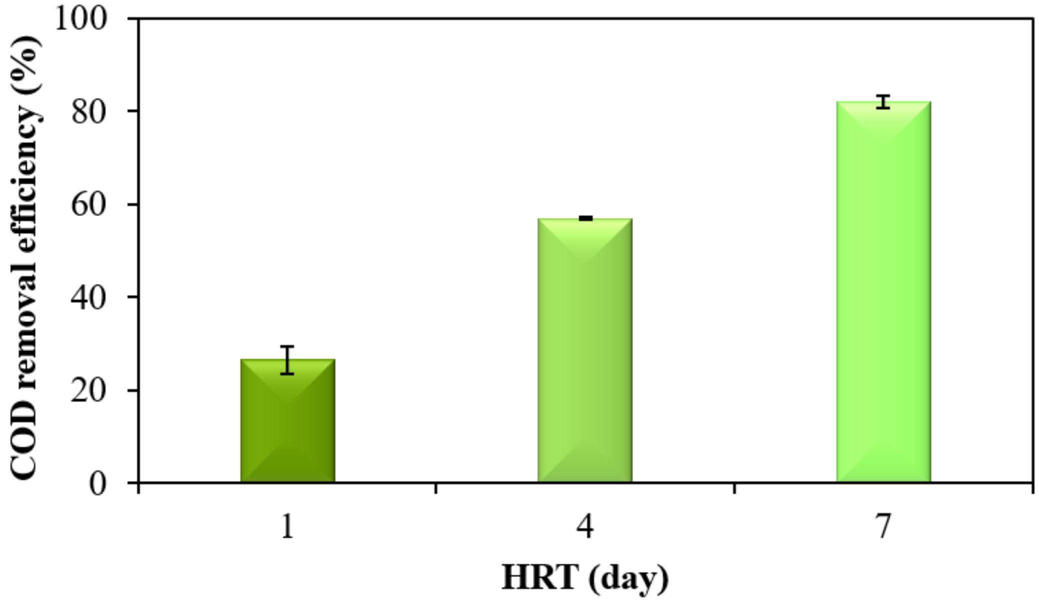

Figure 4.5: Effect of HRT on the removal of COD in copper CW-MFC under closed circuit connection

In this research, the remarkable COD removal was reported at the longest HRT. The prominent cause of this finding was related to the contact between the substrates and microbes. As the HRT increased from 1 d to 7 d, the contact time increases as well. Hence, sufficient time was available for the EABs to consume the organic pollutants that enhanced the removal rate in addition the bioelectricity generation (Jingyu et al., 2020; Yang et al., 2022). Additionally, the extension of HRT led to a higher microbial diversity. This finding was supported by Song et al. (2018). The microbial community also required adequate time to adapt to the environment. Hence, sufficient HRT promoted the growth and stability of the community and thus enhanced the organic matter degradation (Gupta and Nguyen, 2022). To achieve the optimum performance in CW-MFC, HRT of seven days was recommended.

Similar tendencies were reported by other researchers. Despite that, the removal rates in this research exceeded the values obtained by Fang et al. (2015). With the HRT of 1.5 d and 4 d, the up-flow CW-MFC removed approximately 58.9 % and 79.4 % of COD from the synthetic textile wastewater. A further improvement of the pollutant removal was achieved by Wang et al. (2019a) and Yang et al. (2022), in which the removal rates exceeded 80.0 % in less than three days.

4.3.3 Effect of External Resistance

External resistance across the electrodes has significant impacts on the microbial growth and treatment efficiency in addition to the bioelectricity generation (Ebrahimi, Sivakumar and McLauchlan, 2021). As shown in Figure 4.6, the COD removal efficiency decreased slightly with the increase of the external resistance. The peak performance of 89.2 % was achieved when the CW-MFC was connected through the least resistance (110 Ω). About 88.3 % and 87.7 % of COD were removed from the wastewater when connected to the respective resistor of 560 Ω and 1000 Ω. The initial COD concentrations ranged from 81.0 mg/L to 130 mg/L. As the resistance extended, the respective COD concentration dropped to 9 mg/L, 13 mg/L, and 16 mg/L. These findings implied that the application of minimal external resistance (110 Ω) possessed the best treatment capability among the resistors.

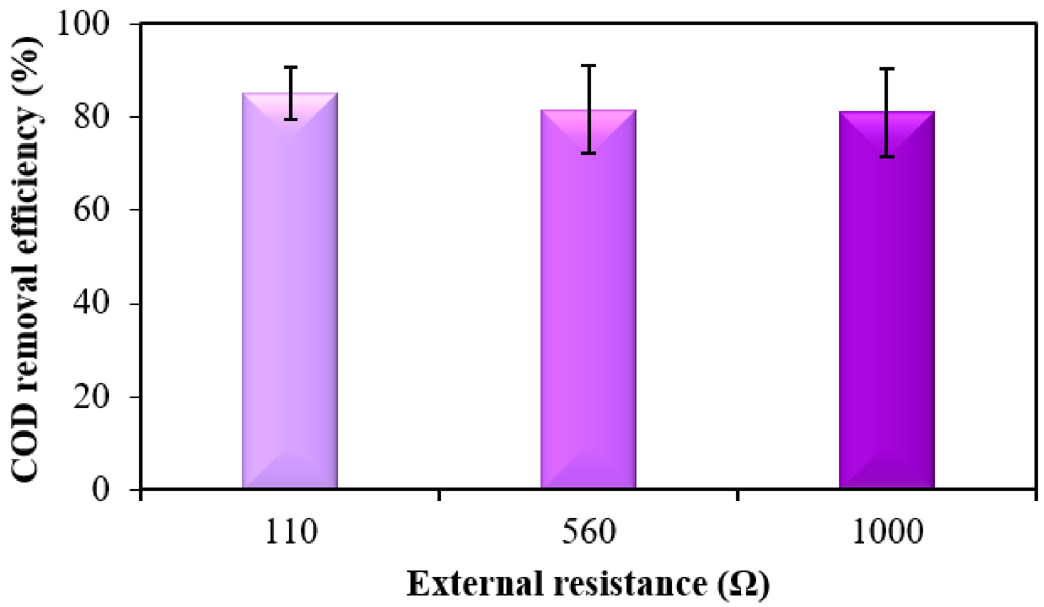

Figure 4.6: Effect of external resistance on the removal of COD in copper CW-MFC under closed circuit connection

The degradation of the pollutant was correlated to the initiation of anode potential. When the external resistance increased from 110 Ω to 1000 Ω, the declining potential reduced the energy gained by the bacteria, resulting in lower COD removal efficiency (Mohan, Pandey and Varjani, 2018). Moreover, the reduction of anode potential could degrade the redox potential, making it an unfavourable electron acceptor (Lyon et al., 2010). Lefebvre, Jiménez and Cabañas (2015) also supported that the influence of external resistance on the anode potential would affect the metabolism rate of the anaerobes. The remarkable performance at 110 Ω was caused by the favourable consumption of the organic pollutants by EAB due to the high current generated (Wang et al., 2019b). The high rate of electron transfer eventually enhanced both the cathodic reaction and the microbial activity in the copper CW-MFC (González del Campo et al., 2016).

The results demonstrated similar trend as the previous literature reports. For instance, Wang et al. (2019a) revealed that the removal of COD declined from 86.39 % to 78.53 % as the external resistance increased from 500 Ω to 2000 Ω, while the performances for the resistances of 50 Ω (87.0 %) and 150 Ω (88.8 %) were relatively stable. Under the similar operation, Yang et al. (2022) obtained a reduction in the removal efficiency from 85.6 % to 82.7 % as the resistance increased from 200 Ω to 2000 Ω. Improvement of the treatment were later achieved by Tamta, Rani and Yadav (2020) by using a pilot, stacked CW-MFC. However, the removal efficiency greatly reduced when the resistance was further increased.

4.4 Optimum Bioelectricity Generation

Similar to the COD removal efficiency, the optimal bioelectricity produced by CW-MFC was dependent on the operational conditions. In contrast to iron, a copper CW-MFC system generated a much greater average power density, which was 164 mW/m^2. Apart from the electrode material, the HRT and the external resistance also influenced the generation of bioelectricity of the system. In this study, the maximum voltage of 322 mV was obtained by the copper CW-MFC when it was connected to an external resistance of 1000 Ω on day 7. Figure 4.7 shows the continuous rise of voltage with the time and had a potential to rise further. The voltage escalated at the greatest applied resistance, and it grew slower as the resistances reduced. Nonetheless, the maximum current and power density was generated with the lowest external resistance of 110 Ω resistor, which were 191 mA/m^2 (1.92 mA in total) and 40.3 mW/m^2 (Figure 4.8). Likewise, the power density declined with increase of the resistance.

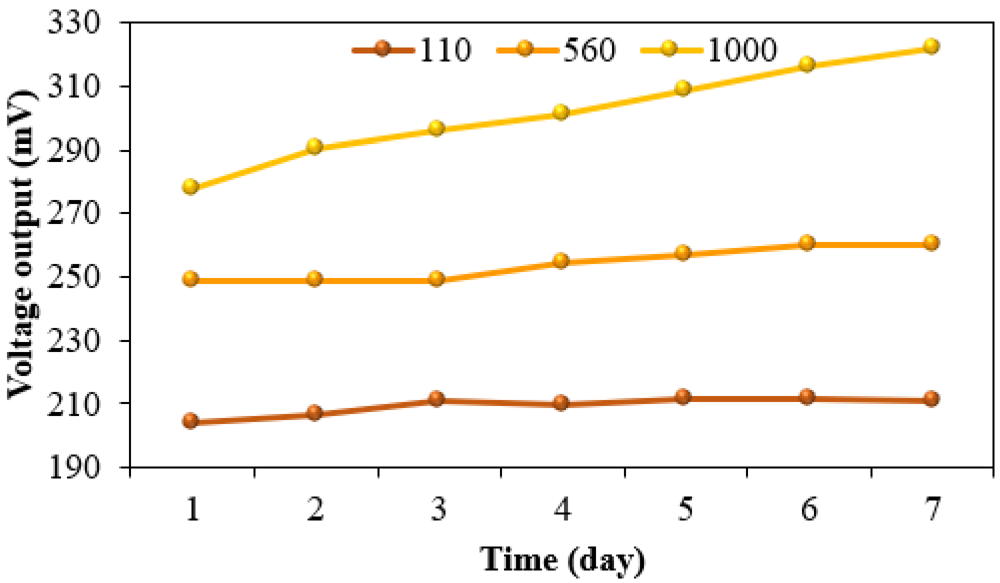

Figure 4.7: Effect of external resistance on voltage output

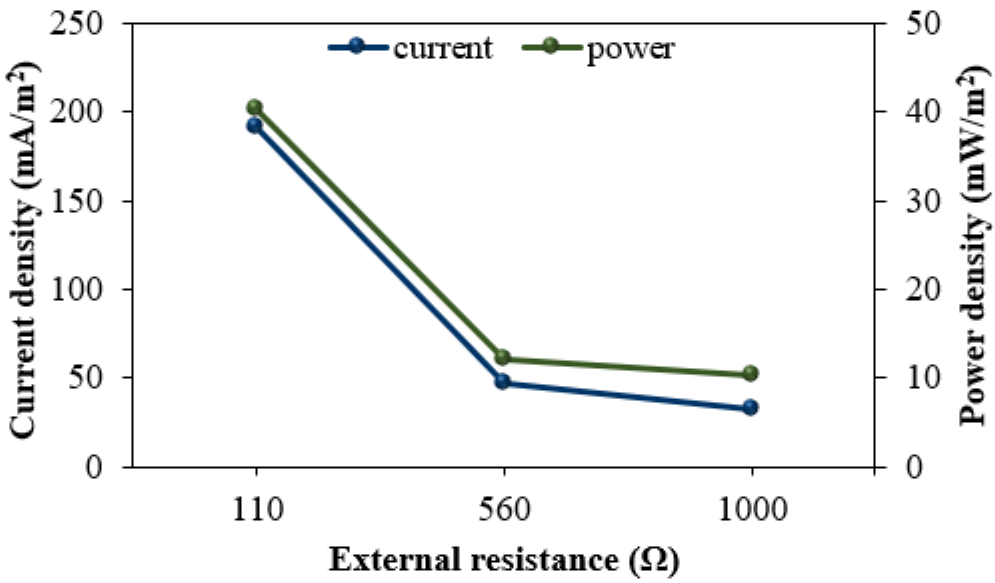

Figure 4.8: Effect of external resistances on generation current and power density

The electrode material of copper was highly conductive compared to the iron as discussed in Section 4.3.1. The copper CW-MFC thereby achieved a higher rate of electron transfer, resulting in the increase of power generation. On the other hand, the extension of HRT led to the higher voltage output and the power density. This outcome was attributed to the parallel increment of the internal resistance that stabilized the increasing voltage output (Shi et al., 2018). The internal resistance existed in the transfer pathway of the hydrogen ions generated from the oxidation process toward the cathode across the gravels in the system (Ghoniem, 2021).

The possible cause of the varying voltages with respect to the external resistances was the activation losses or activation overpotential. To initiate the electron transfer, sufficient energy from the redox processes was essential to overcome the energy barrier, which commonly happened at low current. The voltage drop was typically caused by the resistance that hindered the electron transfer through the electrodes and the external circuit. This phenomenon was known as ohmic loss. With the identical concept of MFC, the peak performance could be achieved if the external and internal resistances were equivalent (Kumar and Kuppam, 2021).

Ideally, the equilibrium of external and internal resistances led to the maximum energy production (González del Campo et al., 2016). However, losses of voltage would occur in actual life. The voltage loss also might be correlated to the microbial metabolism. Microbes required energy to deliver the released the electrons from the breakdown of the substrate to the anode of low potential. Hence, the great difference between the redox potential of the substrate and the anode potential could reduce the voltage output though higher microbial energy was attained (Chan and Li, 2014; Krishnaraj and Sani, 2019b). The anode potential which indicated anode availability as electron acceptors was affected by the external resistance, resulting in different activation losses (Shah et al., 2022).

4.5 Costing Analysis

Costing analysis or cost-benefit analysis (CBA) was established to determine the necessary costs for and evaluate the effectiveness of this research project in term of economic. Table 4.1 compiles all the relevant costs that were required for this study. For the whole project, a total amount of RM 100.80 was required with only RM 50.20 per CW-MFC system. No costs were allocated for the electrodes as they were made of the waste materials donated by the consumers. As a result, the total costs spent for this research was relatively low. From this analysis, it was revealed that CW-MFC is a cost-effective system for the degradation of organic pollutants and bioelectricity generation.

Table 4.1: Costs required for the development of each CW-MFC in this study

Item	Function	Quantity	Amount (RM)
Chlorophytum comosum	Used as plant.	1 pot	3.60
Gravels	Used as filtration media.	6 kg	24.00
Chamber	Used to contain the system.	1 unit	15.50
Copper / iron waste	Used as the electrode material.	1 set	0
Silicone glue	Used to secure water tap.	1 unit	7.10
		Total cost per system	50.20 × 4
		Total project cost	200.80

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study was in line with the objectives. First and foremost, the incorporation of waste materials as the electrodes in CW-MFC systems were successfully conducted for municipal wastewater treatment and power generation. In more specific, the metallic wastes, which were iron strip and copper wire, were used to create the electrode pair in the respective system, allowing the electron flow through the external circuit. In contrast to the iron CW-MFC, the copper CW-MFC removed the COD more effectively due to its higher biocompatibility towards EAB and larger surface area. The average removal efficiencies achieved by the copper and iron CW-MFCs were 82.0 % and 67.2 % respectively. The utilisation of the wastes as the electrode materials in the respective CW-MFC also reduced the cost of this project.

Apart from the electrode material, the effects of the parameters called HRT and external resistance on the performance of the closed circuit CW-MFC with the copper electrodes were investigated. Among the parameters, HRT was the most significant parameter that influenced the municipal wastewater treatment because of the sharp increase in or greatest difference of the COD removal efficiency. The HRT varied between 1 d, 4 d, and 7 d. The average removal efficiencies obtained were 26.4 %, 56.9 %, and 82.0 % for the corresponding HRT which was correlated with the contact time between the microbes and the organic pollutants. Besides, the population of microbes expanded with the time that increased the rate of biodegradation. Meanwhile, the power density also would raise. For the third parameter, the effect of external resistance on the treatment efficiency of CW-MFC was investigated by increasing the resistance from 110 Ω to 1000 Ω. Unlike the previous results, the average removal efficiency declined gradually from 85.1 % to 81.1 %, with the resistance increment. The main root cause of the declined treatment performance was the reduction of anode potential at higher external resistance that lessened the energy gained by the bacteria for breaking down the pollutants.

Finally, the optimum COD removal efficiency and power density of the CW-MFC were obtained, which were 85.1 % and 40.3 mW/m^2 on average. The optimum performance was obtained when the CW-MFC employed with copper electrodes and the lowest external resistance of 110 Ω at the HRT of 7 d. The optimisation procedure improved the treatment efficiency of the system by 8.1 % in maximum. In short, the optimised CW-MFC system in this study could be considered as a cost-effective and sustainable system for municipal wastewater treatment. Apart from wastewater treatment, the CW-MFC system also generated bioelectricity.

5.2 Recommendations

Future investigations should be made to validate the outcomes of this study. Apart from that, the future research could consider the following aspects for improvement of the CW-MFC system in this study.

1. Since the metallic electrode material might be toxic, the carbon waste materials could be explored and replaced the existing electrode to improve the water quality. Besides, alternative waste material could be used as the chamber to further reduce the cost required for the operation of the system.
2. The wastewater strength in this present study was low. Hence, the COD removal efficiency from the raw municipal wastewater or the primary effluent with higher

organic loading could be examined to analyse the COD removal efficiency and bioelectricity generation of the current CW-MFC.

3. Other water quality parameters could be investigated by using the optimal system in this study, including the ammoniacal nitrogen, TSS, DO, and the heavy metals.
4. Other potential operational parameters such as electrode spacing, type of wetland plant, and filtration media could be investigated to further optimize the current system.
5. The present scale of the system in this study was incapable of providing the long-term sustainability and supporting the real application. Therefore, the performance of the scaled-up system could be assessed in the future.

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APPENDICES

APPENDIX A: Figures of Experiment

Figure A1: Electrical cables collected from friends and Green Bank

Figure A2: Iron strip from engineering workshop of UTAR

Figure A3: Drilling holes through PVC pipe by using drill press

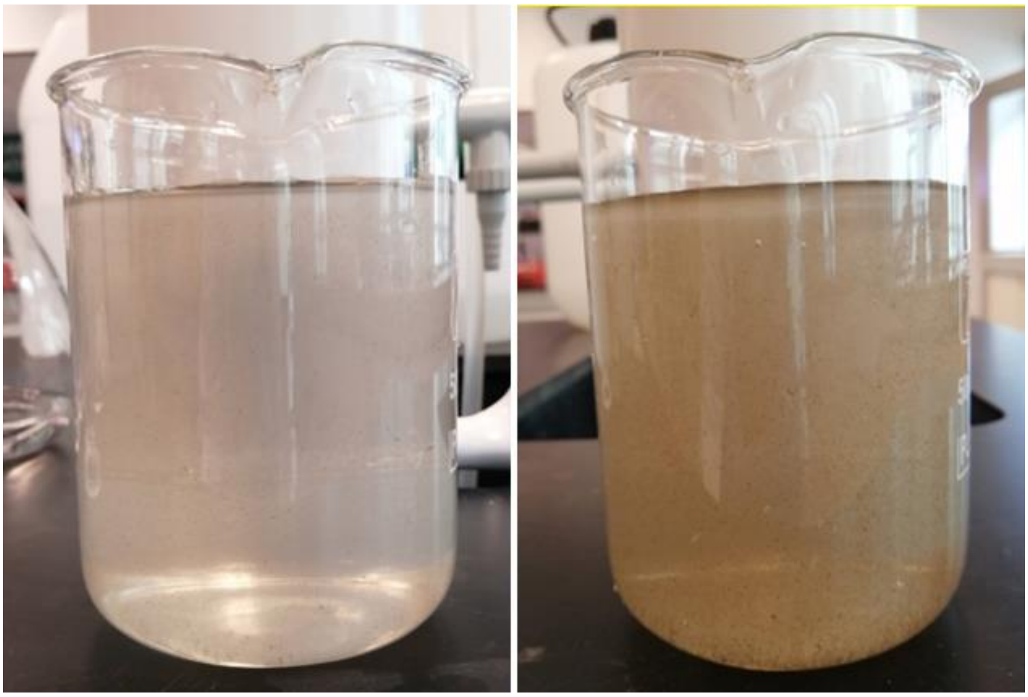

Figure A4: Activated sludge before (left) and after (right) anaerobic pre-treatment

Figure A5: Removing impurities on electrode surface

Figure A6: Placement of copper (left) and iron (right) anodes in the respective CW-MFC systems

Figure A7: Placement of cathode on the surface of filtration media or gravels

Figure A8: Plants employed in the experiment

Figure A9: Completed setup of copper and iron CW-MFCs under different circuit connections

Figure A10: Voltage measurement

APPENDIX B: Competition

Penang International Invention, Innovation and Design (PIID 2023) – Category B (UNI): University and Technical Institution Student

Title of Project:	Constructed Wetland Integrated Microbial Fuel Cell for Simultaneous Wastewater Treatment and Electricity Production
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Status:	Registered. In progress of preparation.