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Isolation and Characterization of Electroactive Bacteria for Electricity Generation in Microbial Fuel Cell (MFC) Supplemented with Palm Oil Mill Effluent (POME)

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A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Bioindustrial Technology) with Honours

FACULTY OF BIOENGINEERING AND TECHNOLOGY

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

_____

Student

Name : CHEE YU CHENG

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I certified that the report of this final year project entitle "Isolation and Characterization of Electroactive Bacteria for Electricity Generation in Microbial Fuel Cell (MFC) Supplemented with Palm Oil Mill Effluent (POME)" by CHEE YU CHENG, matric number J20A0430 has been examined and all the corrections recommended by examiners have been done for the degree of Bachelor of Applied Science (Bioindustrial Technology) with Honours, Faculty Bioengineering and Technology, University Malaysia Kelantan.

Approved by:

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Supervisor

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Pengasingan dan Pengklasifikasian Bakteria Elektroaktif untuk Penghasilan Elektrik dalam Sel Bahan Bakar Mikrob (MFC) Ditambah dengan Efluen Kilang Minyak Kelapa Sawit (POME)

ABSTRAK

Sel bahan api mikrob (MFC) boleh menukarkan tenaga kimia kepada tenaga elektrik dengan menggunakan bakteria elektroaktif (EAB). MFC berkemampuan untuk menjana elektrik dan merawat air kumbahan pada masa yang sama. Efluen kilang minyak kelapa sawit (POME) adalah sisa organik yang boleh bertindak sebagai penderma elektron untuk EAB seperti *Shewanella oneidensis* MR-1 untuk menghasilkan elektrik. Disebabkan POME yang dikumpulkan itu ialah pH 10, eksperimen dijalankan dengan menggunakan MR-1 sebagai kawalan untuk membandingkan penghasilan elektrik dengan pengkayaan sampel tanah beralkali. Dari persediaan MFC ini, teknik isolasi dan pengklasifikasian telah dilakukan untuk mengasingkan EAB alkali dari POME. Penghasilan elektrik dalam MFC oleh MR-1 sangat rendah berbanding dengan MFC yang diperkaya oleh sampel tanah alkali. Penghasilan elektrik tertinggi oleh MFC dengan pengkayaan pertama oleh sampel tanah adalah 8.910 W/m^2 manakala ketumpatan kuasa pengkayaan kedua adalah 9.119 W/m^2. EAB diasingkan dari sampel pengkayaan kedua melalui pencairan berturutan dan dicirikan dengan menggunakan teknik-teknik seperti pewarnaan Gram, ujian katalase, dan ujian oksidase. DNA EAB yang diasingkan diekstrak untuk penjujukan DNA untuk pengenalpastian jenis baktieria pada masa depan. Kajian ini melaporkan bahawa MR-1 tidak sesuai untuk penjanaan elektrik dalam persekitaran beralkali dan EAB alkali perlu diasingkan. EAB alkali yang diasingkan, GR-1, dapat menghasilkan lebih banyak elektrik dalam POME alkali dan dengan itu meningkatkan jangka masa untuk penghasilan elektrik. Hasil ujian biokimia menunjukkan bahawa GR-1 adalah bakteria bentuk kokus Gram negatif dan kedua-dua ujian katalase dan oksidase menunjukkan keputusan negatif. Penemuan ini menyediakan pandangan yang berharga bahawa lebih banyak ekstremofil perlu diasingkan supaya ia dapat meningkatkan prestasi MFC dalam apa jua situasi ekstrem.

Kata kunci: MFC, POME, bakteria elektroaktif, *Shewanella oneidensis* MR-1, elektrik

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Isolation and Characterization of Electroactive Bacteria for Electricity Generation in Microbial Fuel Cell (MFC) Supplemented with Palm Oil Mill Effluent (POME)

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ABSTRACT

Microbial fuel cell (MFC) converts chemical energy into electrical energy by using the electroactive bacteria (EAB). MFC is capable to generate electricity and treat the wastewater simultaneously. Palm oil mill effluent (POME) is the organic waste which can act as the electron donor for the EAB like *Shewanella oneidensi* MR-1 to generate electricity. Since the collected POME was pH 10, the experiment was carried out by using the MR-1 as the negative control to compare the electricity generation by the enrichment of alkaline soil sample. From this MFC setup, isolation and characterization have been done to isolate the alkaliphilic EAB from the POME. The electricity generated in MFC by MR-1 was very low compared to the MFC that enhanced by the alkaline soil sample. The highest electricity generated by the MFC with first enrichment by the soil sample was 8.910 W/m^2 whereas the second enrichment was 9.119 W/m^2 power density. The EAB was isolated from the second enrichment sample by serial dilution and characterized by using techniques such as Gram staining, catalase test and oxidase test. The DNA of the isolated EAB were extracted for DNA sequencing for future identification. This study reported that MR-1 was not suitable for electricity generation in the alkaline environment and alkaliphilic EAB need to be isolated. The isolated alkaliphilic EAB, GR-1 can generate more electricity in the alkaline POME and hence increase the time-interval for the electricity generation. The result of the biochemical test showed that GR-1 was Gram negative cocci shape bacteria and both catalase and oxidase tests showed negative result. This finding provides valuable insights into more extremophiles need to be isolated so it can increase the performance of MFC in any extreme situation.

Keywords: MFC, POME, electroactive bacteria, *Shewanella oneidensis* MR-1, electricity

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LIST OF ABBREVIATIONS

MFC	Microbial Fuel Cell
MPOC	Malaysia Palam Oil Council
COD	Chemical oxygen demand
BOD	Biological oxygen demand
Zn	Zinc
Fe	Iron
EAB	Electroactive bacteria
S. oneidensis MR-1	*Shewanella oneidensis* MR-1
DMRB	Dissimilatory metal reducing bacteria
Fe (III)	Iron (III) ion
Fe (II)	Iron (II) ion
U (VI)	Uranium (VI) ion
Cr (VI)	Chromium (VI) ion
Mn (VI)	Manganate (VI) ion
SEM	Scanning electron microscope
PEM	Proton exchange membrane
OMCs	Outer membrane cytochromes
TSS	Total suspended solid
FFB	Fresh fruit bunch
GF	Graphite felt
CC	Carbon cloth
ACFF	Activated carbon fiber felt

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GP	Graphite paper
AS	Aluminium sheet
DLs	Diffusion layers
PTFE	Polytetrafluoroethylene
H_2O_2	Hydrogen peroxide
LB agar	Luria Bertani agar
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
KCl	Potassium chloride
ATCC	American Type Culture Collection
N	North
S	South
$K_3[Fe(CN)_6]$	Potassium ferricyanide
TRMS	True root mean square
Cyt-c	Cytochrome-*c*
GR-1	Gunung Reng-1

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LIST OF SYMBOLS

%	Percentage
mg/L	Milligram per liter
°C	Degree Celsius
W/m^3	Watt per cubic meter
W/m^2	Watt per square meter
mW/m^2	Milliwatt per square meter
M	Molar
g	Gram
g/L	Gram per liter
mL	Milliliter
P	Power
I	Current
V	Voltage
w/v	weight per volume
mA	Milliampere

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Microbial fuel cell (MFC) is a device that can convert chemical energy into electrical energy by using electroactive bacteria. In MFC, the electrons can produce via the microbial catabolic activities from different wastes such as complex organic wastes, biomass wastes, and industrial wastes (Watanabe, 2008) .Although the idea "animal electricity" was founded by Galvani through the experiments with frog legs, the pioneer of generating electricity by using microorganisms was attributed to Potter in 1911 (Santoro et al., 2017). The microorganisms will utilize the nutrient from the wastes and produce electricity through some electrons transfer mechanisms.

According to Matsumoto et al., they used MFC to generate electricity in rice paddy fields. Based on previous studies, they are a lot of organic nutrients that will be excreted by the plants via roots into the soils and these organic compounds will be served as the nutrients for the rhizosphere microbes (Matsumoto et al., 2020) .The electrodes will be constructed in the paddy field and the electroactive microbes will produce electrons and attach to the electrodes. After the microbes attaching to the electrodes, it will form biofilm.

According to Malaysia Palam Oil Council (MPOC), Malaysia has been contributed about 25.8% and 34.3% of the world's palm oil and exports respectively in 2020. The palm oil industries in Malaysia are increasing. However, behind this potential, the amount of liquid waste that will be discharged by the industries which is called palm oil mill effluent (POME) is also increasing. In average, POME contains high COD and BOD, which are the range 3500 mg/L to 8200 mg/L and 15103 mg/L to 65100 mg/L respectively (Leela et al., 2018). Recently, the MFC technology was implemented in treating the POME by reducing the BOD and COD concentration. The electroactive

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microorganisms such as *Geobacter* and *Shewanella* species will convert the chemical energy that stored in the chemical bonds in organic matter in the POME into electricity. The microorganisms will oxidize the organic matter in the POME and the electrons will be transferred to the electrode (Cheng et al., 2010). The organic matter in the POME will act as the electron donors to the electroactive bacteria. Hence, electricity will be produced.

According to the previous study, the MFC technology was not drastically developed because the power generation capacity is still deficient compared to the chemical fuel cell. However, nowadays, the research on this MFC technology is increasing because this MFC technology is capable to generate electricity and treating the organic pollutants in the wastewater simultaneously (Nguyen et al., 2020). As a result, the MFC that is constructed in the palm oil mill can help to treat POME and at the same time it can generate electricity. The electroactive microorganisms will act as biocatalyst to oxidize the organic nutrients in the anode chamber and the electrons will flow to the cathode chamber via external circuit (Jatoi et al., 2021). Although the MFC can provide a lot of contributions to the industries, but the setup of the MFC is very costly.

From the research, there are several factors that will affect the performance of MFC such as the MFC design, the electrodes' materials, operating condition, type of membrane, type of substrates and so on (Aghababaie et al., 2015). In this study, the POME pH is the most crucial factor that affects the performances of the MFC. The pH of the POME is very basic (about pH 10) and the catabolic activity of *Shewanell oneidensis* MR-1 is very low. Therefore, this becomes one of the crucial challenges that faced by the palm oil mill industries in our countries. This experiment is carried out to isolate the electroactive bacteria that can survive in the high pH POME and hence to generate higher electricity from the MFC. The experiment is set up by adding the alkaline soil into the POME.

1.2 Problem Statement

Recently, the increment of the POME wastewater has polluted the environment, especially the water pollution. For example, there are many total suspended solids, biochemical oxygen demand (BOD), chemical oxygen demand (COD), oil, and grease detected in the fresh POME. Besides, POME also contains some heavy metals that are

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toxic and non-biodegradable such as zinc (Zn) and iron (Fe). This heavy metal can harm aquatic animals and human health (Jumadi et al., 2020). The POME can undergo some treatments to produce biogas and it can also be the raw material of the biodiesel production (A Aziz et al., 2020; Leela et al., 2018). The nutrients in the POME can be used as the raw materials for the fermentation process. Consequently, the nutrient in POME can be used in the MFC technology as a substrate for the electroactive bacteria to generate electricity. However, the efficiency of the electricity generation in the MFC is very low and it is also will cause the discontinuous of power generation after a specific time interval (Maddalwar et al., 2021). This is maybe because the pH of the POME is affecting the redox reaction of the electroactive microorganisms in the MFC. Therefore, this study aims to isolate and characterize the electroactive bacteria for electricity generation in MFC supplemented with POME. This can help to reduce the cost of neutralizing the high pH POME in the industries instead of using the alkaliphilic bacteria.

1.3 Objectives

The objectives of this study are:

1. To isolate the electroactive bacteria for generation of electricity in MFC supplemented with POME as substrate.

2. To characterize the electroactive bacteria isolated from MFC supplemented with POME.

1.4 Scope of Study

In this research, the raw material that use in MFC is POME. The POME will be collected from the nearby palm oil mill. To run the MFC, the dual chamber MFC is chosen in this experiment. After that, the pH of the POME is measured first and then observed

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the electricity generation by the MFC supplemented with the original POME using *Shewanella oneidensis* MR-1. To optimize the electricity generation in the MFC, the soil which have same pH with the POME will be added into the anode chamber. Then, the electricity generation by the MFC will be measured and isolate the electroactive bacteria from the POME.

1.5 Significances of Study

The findings of this study will attribute to the benefits of government considering the pollution by the POME is increasing. In this research, the alkaliphilic electroactive bacteria (EAB) need to be isolated to generate more electricity in the high pH POME. It is because MR-1 is neutrophilic bacteria and hence the performance of the MFC is low. EAB will utilize the nutrients in the POME as the electron donor and this can treat the POME. The technology of MFC can generate electricity by using electroactive bacteria and treat the wastewater simultaneously. This can be one of the green energy technologies in the future so this can help to achieve the Net Zero Carbon by 2050 (Fankhauser et al., 2022).

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter present the effect of POME pH on electricity generation in microbial fuel cell (MFC). MFC can generate the electricity by utilizing the electrons from the organic or inorganic compounds to complete the metabolism by bacteria. During the metabolism activity of the bacteria, the bacteria will accept the electron from the substrate and the electrons will flow from the bacterial to the anode. The electron will then flow to the cathode and the electrons will be flow to the electron acceptor. Therefore, the flow of the electrons will generate the electricity.

2.2 Dissimilatory metal reducing bacteria (DMRB)

Dissimilatory metal-reducing bacteria (DMRB) is the bacteria that can utilize the metal ions as the terminal electron acceptor in the anaerobic condition instead of oxygen (O_2), which performed as the terminal electro acceptor in the aerobic conditions. DMRB play an important role in some important environment processes, such as biogeochemical cycles, bioremediation, and electricity generation in the microbial fuel cells (Wee et al., 2014). This DMRB can be isolated from the natural environment including freshwater sediments, mining area, and estuary. The dissimilatory metal reduction is the process where bacteria can reduce a variety of metal ions in their metabolic pathway enzymatically. DMRB can conserve energy to growth in anaerobic conditions by coupling oxidation of organic acid to the reduction of the Fe(III) ions into Fe(II) ions (Lovley, 1993). The major difference between dissimilatory and assimilatory Fe(III) reduction is

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the iron compound will be assimilated into enzymes, cofactor and magnetosomes in assimilatory Fe(III) reduction (Lovley et al., 2004).

2.3 Genus Shewanella

The *Shewanella* has been classified as proteobacteria phylum. The *Shewanella* are the members of the order Alteromonadales, family *Shewanellacea*, and class γ-proteobacteria (Figure 2.1). They are gram negative in rod shape (2-3 µm in length, 0.4-0.7 µm in diameter), and motile by single, unsheathed, polar flagellum (Hau & Gralnick, 2007; Satomi, 2014). The *Shewanella* can be isolated from the anaerobic environment such as deep marine sediments and rich metal oxide sediments. Most of this *Shewanella* can be cultured easily in the laboratory with common growth media by enrichment of environmental samples. The *Shewanella* are facultative anaerobes because they have extreme respiratory versatility which act as electron acceptors including oxygen, radionuclides, and metal oxides. They are able to use the metals as terminal electron acceptors, and this has led to the research on electricity generation by *S. oneidensis* (Bertling et al., 2021).

In 1931, the first *Shewanella* was isolated from the butter putrefaction as it is one of several contaminating microorganisms. This *Shewanella* spp. is renamed as *Achromobacter putrefaciens* but the taxon was renamed to *Pseudomonas* as the further growth of biochemical characterizations. In 1972, the *Pseudomonas* genus is then classified into the newly genus, *Alteromonas*, based on the GC content of DNA. Finally, based on the 5S rRNA sequence data, the genus *Alteromonas* is then reclassified into a new genus, *Shewanella*. *Shewanella* is used to honour Dr. James Shewan's work in fisheries microbiology (MacDonell & Colwell, 1985). Based on DNA:DNA hybridization and 16S rRNA sequencing, there are about 40 species assigned to the genus *Shewanella* (Irshaid, 2013).

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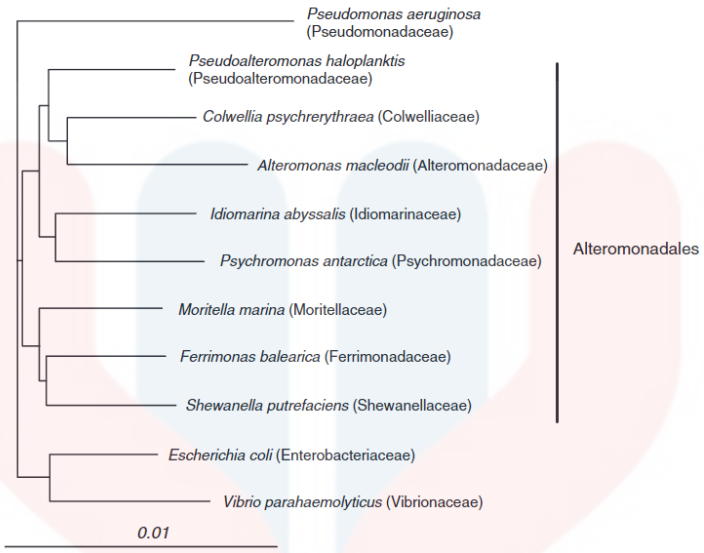

Figure 2.1: Phylogenetic tree of the Alteromonadales and some close-related families based on 16S rRNA gene sequences. The tree was constructed using the neighbor-joining (NJ) method, and genetic distances were computed by Kimura's model. The scale bar indicates the genetic distance of 0.01. The tree was grouped with Pseudomonas aeruginosa.

Source: (Satomi, 2014)

2.3.1 *Shewanella oneidensis* MR-1

Shewanella oneidensis MR-1 is previously named as *Alteromonas putrefaciens* MR-1. *S.oneidensis* MR-1 is rod shaped gram negative γ-proteobacteria with high respiratory versatility. *S.oneidensis* MR-1 is mesophilic bacteria that can grow around 35°C. However, it can grow in a wide range of temperatures, but the doubling times will be affected by the temperature. The name *S.oneidensis* MR-1 was given because it was isolateed from the sediments of Lake Oneida in New York State. It is able to reduce metal ions such as Fe(III), U(VI), Cr(VI), and Mn(VI) metabolically so it is a good candidate for bioremediation of subsurface metal-contaminated areas. Therefore, *S. oneidensis* MR-1 is the iron reducing bacteria model.

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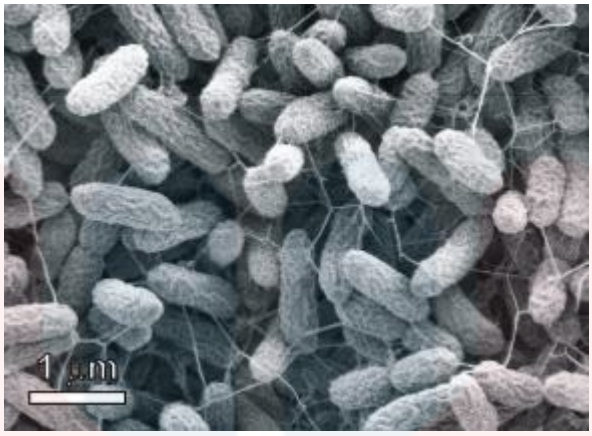

Figure 2.2: SEM image of *S.oneidensis* MR-1cultivated under electron-acceptor limited conditions expressing high amounts of nanowires connecting neighbouring cells.

Source: (Leung et al., 2013)

2.4 Microbial Fuel Cell (MFC)

MFC is one of the devices that can conserve energy continuously in the form of electricity by using the substrate from the wastewater. MFC can convert the chemical energy into the electric energy with the aid of catalytic activity of electroactive microorganisms (Juliastuti et al., 2018). The use of substrate from the wastewater in MFC makes it an eco-friendly device that gives advantages in electricity generation and wastewater treatment (Obileke et al., 2021).

MFC is made up of two electrodes which are anode and cathode electrodes separated by proton exchange membrane (PEM). The electroactive microorganisms and the substrate will be held in the anode with anaerobic condition. The cathode usually is filled with saltwater solutions or air as the terminal electron acceptor. The microorganisms will use the organic materials in the wastewater as substrates for the catalytic activities to generate electricity. The catalytic process involves redox reactions. The electrons will be transferred from the anode to the cathode via copper wire outside the chamber. In addition, the protons will pass through the PEM to the cathode chamber to reduce the oxygen into water molecules. The oxygen acts as the terminal electro acceptors. Commonly, microorganisms use acetate as the substrate and the reaction (1) to (3) are presented (Saravanan et al., 2023):

Anodic reaction: $CH_3COOH + H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-$ Equation 2.1

Cathodic reaction: $8H^+ + 8e^- + 2O2 \rightarrow 4H_2O$ Equation 2.2

Overall reaction: $CH_3COOH + 2O_2 \rightarrow 2H_2O + 2CO_2$ Equation 2.3

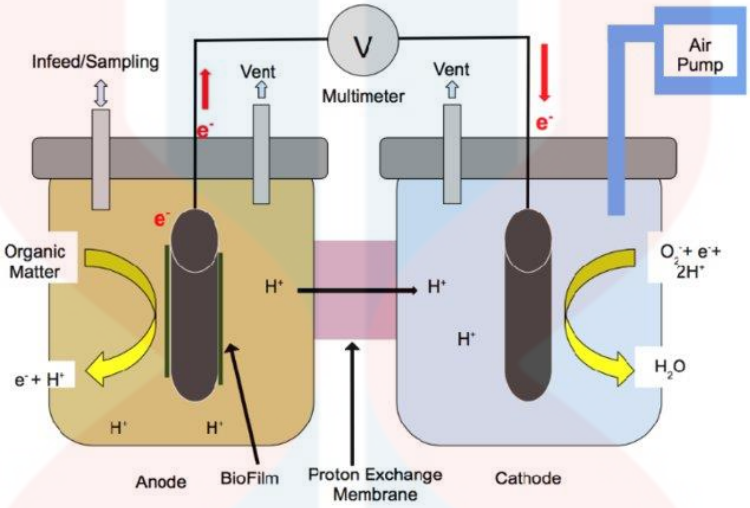

Figure 2.3: The schematic diagram of the MFC design.

Source: (Pradhan & Pradhan, 2020)

2.4.1 Mediator-less MFC

In mediator-less MFC, microorganisms can generate electricity without the aid of mediators. No external mediators such as neutral red, humic acid, and anthraquinone-2,6-disulphonate do not required to transport the electrons from the bacteria to the anode. This will help to increase the efficiency of the electricity generation (Mane et al., 2022; Obileke et al., 2021). On the other hand, the mediator-less needs a specific bacteria like *S.oneidensis* MR-1. It is because the MR-1 utilizes the substrates and directly produces the electrons to the anode and H^+ to the cathode through PEM in the MFC. In this situation, no dissolved redox species are involved in the anode chamber for the electron transfer. However, the electron is transferred directly via the outer membrane cytochromes (OMCs), conductive pili and nanowires. The redox protein like C-type cytochrome which occurs in the outer membrane of the bacteria will facilitate the electrons transfer to the anode electrode. These C-type cytochromes have a redox potential about 1 V and they are very stable against chemical modification (Aiyer, 2020). As a result, the electrons will transfer directly to the anode with nanowires comprising of

MtrC and Omc A. Therefore, electricity will be produced using long appendages called nanowires.

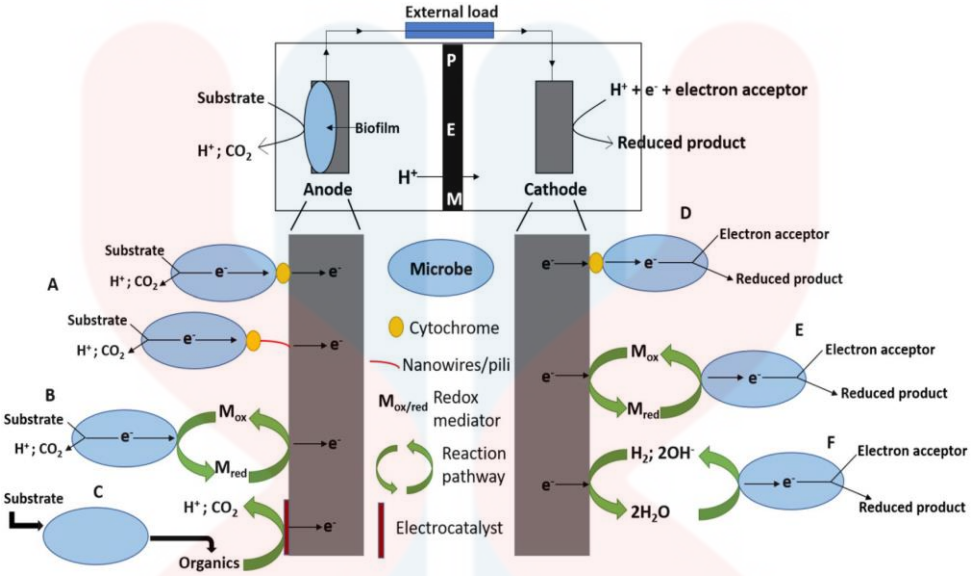

Figure 2.4: Schematic diagram illustrating anodic and cathodic electron transfer mechanisms: **a** direct electron transfer from microbe to anode; **b** mediated electron transfer involving a redox mediator; **c** electron transfer via reduced metabolites; **d** direct electron transfer from cathode to microbe; **e** indirect electron transfer from cathode to microbe involving a redox mediator; **f** electron transfer to microbe via oxidation of hydrogen.

Source: (Aiyer, 2020)

2.4.2 Potential of MFC

MFC is one of the green energies. It can be said that because the electricity that is generated by MFC is renewable. MFC will convert the chemical energy from the organic materials in the wastewater into electricity instead of using unrenewable energy such as petroleum (Koleva et al., 2022). Electroactive microorganisms will be the important alternative source to produce the energy from fossil fuels. In addition, using MFC in the wastewater treatment system will bring a lot of benefits in different aspects. The use of MFC in wastewater treatment will decrease carbon emission and reduce the amount of sludge production. In addition, MFC can also recover the energy by using the organic material in the wastewater in lower operation costs (Koleva et al., 2022).

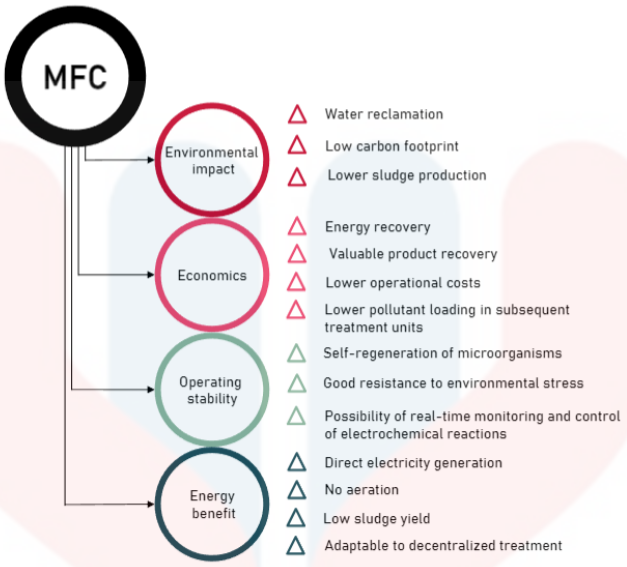

Figure 2.5: Potential benefits of MFCs for energy, environmental, operational, and economic sustainability.

Source: (Nosek et al., 2020)

2.5 Palm Oil Mill Effluent (POME)

POME is the wastewater that is generated by the palm oil industry during the crude palm oil extraction process from fresh fruit bunch. Every ton of the fresh fruit bunch process will produce 58.4% of the POME. As the POME is highly acidic, it is 100 times more polluting than the domestic waste and it will pose a threat to the environment if it is directly disposed into the water body (Nuaini et al., 2018; Tan et al., 2017). In addition, the POME also contains high oil and grease and total suspended solids (TSS). However, the oil and grease in the POME can be used as the raw material for biofuel production and the carbon source for the microorganisms in the MFC.

2.5.1 Characterization of POME

The characteristics of POME may differ due to the quality of the production process in the palm oil mill. There are some parameters measured for the POME and the regulatory discharge limits are shown in Table 2.1.

Table 2.1: POME characteristics and the discharged limits regulated by the Department of Environment.

Source: (Akhbari et al., 2020)

Parameter	Concentration	Regulatory discharge limits	Unit
Temp	80-90	40	℃
pH	5 ± 0.2	6-9	-
Oil and grease (O&G)	4,000 ± 20	1	mg/L
Biological oxygen demand (BOD)	25,000 ± 1,000	100	mg/L
Chemical oxygen demand (COD)	50,000 ± 2,000	50	mg/L
Total solid (TS)	40,000 ± 1,000	-	mg/L
Total suspended solid (TSS)	18,000 ± 500	50	mg/L
Total volatile solid (TVS)	34,000 ± 800	-	mg/L
NH3-N	35 ± 1	-	mg/L
TKN	750 ± 5	-	mg/L
Turbidity	664 ± 4	-	NTU

POME is produced from the processes of the palm oil mill industries including sterilization of fresh fruit bunch (FFB), clarification, hydroclones and separation processes. The discharged POME is high in biodegradable organics matters and this will adversely affect the environment. The processes in the palm oil mill are just using water in every process and no chemical agent is added to the extraction process. Therefore, the effluent is non-toxic. However, the organic matters in the POME can reduce the dissolved oxygen in the water body and hence harm the harm the aquatic animals (Akhbari et al., 2020).

2.5.2 Advantage of POME

The raw POME contains a lot of organic matters that can further utilize to produce product through biotransformation. In POME, it contains carbohydrates, protein, nitrogenous compounds, minerals, and lipids in high concentration. These nutrients in the POME are good raw material for bioconversion to produce valuable products including citric acids, bioethanol, bioplastics, biohydrogen, biofertilizers, and some enzymes production (Salihu & Alam, 2012).

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Table 2.2: The proximate composition and mineral contain of raw POME.

Source: (Salihu & Alam, 2012)

Major constituents	Composition (%)	Macro-minerals	Composition (µg/g dry weight)	Micro-minerals	Composition (µg/g dry weight)
Moisture	6.99±0.14	K	8951.55±256.45	Fe	11.08±2.20
Crude protein	12.75±1.30	Na	94.57±6.45	Cu	10.76±1.04
Crude lipid	10.21±1.24	Ca	1650.09±160.45	Zn	17.58±2.10
Ash	14.88±1.35	Mg	911.95±95.50	Mn	38.81±3.65
Carbohydrate	29.55±2.44	P	14377.38±1206.88	Mo	6.45±0.40
Nitrogen free extract	26.39±2.33	S	13.32±1.45	Cr	4.02±0.44
Total carotene	0.019±0.001			Co	2.40±0.35
				Ni	1.31±0.30
				Se	12.32±1.35
				Si	10.50±1.80
				Sn	2.30±0.30
				Al	16.60±1.44
				B	7.60±0.60
				As	9.09±0.65
				V	0.12±0.02

From the previous study, the biomass in the POME is digested by electroactive bacterial anaerobically via hydrolysis, acidogenesis, acetogenesis and methanogenesis to generate electricity in MFC. During these processes, the nutrients in POME will be broken down into simple compounds that needed by the bacteria (Alkhair et al., 2018). Therefore, POME gives a lot of benefits in the MFC development.

2.6 Factors that affecting the performance of MFC

2.6.1 pH

pH of the microenvironment in the POME will affect the catalytic activities of the bacteria and hence affect the performance of MFC. According to (Foad Marashi & Kariminia, 2015), three different pH of the POME were conducted including 8.5, 7.0, and 5.4 periodically based on the optimal range of pH for the methane-producing bacteria. From this experiment, at pH 8.5, the production of higher power density is observed which are 40% and 66% higher than at pH 7.0 and pH 5.4 respectively. This result is represented to the methane-producing bacteria.

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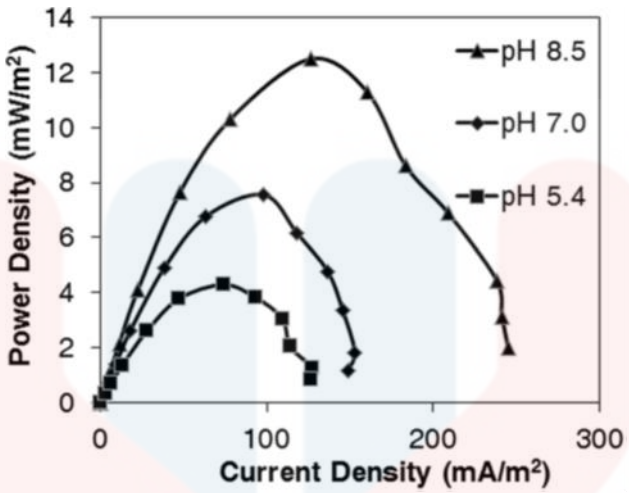

Figure 2.6: Power density curves at different pH values.

Source: (Foad Marashi & Kariminia, 2015)

For the iron reducing bacteria like *S. oneidensis* MR-1, it is neutrophilic gammaproteobacterial (Fauque & Barton, 2012). According to (Biffinger et al., 2008) research, the power generation by the MFC is higher in neutral pH compared to in acidic environment. 10.0 W/m^3 and 9.2 W/m^3 power were generated in the pH 6 and pH 7 environment respectively. From this research, we can assume that the MFC can generate the optimum electricity in the pH range 6-8.

Table 2.3: Electrical parameters from mini-MFC containing *Shewanella oneidensis* MR-1 and DSP10.

Source: (Biffinger et al., 2008)

pH	DSP10			MR-1		
	OCV (V)	I_{sc} (mA)	Power density (W/m^3)	OCV (V)	I_{sc} (mA)	Power density (W/m^3)
5	390	0.06 ± 0.02	3.0 ± 1	450	0.22 ± 0.03	6.0 ± 1
6	490	0.36 ± 0.03	12 ± 1	530	0.31 ± 0.03	10 ± 1
7	460	0.33 ± 0.05	14 ± 2	470	0.27 ± 0.02	9.2 ± 2

I_{sc}: short circuit current; OCV: open circuit voltage.

2.6.2 Substrate

There are many organic matters that can be used as the substrates for the microorganisms in the MFC including glucose, acetate, molasses, sugar, palm sugar, and so on. Based on the (Khoirunnisa et al., 2021), it showed that the molasses as the substrates for the microorganism can generate the highest electricity in the MFC. From the result, the electricity generation potential is still high at the end of the incubation

period. The molasses is the best substrate for the electricity generation in the MFC because the electricity output achieved the highest value as presented in the table below.

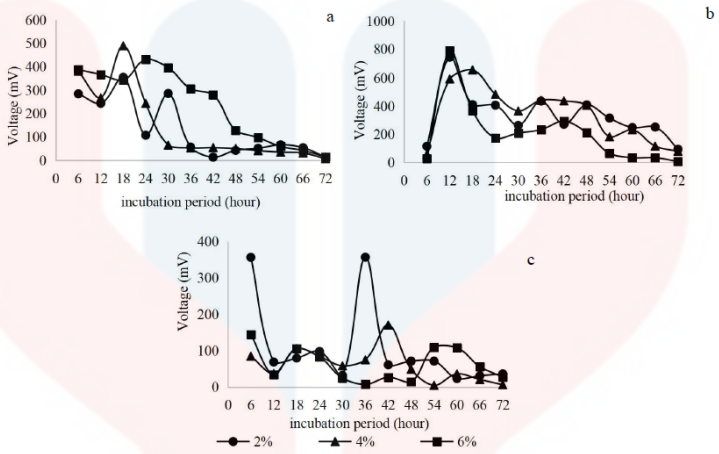

Figure 2.7: The voltage as a function of time from the MFC for different organic substrates: sugar (a), molasses (b), palm sugar (c)

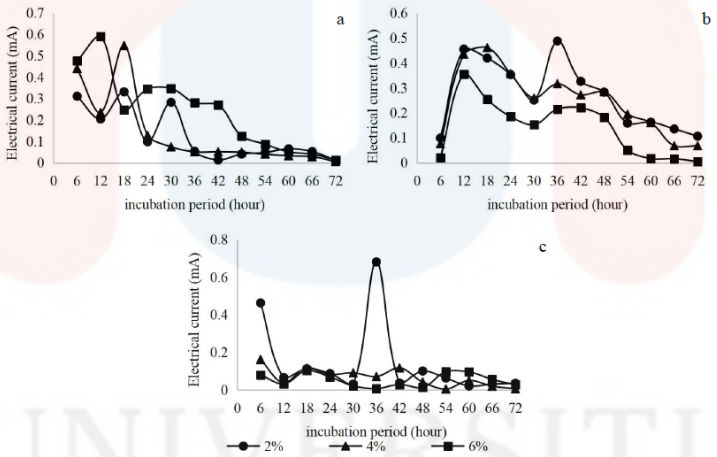

Figure 2.8: The electrical current as a function of time from the MFC for different organic substrates: sugar (a), molasses (b), palm sugar (c)

Source: (Khoirunnisa et al., 2021)

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2.6.3 Anode

The anode in the MFC plays an important role in electricity generation. The anode is the habitat of the electroactive bacteria and the place where the electrons are generated. The anode material must have good biocompatibility, a good electric conductor, and a large specific surface area. Due to the variety of organic matters in the anode chamber, the anode should be chemically stable and corrosion resistant. According to (Yu et al., 2021) research, graphite felt (GF), carbon cloth (CC), activated carbon fiber felt (ACFF), graphite paper (GP), and aluminium sheet (AS) were used as the anode electrode. The output of electricity generation by different anodes is different. Based on Figure 2.9, the graph shows that the GF produced the highest voltage output compared to other anode materials and it can keep maintain the voltage output with the duration of 84 days.

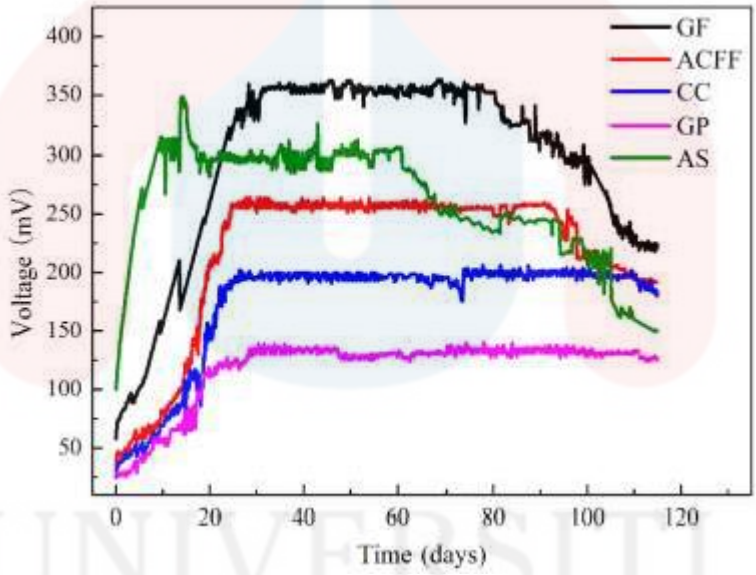

Figure 2.9: Voltage output of five SMFCs with different anode materials.

Source: (Yu et al., 2021)

On the other hand, copper is not suitable to use as the anode as it is toxic to the microbial community. Compared with (Aghababaie et al., 2015) study, copper is more suitable to use as the anode, however it has low electron transfer conductivity. In addition, the carbon anode can generate more electricity than the copper anode which is 2 mW/m^2 and 880 mW/m^2 respectively. Besides, graphite rods are more suitable to use as anodes in the MFC because it is more versatile because of its simple handling and low cost. Therefore, the most familiar anode material is made of graphite because it is inexpensive and can generate more electricity (Aghababaie et al., 2015).

2.6.4 Cathode

There are 2 important components in cathode chamber including cathode and electron acceptor. Commonly, the air-cathode is always used in the cathode chamber. Most of the air-cathodes are made of carbon cloth, and diffusion layers (DLs) as polytetrafluoroethylene (PTFE). This DLs are used to control the oxygen intrusion and elimination of water loss (Anjum et al., 2021). Electrode acceptors that can be utilized in the cathode chamber including oxygen, ferricyanide, ferric iron, hexacyanoferrate, manganese, H_2O_2 and nitrobenzene. However, oxygen is the best choice of electron acceptor because it is sustainable, available, and environmentally friendly. In addition, oxygen is also having a very electropositive redox potential, and this can increase the oxidation rate of the reaction (Anjum et al., 2021; Samuel Reinhard Ignatius Sitorus, 2020) .

According to (Aghababaie et al., 2015) research, the electricity generation in MFC is affected by the differences of the redox potential of anode and cathode. The electrons will be transferred from the organic substrates to the anode via a cascade of complex respiratory chain. The electrons will be transferred to the cathode through external circuit and the electrons will meet the terminal electrons acceptor. Based on the redox tower (Table 4), oxygen is the most suitable electrons acceptor as it has the highest positive E_0' value. In addition, this can form a bigger gap between the organic substrates and the oxygen. The bigger the gap between the organic substrates and the oxygen, it will produce a higher redox potential and hence generate higher electricity.

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Table 2.4: Redox potential of various reactions in MFC electrodes.

Source: (Aghababaie et al., 2015)

Oxidation/reduction pairs $E^{o\prime}$	(mV)
$2H^+/H_2$	−420
Ferredoxin(Fe^{3+})/ferredoxin(Fe^{2+})	−420
NAD + /NADH	−320
S/H_2S	−274
S_0/HS^-	−270
$SO4^{2-}/H_2S$	−220
Pyruvate^{2-} + $2H^+$ + $2e^-$ → lactate^{2-}	−185
2,6-AQDS/2,6-AHQDS	−184
FAD/FADH2	−180
Menaquinione ox/red	−75
Pyocyanin ox/red	−34
Humic substances ox/red	−200 to +300
Methylene blue ox/red	+11
Fumarate^{2-}/succinate^{2-}	+31
Thionine ox/red	+64
Cytochrome *b*(Fe^{3+})/cytochrome *b*(Fe^{2+})	+75
Fe(III) EDTA/Fe(II) EDTA	+96
Ubiquinone ox/red	+113
Cytochrome *c*(Fe^{3+})/cytochrome *c*(Fe^{2+})	+254
O_2/H_2O_2	+275
Fe(III) citrate/Fe(II) citrate	+385
Fe(III) NTA/Fe(II) NTA	+385
NO_3^-/NO_2^-	+421
$[Fe(CN)_6]^{3+}/[Fe(CN)_6]^{4+}$	+430
NO^{2-}/NH^{4+}	+440
MnO_2/Mn^{2+}	+600
Fe^{3+}/Fe^{2+}	+771
O_2/H_2O	+820

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CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

In this chapter, materials and methods are briefly presented for effect of POME pH on electricity generation by *Shewanella oneidensis* MR-1 in MFC. The stages involve in this study include the material used, the preparation of seed culture and culture medium, MFC setup, and electrochemical measurement.

3.2 Materials

The materials and apparatus that need to be used in the research are presented in Table 3.1.

Table 3.1: The materials and apparatus used in the research.

Bacteria strain	*Shewanella oneidensis* MR-1 (Dai et al., 2021)
Chemical reagent	Luria Bertani agar (LB agar), Luria Bertani Broth (LB Broth), 1.0 M hydrochloride acid (HCl), 1.0 M sodium hydroxide (NaOH), distilled water, 1% potassium chloride (KCl), agar powder
Apparatus	Petri dish, Schott bottle, dropper, glass rod, glass rod, spatula, beaker, conical flasks, parafilm, copper wires, crocodile clip, pH meter, carbon rod as electrodes, PVC clear hose, TRMS multimeter, scoop, sampling bag

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3.3 Methods

3.3.1 Preparation of LB medium and LB broth

To prepare LB medium, 10g of tryptone, 5g of yeast extract, 5g of NaCl, 15g of agar, and 1 liter of distilled water were required. Furthermore, to prepare the LB broth, the same components as in the LB medium were added to a serum bottle and gently swirled. The cap of the serum bottle was sealed with aluminum foil, and the mixture was autoclaved at a temperature of 151°C and a pressure of 15 psi for approximately 20 minutes. After autoclaving, the serum bottle was cooled and stored at 4°C. For the preparation of LB agar plates, the LB agar medium was autoclaved and poured into sterile petri dishes. Subsequently, the dishes were sealed and kept in a chiller at 4°C.

3.3.2 Preparation, Inoculation, and Cultivation of Bacterial Strain

The *S. oneidensis* MR-1 strain was retrieved from the American Type Culture Collection (ATCC) and stored at -80°C prior to use. The stock culture of MR-1 was cultured aerobically on Luria-Bertani (LB) agar, which contained 10g/L NaCl, 5g/L yeast extract, 10g/L tryptone, and 16g/L agar. The culture was incubated at 30°C for 2 days. After the incubation period, a single colony of MR-1 was inoculated into a test tube containing 5 mL of LB broth, consisting of 10g/L NaCl, 5g/L yeast extract, and 10g/L tryptone. The culture was grown under aerobic conditions in an orbital shaker at 150 rpm and 30°C. Subsequently, the culture was transferred into a 50 mL volume of LB broth in conical flask, serving as a new nutrient source, and cultivated for approximately one day. This culture served as the source of electroactive bacteria for the microbial fuel cell (MFC) (Wu et al., 2019).

3.4 POME treatment

Raw POME was collected from the nearby palm oil mill industry (Solid Orient Holdings Sdn. Bhd.). The samples were transported to the laboratory in plastic bottles and stored at 4°C until used. The pH of the POME was alkaline, about pH 10.

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3.5 Soil sampling

Several sampling points were selected to collect the soil samples. The soil samples were collected around the Gunung Reng compound. The pH of the soil samples was tested and the soil samples around pH 9 were collected by using a scoop and kept in the sampling bag in the chiller.

Table 3.2: The location coordinates and the pH of the collected soil samples.

Samples	Coordinate	pH
1	5° 42' 52.7112" N 101° 44' 55.9428" E	9.04
2	5° 42' 55.0872" N 101° 44' 43.4976" E	9.12
3	5° 42' 54.7632" N 101° 44' 42.3348" E	9.05

3.6 MFC Construction

A two-chambered MFC reactor was constructed in this study. The MFC reactor was composed of two conical flasks and a salt bridge is used as a medium for the protons flow within two chambers. Each chamber of the double chamber had two holes, one with a diameter of 6 mm and the other with a diameter of 1.5 mm, on the lids for the insertion of the electrodes. It was prepared by using a mixture of 1% KCl solutions and agar in a ratio of 1:1 (Jalilluddin et al., 2015). Next, 250 µL of the *S. oneidensis* MR-1 inoculum was filled in the anode compartment of the MFC reactor. The compartment was then filled with POME until it reached a volume of 250 mL. The cathode chamber is filled up with 250 mL of potassium ferricyanide ($K_3[Fe(CN)_6]$) as the electron acceptor to complete the circuit. Carbon rod with 0.5 cm diameter and 10 cm length were used as electrodes in the MFC. To establish a closed circuit, copper wires were used to connect the electrodes externally. Afterwards, the MFC was sterilized using ethanol to minimize contamination before transferring the inoculum to the anode chamber. Once the sterilization process was complete, the MR-1 culture was transferred into the anode chamber. The MFC was then

operated for approximately one week to measure electricity generation (Jamlus et al., 2021).

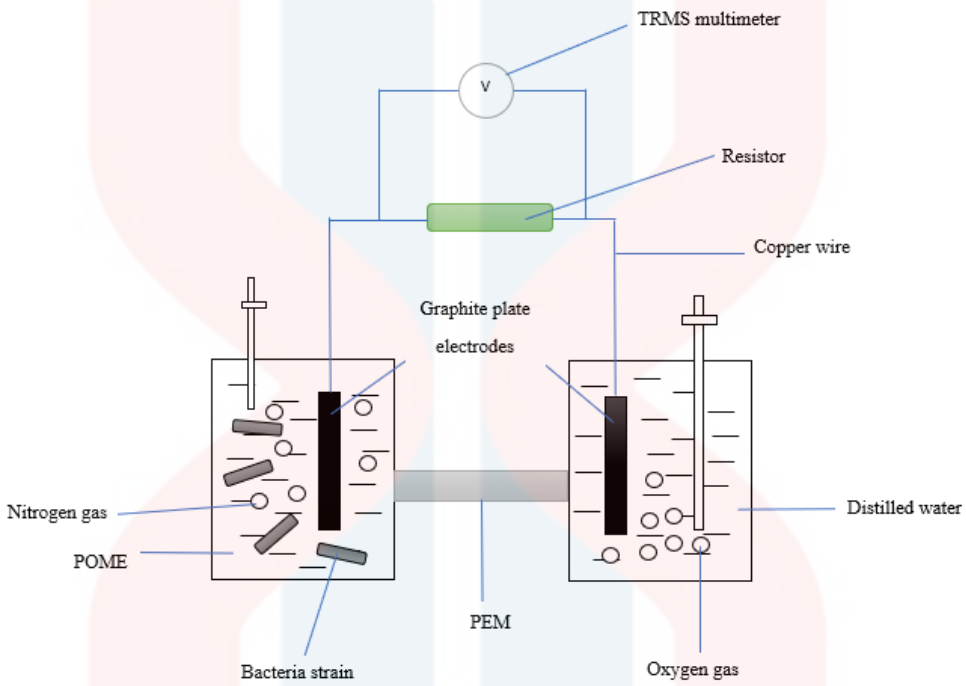

Figure 3.1: The schematic diagram of MFC setup.

3.6.1 Enrichment of MFC with alkaline soil samples

A MFC was setting up by adding 20g of soil sample and 280 mL of POME into the anode chamber. Then, the setup up was same as the method in section 3.6.

3.7 Electrochemical Measurement

3.7.1 Voltage Production

During the incubation period, the voltage measurement was carried out using a wireless true root mean square (TRMS) multimeter (brand). The voltage production was recorded everyday using the TRMS multimeter with a data logger.

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3.7.2 Current Production

After measuring the voltage production, the current production from the MFC was measured using the TRMS multimeter. The current production was recorded every day with a data logger on the TRMS multimeter.

3.7.3 Power production

The electricity generation by the MFC was measured once the voltage output reached a steady state. The steady state was determined when the voltage readings became stable after the operation. The current and voltage were monitored daily using the TRMS multimeter for a period of 72 hours. These measurements were used to calculate the power according to Equation (3.2):

$$P = IV \qquad \text{Equation 3.1}$$

where P is power in Watt (W), I is current in Ampere (A) and V is voltage in Volt (V).

The magnitude of power production is then divided by the surface area of the electrode to calculate the power density.

$$\textit{Power density} = \textit{Power} / \textit{surface area of the electrode} \qquad \text{Equation 3.2}$$

3.8 Bacteria Identification and Characterization

3.8.1 Gram Staining

The cells from the fresh cultures were used for Gram staining. The morphology of the samples was determined using light microscope after the gram staining procedures. The steps involved in Gram staining were the application of the primary stain which in this case was the crystal violet, application of iodine solution, the decolorization step and last step was the application of red stain, safranin. Gram positive bacteria will result in purple stains while Gram negative bacteria will result in red or pink stains.

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3.8.2 Catalase Test

Catalase is a universal antioxidant enzyme which can degrade the hydrogen peroxide H_2O_2 into oxygen and water (Iwase et al., 2013). To determine the capability of bacterial isolate in producing catalase enzyme, a small amount of bacterial colony was transferred to a clean and dry glass slide using an inoculum loop and a drop of 3% H_2O_2 is dropped on the slide.

3.8.3 Oxidase Test

Oxidase test is used to identify the bacteria that can produce the cytochrome c oxidase which is an enzyme that takes part in the bacterial electron transport chain. In the oxidase test, the bacterial colony was smeared with the sterile cotton swab on the filter paper and dropped some substrate tetramethyl-p-phenylenediamine dihydrochloride onto the colony. The colour change within 10 to 30 seconds was observed.

3.9 Identification of bacteria strain

The isolated electroactive bacteria were identified by observing the morphological and biochemical test such as the appearance of the colony in the agar plate, Gram-stanning, catalase taste, oxidase test, and 16s RNA identification.

3.9.1 DNA extraction from the enriched MFC

After the incubation period of the enriched MFC, serial dilution process was carried out to get a single colony of the bacteria from the alkaline POME. The bacteria were grown on LB plate for 24 hours, and then transferred into LB broth for overnight. The DNA was extracted from the overnight culture following the protocol provided by Macherey-Nagel DNA extraction kit.

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3.9.2 Gel electrophoresis

The extracted DNA was tested by using the gel electrophoresis. The DNA was loaded into 1% w/v agarose gel with HindIII marker. The sample was run at 80V and 200mA for 30 minutes. The band appeared on the agarose gel indicated the presence of extracted DNA for the isolated electroactive bacteria. The gel was observed under UV light.

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

Shewanella oneidensis MR-1 can generate electricity in the MFC which supplemented with the alkaline POME. However, the POME which enriched by the alkaline soil sample can produce higher electricity. Therefore, the electroactive bacteria (EAB) were isolated from the POME in the anode chamber.

4.2 Electricity Generation in the MFC supplemented by alkaline POME

Double chambered MFC was constructed using the conical flasks (Figure 3.1). *Shewanella oneidensis* MR-1 was used as the electrogen to produce electricity in the MFC. The MR-1 was inoculated in the anode chamber and incubated for one week. The voltage and current reading were taken every day to calculate the power density. The electricity that generated by the EAB was depicted in the Figure 4.1.

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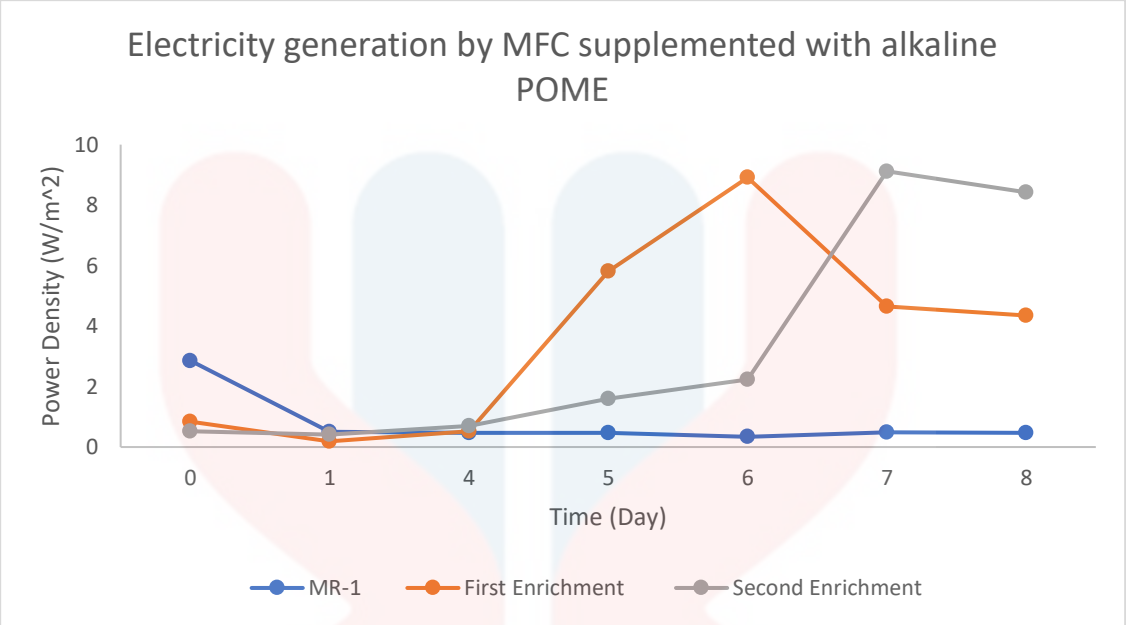

Figure 4.1: The power density that produced by MR-1 and the enrichment by the soil sample.

According to the Figure 4.1, the power density that produced by MR-1 was very low compared to the enrichment by the soil samples. In the alkaline anode chamber, MR-1 generated 2.859 W/m^2 power density on the Day-0. After Day-0, the power density generation was reduced and remain the same at the rage of 0.300-0.600 W/m^2 until Day-8. The power density dropped about 83.57% from Day-0 until Day-8. The electricity generation by MR-1 was low because the MR-1 is neutrophilic bacteria, and this will result in reduction of the metabolic activity. According to (Fathollahi & Coupe, 2021), the optimum pH for the biofilm formation was at pH 7 but the biofilm formation in alkaline condition at alkaline pH was better than acid conditions. Therefore, increasing the pH in the POME has a positive effect on the current density because the electron transfer is easier but not much (Malekmohammadi & Ahmad Mirbagheri, 2021).

To isolate the EAB that can generate more electricity in the alkaline condition, the alkaline soil which collected from the Gunung Reng area was added into the anode chamber. This was because there might be more bacterial community that capable to produce more electricity in the alkaline condition. In the first enrichment setup, the highest power density that generated was 8.910 W/m^2. The highest power density was achieved on Day-6 because the bacterial communities in the soil sample need to take time to adapt to the new environment. The substrate in the POME was act as electron donor to the bacteria in the soil. The alkaliphilic bacteria accepted the electrons and the electrons

transferred to the electrode to produce electricity. After Day-6, the electricity generation dropped to 0.470 W/m^2 maybe because the nutrients in the POME was reduced.

When the electricity generation achieved the peak of the graph, 10 mL of the sample from anode was transferred to a new POME for the second enrichment. This step was conducted to enhance the growth of the alkaliphilic bacteria and generate more electricity in the second enrichment, it took a day longer in the lagging phase to generate electricity. On Day-0, the power density was 0.520 W/m^2 and it continued increasing gradually until it achieved the peak on Day-7. The highest power density that generated in the second enrichment was 9.119 W/m^2. After it reached the highest peak of the performance, it began to drop. Compared to Figure 4.6, the voltage generation in this setup was higher after the first enrichment but only a slightly difference which was 5.64%. The time interval for the electricity generation in the second enrichment was longer than the first enrichment setup.

4.3 Isolation of microorganisms

Enrichment technique and serial dilution method were used to isolate the electroactive bacteria (EAB) from the anode chamber in the MFC which supplemented with alkaline POME and soil sample. In the first enrichment, 20 g of soil sample was added into the POME in the anode chamber, the electricity generation was higher than the MFC supplemented with alkaline POME and MR-1. After a week, 10 mL of the sample from the first enrichment was transferred to the new anode chamber of POME for the second enrichment. After a week, the highest electricity generation was 9.119 W/m^2 higher than the first enrichment about 2.28%. It was maybe because the mesophilic bacteria were died during the first enrichment and only left the alkaliphilic bacteria. In the second enrichment, there left only alkaliphilic bacteria and this caused more electricity was generated.

After the second enrichment, serial dilution was conducted to isolate the bacteria. 1 mL of sample from the second enrichment was transferred to the test tube for the serial dilution. The 10^{-2} was used for the further investigation. The 10^{-2} sample were circular, milky yellow, smooth, and opaque. Only one type of colony occurred, and the colony was streak on the new LB agar plate to get the pure culture.

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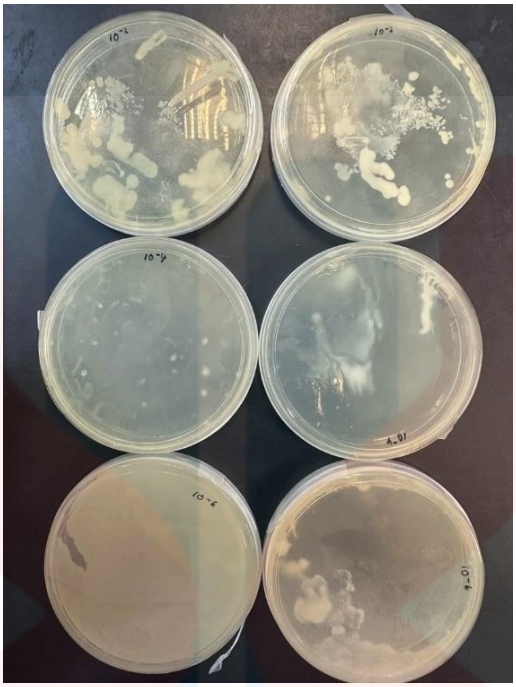

Figure 4.2: The bacteria colonies isolated from the POME by serial dilution.

4.4 Identification of Microorganisms

4.4.1 Gram staining

Gram staining was done to identify whether the isolated EAB was Gram positive or Gram negative and the morphology of the sample. The observation was done under a camera light microscope at 100x magnification with the aid of oil immersion.

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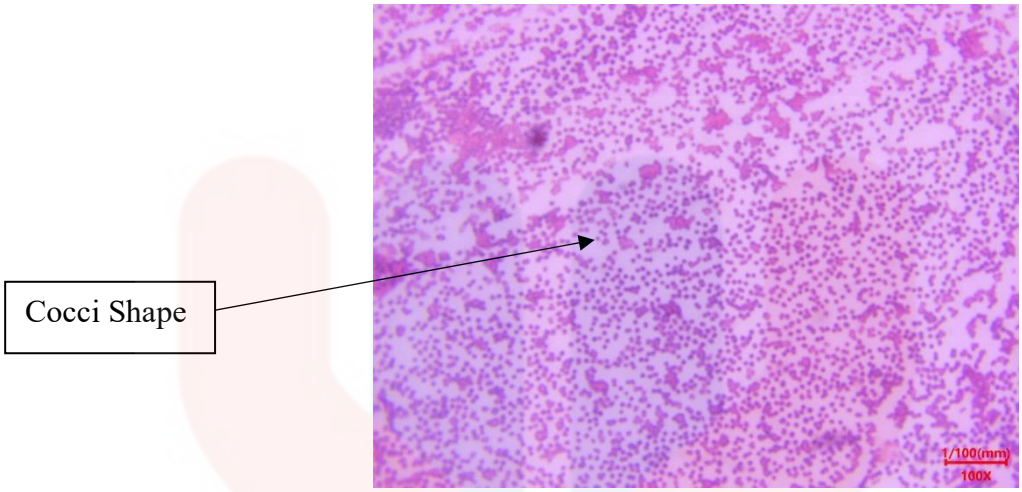

Figure 4.3: The morphology of Gram-negative electroactive bacteria isolated from alkaline POME under 100X camera light microscope.

Figure 4.3 indicated that the isolated EB, also known as GR-1, was pink colour stain and cocci shape. The pink colour stain showed that the bacteria were in Gram negative. The Gram-negative bacteria have a thin peptidoglycan layer and high lipid content cell walls. During the Gram staining process, the purple crystal violet stain was decolorized by the acetone and the positively charged pink safranin was added to counterstain the Gram-negative bacteria.

4.4.2 Catalase Test

In the catalase test, it showed negative result which was no bubbles produced after added the hydrogen peroxide, H_2O_2 on the single colony of GR-1. This was because the isolated does not produce catalase enzyme that can protect the bacteria from H_2O_2. Catalase can degrade the H_2O_2 into water and oxygen.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Bacteria lacking catalase activity can exhibit anaerobic characteristics, functioning either as obligate anaerobes or as facultative anaerobes exclusively engaging in fermentation while abstaining from aerobic respiration, wherein oxygen serves as the terminal electron acceptor.

Figure 4.4: Catalase test. Absence of bubbles formation indicated the negative catalase test activity of GR-1.

4.4.3 Oxidase Test

In the oxidase test, the tetramethyl-p-phenylene diamine dihydrochloride (TMPD) is used as the basic dye which function as biological electron donors (Jurtshuk & McQuitty, 1976). This test is used to detect the cytochrome-*c* (cyt-*c*) as well as oxidase enzyme. Cyt-*C* is a small globular protein that act as electron transporter in aerobic as well as anaerobic respiration (Zaidi et al., 2014). From the oxidase test, GR-1 did not change to dark blue colour and this indicates that GR-1 was oxidase negative. It was because GR-1 does not contain Cyt-*C* and the electrons cannot flow to reduce the oxygen. Therefore, the negative oxidase test indicated that GR-1 might be anaerobic.

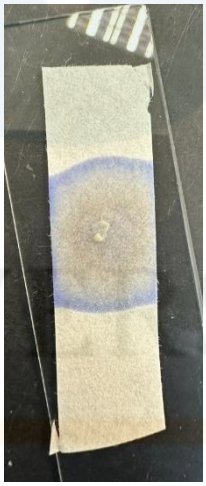

Figure 4.5: Oxidase Test. Negative oxidase test of GR-1.

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4.5 Soil DNA Extraction

Figure 4.6 showed the result of the DNA extraction on the agarose gel. Compared to the DNA ladder, the DNA sizes were about 9400 bp.

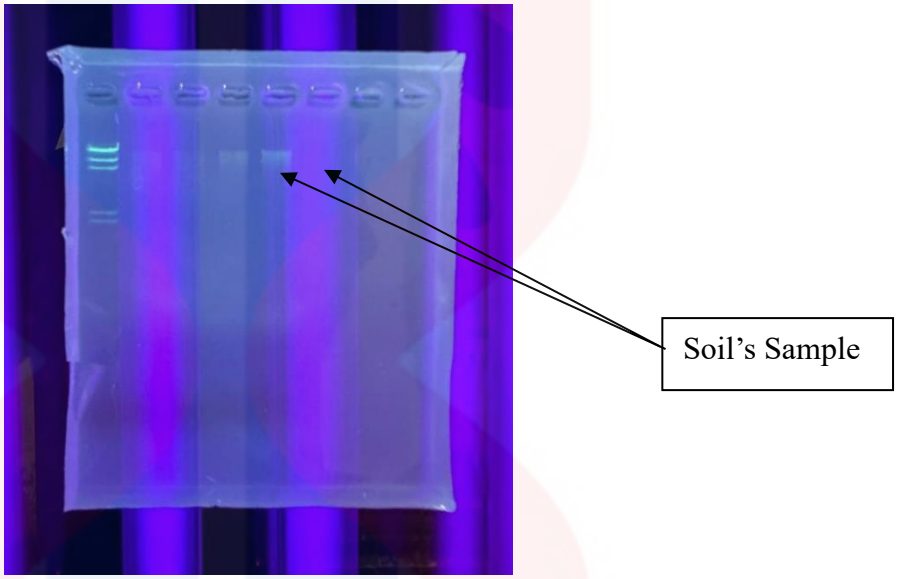

Figure 4.6: The DNA bands of the soil's sample under UV light.

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CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In this research, this research is carried out to isolate and characterize the electroactive bacteria for generation of electricity in MFC supplemented with POME as substrate. The MFC can generate electricity and the electricity was measured by using the true root mean square (TRMS) multimeter. MR-1 and the soil sample were used as the electrogens to produce electricity. Conclusions that can retrieved from this research were:

1) *Shewanella oneidensis* MR-1 is not suitable for electricity generation in the MFC supplemented with high pH POME.
2) The alkaline soil sample produced more electricity in the MFC supplemented with the high pH POME.
3) The isolated GR-1 was Gram negative cocci shape, catalase, and oxidase negative.

We successfully isolated the EAB that is suitable for application in MFC by using alkaline POME. Further research needs to be done to identify the strain of the isolated EAB.

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5.2 Recommendations

It is highly recommended that a better setup of the MFC container must be applied and continuous nitrogen sparging to maintain the anaerobic condition in the anode chamber. The anaerobic condition in anode chamber was implemented to avoid competitive utilization of electron acceptor with oxygen. This is because the oxygen is more electropositive than other organic substrate in the POME and hence the electroactive bacteria in the MFC cannot accept the electrons from the organic substrate. Apart from that, the voltage generated is very low, so different substrate can be used in the anode chamber to increase the electricity generation. In addition, the different extremophile bacteria such as thermophile, acidophile, alkaliphile, and others need to be isolated so the bacteria can be implemented in different conditions of wastewaters. Further research needs to be done to increase the time interval for the electricity generation by the MFC in the future.

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REFERENCES

A Aziz, M. M., Kassim, K. A., ElSergany, M., Anuar, S., Jorat, M. E., Yaacob, H., Ahsan, A., Imteaz, M. A., & Arifuzzaman. (2020). Recent Advances On Palm Oil Mill Effluent (POME) Pretreatment And Anaerobic Reactor For Sustainable Biogas Production. *Renewable and Sustainable Energy Reviews*, *119*, 109603. https://doi.org/https://doi.org/10.1016/j.rser.2019.109603

Aghababaie, M., Farhadian, M., Jeihanipour, A., & Biria, D. (2015). Effective Factors On The Performance Of Microbial Fuel Cells In Wastewater Treatment – A Review. *Environmental Technology Reviews*, *4*(1), 71-89. https://doi.org/10.1080/09593330.2015.1077896

Aiyer, K. S. (2020). How Does Electron Transfer Occur In Microbial Fuel Cells? *World J Microbiol Biotechnol*, *36*(2), 19. https://doi.org/10.1007/s11274-020-2801-z

Akhbari, A., Kutty, P. K., Chuen, O. C., & Ibrahim, S. (2020). A Study Of Palm Oil Mill Processing And Environmental Assessment Of Palm Oil Mill Effluent Treatment. *Environmental Engineering Research*, *25*(2), 212-221.

Alkhair, K. B., Hassan, O. H., Aminah, S., YapKian, C., Rahman, Z. A., & Yahya, M. Z. A. (2018). Palm Oil Mill Effluent's Microbial Fuel Cell's Optimisation Procedure By Using Two-Level Factorial Design Method And Chemical Oxygen Demand Treatment. *Journal of Oil Palm Research*.

Anjum, A., Ali Mazari, S., Hashmi, Z., Sattar Jatoi, A., & Abro, R. (2021). A Review Of Role Of Cathodes In The Performance Of Microbial Fuel Cells. *Journal of Electroanalytical Chemistry*, *899*, 115673. https://doi.org/https://doi.org/10.1016/j.jelechem.2021.115673

Bertling, K., Banerjee, A., & Saffarini, D. (2021). Aerobic Respiration and Its Regulation in the Metal Reducer *Shewanella oneidensis*. *Front Microbiol*, *12*, 723835. https://doi.org/10.3389/fmicb.2021.723835

Biffinger, J., Pietron, J., Nadeau, L., Johnson, G., Williams, C., Nealson, K., & Ringeisen, B. (2008). The Influence Of Acidity On Microbial Fuel Cells Containing *Shewanella oneidensis*. *Biosensors & bioelectronics*, *24*, 906-911. https://doi.org/10.1016/j.bios.2008.07.034

Cheng, J., Zhu, X., Ni, J., & Borthwick, A. (2010). Palm Oil Mill Effluent Treatment Using A Two-Stage Microbial Fuel Cells System Integrated With Immobilized Biological Aerated Filters. *Bioresource Technology*, *101*(8), 2729-2734. https://doi.org/https://doi.org/10.1016/j.biortech.2009.12.017

Dai, H. N., Duong Nguyen, T.-A., My Le, L.-P., Tran, M. V., Lan, T.-H., & Wang, C.-T. (2021). Power Generation Of *Shewanella oneidensis* MR-1 Microbial Fuel Cells In Bamboo Fermentation Effluent. *International Journal of Hydrogen Energy*, *46*(31), 16612-16621. https://doi.org/https://doi.org/10.1016/j.ijhydene.2020.09.264

Fankhauser, S., Smith, S. M., Allen, M., Axelsson, K., Hale, T., Hepburn, C., Kendall, J. M., Khosla, R., Lezaun, J., Mitchell-Larson, E., Obersteiner, M., Rajamani, L., Rickaby, R., Seddon, N., & Wetzer, T. (2022). The Meaning Of Net Zero And How To Get It Right. *Nature Climate Change*, *12*(1), 15-21. https://doi.org/10.1038/s41558-021-01245-w

Fathollahi, A., & Coupe, S. J. (2021). Effect Of Environmental And Nutritional Conditions On The Formation Of Single And Mixed-Species Biofilms And Their Efficiency In Cadmium Removal. *Chemosphere*, *283*, 131152. https://doi.org/https://doi.org/10.1016/j.chemosphere.2021.131152

Fauque, G. D., & Barton, L. L. (2012). Chapter 1 - Hemoproteins In Dissimilatory Sulfate- And Sulfur-Reducing Prokaryotes. In R. K. Poole (Ed.), *Advances in Microbial Physiology* (Vol. 60, pp. 1-90). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-398264-3.00001-2

Foad Marashi, S. K., & Kariminia, H.-R. (2015). Performance Of A Single Chamber Microbial Fuel Cell At Different Organic Loads And Ph Values Using Purified Terephthalic Acid Wastewater. *Journal of Environmental Health Science and Engineering*, *13*(1), 27. https://doi.org/10.1186/s40201-015-0179-x

Hau, H. H., & Gralnick, J. A. (2007). Ecology And Biotechnology Of The Genus *Shewanella*. *Annu Rev Microbiol*, *61*, 237-258. https://doi.org/10.1146/annurev.micro.61.080706.093257

Irshaid, F. (2013). A New *Shewanella putrefaciens* Strain Isolated From The Dead Sea Of Jordan. *Malaysian Applied Biology Journal*, *42*.

Iwase, T., Tajima, A., Sugimoto, S., Okuda, K., Hironaka, I., Kamata, Y., Takada, K., & Mizunoe, Y. (2013). A Simple Assay For Measuring Catalase Activity: A Visual Approach. *Sci Rep*, *3*, 3081. https://doi.org/10.1038/srep03081

FYP FBKT

Jalilluddin, A., Tay, C., & Abdul-Talib, S. (2015). Performance of Two-Chambered Microbial Fuel Cell (MFC) at Different pH Anode Microenvironment Using Palm Oil Mill Effluent (POME) as Substrate. *Applied Mechanics and Materials*, *773-774*, 511-519. https://doi.org/10.4028/www.scientific.net/AMM.773-774.511

Jamlus, N. I. I. M., Masri, M. N., Wee, S. K., & Shoparwe, N. F. (2021). Electricity Generation by Locally Isolated Electroactive Bacteria in Microbial Fuel Cell. *IOP Conference Series: Earth and Environmental Science*, *765*(1), 012115. https://doi.org/10.1088/1755-1315/765/1/012115

Jatoi, A. S., Akhter, F., Mazari, S. A., Sabzoi, N., Aziz, S., Soomro, S. A., Mubarak, N. M., Baloch, H., Memon, A. Q., & Ahmed, S. (2021). Advanced Microbial Fuel Cell For Waste Water Treatment-A Review. *Environ Sci Pollut Res Int*, *28*(5), 5005-5019. https://doi.org/10.1007/s11356-020-11691-2

Juliastuti, S. R., Darmawan, R., Ayuningtyas, A., & Ellyza, N. (2018). The Utilization Of *Eschericia coli* And *Shewanella oneidensis* For Microbial Fuel Cell. *IOP Conference Series: Materials Science and Engineering*, *334*(1), 012067. https://doi.org/10.1088/1757-899X/334/1/012067

Jumadi, J., Kamari, A., & Wong, S. T. S. (2020). Water Quality Assessment And A Study Of Current Palm Oil Mill Effluent (POME) Treatment By Ponding System Method. *IOP Conference Series: Materials Science and Engineering*, *980*(1), 012076. https://doi.org/10.1088/1757-899X/980/1/012076

Jurtshuk, P., Jr., & McQuitty, D. N. (1976). Use Of A Quantitative Oxidase Test For Characterizing Oxidative Metabolism In Bacteria. *Appl Environ Microbiol*, *31*(5), 668-679. https://doi.org/10.1128/aem.31.5.668-679.1976

Khoirunnisa, N., Anwar, S., Sudadi, U., & Santosa, D. A. (2021). Effect Of Organic Substrate Type In Electricity Production From Microbial Fuel Cell (MFC) Inoculated By *Staphylococcus saprophyticus* ICBB 9554. *IOP Conference Series: Earth and Environmental Science*, *927*, 012028. https://doi.org/10.1088/1755-1315/927/1/012028

Koleva, R., Peeva, G., Yemendzhiev, H., & Nenov, V. (2022). Potential Use of Microbial Fuel Cell Technology in Wastewater Treatment. *Processes*, *10*(3), 486. https://www.mdpi.com/2227-9717/10/3/486

Leela, D., Nur, S. M., Yandri, E., & Ariati, R. (2018, November 01, 2018). Performance of Palm Oil Mill Effluent (POME) as Biodiesel Source Based on Different Ponds. E3S Web of Conferences,

FYP FBKT

Leung, K. M., Wanger, G., El-Naggar, M. Y., Gorby, Y., Southam, G., Lau, W. M., & Yang, J. (2013). *Shewanella oneidensis* MR-1 Bacterial Nanowires Exhibit P-Type, Tunable Electronic Behavior. *Nano Lett*, *13*(6), 2407-2411. https://doi.org/10.1021/nl400237p

Lovley, D. R. (1993). Dissimilatory Metal Reduction. *Annu Rev Microbiol*, *47*, 263-290. https://doi.org/10.1146/annurev.mi.47.100193.001403

Lovley, D. R., Holmes, D. E., & Nevin, K. P. (2004). Dissimilatory Fe(III) and Mn(IV) Reduction. *Adv Microb Physiol*, *49*, 219-286. https://doi.org/10.1016/s0065-2911(04)49005-5

MacDonell, M. T., & Colwell, R. R. (1985). Phylogeny of the Vibrionaceae, and Recommendation for Two New Genera, *Listonella* and *Shewanella*. *Systematic and Applied Microbiology*, *6*(2), 171-182. https://doi.org/https://doi.org/10.1016/S0723-2020(85)80051-5

Maddalwar, S., Kumar Nayak, K., Kumar, M., & Singh, L. (2021). Plant Microbial Fuel Cell: Opportunities, Challenges, And Prospects. *Bioresour Technol*, *341*, 125772. https://doi.org/10.1016/j.biortech.2021.125772

Malekmohammadi, S., & Ahmad Mirbagheri, S. (2021). A Review Of The Operating Parameters On The Microbial Fuel Cell For Wastewater Treatment And Electricity Generation. *Water Science and Technology*, *84*(6), 1309-1323. https://doi.org/10.2166/wst.2021.333

Mane, V. B., Nayakar, R., Khan, S., Dontul, A., & Diwate, S. R. (2022). Waste Management And Energy Generation With The Help Of Microbial Fuel Cell.

Matsumoto, A., Nagoya, M., Tsuchiya, M., Suga, K., Inohana, Y., Hirose, A., Yamada, S., Hirano, S., Ito, Y., Tanaka, S., Kouzuma, A., & Watanabe, K. (2020). Enhanced Electricity Generation In Rice Paddy-Field Microbial Fuel Cells Supplemented With Iron Powders. *Bioelectrochemistry*, *136*, 107625. https://doi.org/https://doi.org/10.1016/j.bioelechem.2020.107625

Nguyen, D.-T., Tamura, T., Tobe, R., Mihara, H., & Taguchi, K. (2020). Microbial Fuel Cell Performance Improvement Based On Flic-Deficient *E. coli* Strain. *Energy Reports*, *6*, 763-767. https://doi.org/https://doi.org/10.1016/j.egyr.2020.11.133

Nosek, D., Jachimowicz, P., & Cydzik-Kwiatkowska, A. (2020). Anode Modification as an Alternative Approach to Improve Electricity Generation in Microbial Fuel Cells. *Energies*, *13*, 6596. https://doi.org/10.3390/en13246596

FYP FBKT

Nuaini, N., Hamdani, H., & Rizal, T. A. (2018). Feasibility Study Of Electricity Power Plant From Biogas By Utilizing The Liquid Waste Of Palm Oil Mill In Aceh Province. *IOP Conference Series: Materials Science and Engineering*, *434*(1), 012170. https://doi.org/10.1088/1757-899X/434/1/012170

Obileke, K., Onyeaka, H., Meyer, E. L., & Nwokolo, N. (2021). Microbial Fuel Cells, A Renewable Energy Technology For Bio-Electricity Generation: A Mini-Review. *Electrochemistry Communications*, *125*, 107003. https://doi.org/https://doi.org/10.1016/j.elecom.2021.107003

Pradhan, B., & Pradhan, R. (2020). Performance Of Inherent Electrogens In Benthic Sediment Mud At Different pH Conditions In Microbial Fuel Cell. *European Scientific Journal*. https://doi.org/10.19044/esj.2020.v16n30p1

Salihu, A., & Alam, M. (2012). Palm Oil Mill Effluent: A Waste Or A Raw Material? *Journal of Applied Science Research*, *8*, 466-473.

Samuel Reinhard Ignatius Sitorus. (2020). *Review on high-performance air cathode microbial fuel cell for power generation and COD reduction*. Penerbit Universiti Kebangsaan Malaysia. http://journalarticle.ukm.my/17192/1/02.pdf

Saravanan, A., Karishma, S., Kumar, P. S., Yaashikaa, P. R., Jeevanantham, S., & Gayathri, B. (2023). Microbial Electrolysis Cells And Microbial Fuel Cells For Biohydrogen Production: Current Advances And Emerging Challenges. *Biomass Conversion and Biorefinery*, *13*(10), 8403-8423. https://doi.org/10.1007/s13399-020-00973-x

Satomi, M. (2014). *Shewanella.* In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of Food Microbiology (Second Edition)* (pp. 397-407). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-384730-0.00307-4

Tan, H. M., Lew, J., Gouwanda, D., & Poh, P. E. (2017). Fuzzy Logic Modelling For Thermophilic Anaerobic Digestion Of Palm Oil Mill Effluent (POME) Treatment. https://doi.org/10.1109/IEA.2017.7939219

Watanabe, K. (2008). Recent Developments In Microbial Fuel Cell Technologies For Sustainable Bioenergy. *J Biosci Bioeng*, *106*(6), 528-536. https://doi.org/10.1263/jbb.106.528

Wee, S. K., Burns, J. L., & DiChristina, T. J. (2014). Identification Of A Molecular Signature Unique To Metal-Reducing Gammaproteobacteria. *FEMS Microbiol Lett*, *350*(1), 90-99. https://doi.org/10.1111/1574-6968.12304

FYP FBKT

Wu, H., Wu, Q., Zhang, J., Gu, Q., Wei, L., Guo, W., & He, M. (2019). Chromium Ion Removal From Raw Water By Magnetic Iron Composites And S*hewanella oneidensis* MR-1. *Scientific Reports*, *9*, 3687. https://doi.org/10.1038/s41598-018-37470-1

Yu, B., Feng, L., He, Y., Yang, L., & Xun, Y. (2021). Effects Of Anode Materials On The Performance And Anode Microbial Community Of Soil Microbial Fuel Cell. *Journal of Hazardous Materials*, *401*, 123394. https://doi.org/https://doi.org/10.1016/j.jhazmat.2020.123394

Zaidi, S., Hassan, M. I., Islam, A., & Ahmad, F. (2014). The Role Of Key Residues In Structure, Function, And Stability Of Cytochrome-C. *Cell Mol Life Sci*, *71*(2), 229-255. https://doi.org/10.1007/s00018-013-1341-1

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APPENDIX A

Figure A.1: *Shewanella oneidensis* MR-1 on LB agar plate.

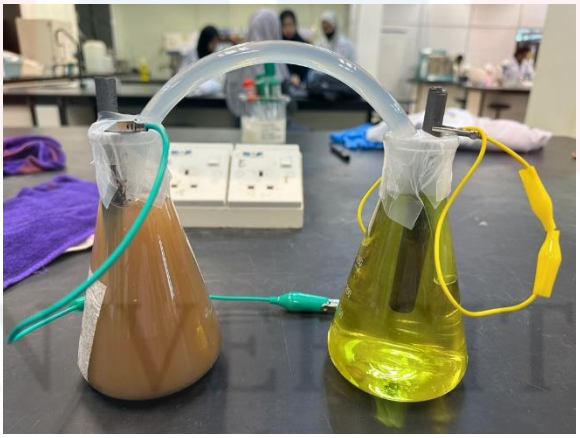

Figure A.2: Dual-chamber microbial fuel cell (DMFC) setup.

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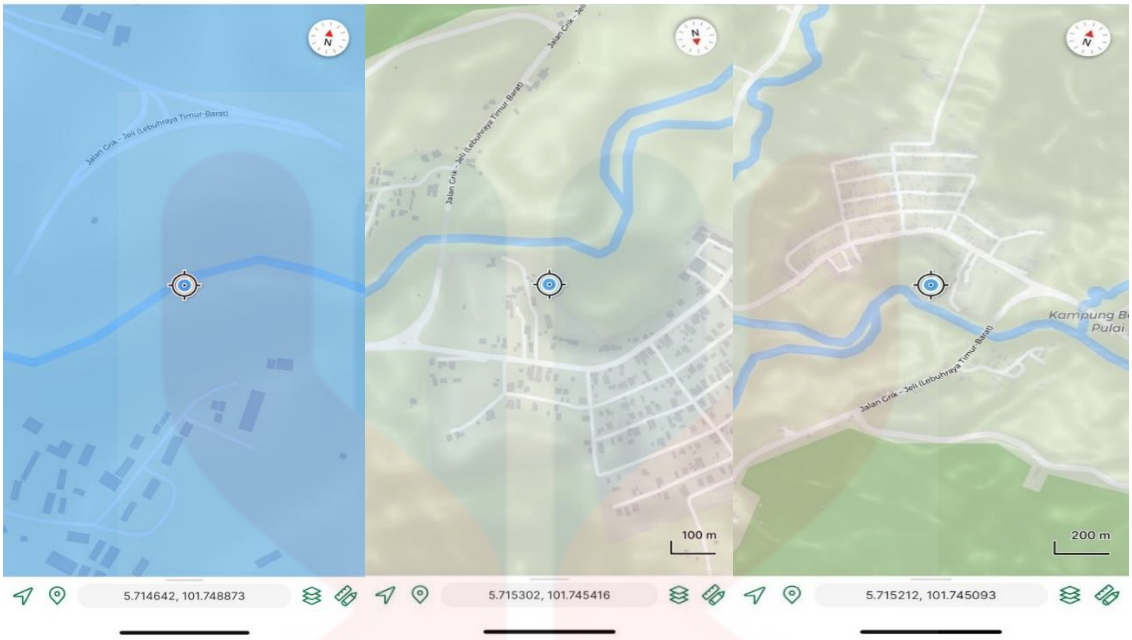

Figure A.3: The locations of the soil sampling.

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