Doctoral Thesis

Development of practical Microbial Fuel Cells for electrical generation and biosensing applications

March 2023

Doctoral Program in Advanced Electrical,
Electronic and Computer Systems
Graduate School of Science and Engineering
Ritsumeikan University

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Doctoral Thesis Reviewed
by Ritsumeikan University

Development of practical Microbial Fuel Cells for electrical generation and biosensing applications

(発電およびバイオセンシングアプリケーションのための
実用的な微生物燃料電池の開発)

March 2023
2023 年3 月

Doctoral Program in Advanced Electrical,
Electronic and Computer Systems
Graduate School of Science and Engineering
Ritsumeikan University
立命館大学大学院理工学研究科
電子システム専攻

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DECLARATION

I, Nakamoto Dung, whose former name was Nguyen Hoang Uyen Dung, declare that this thesis titled “Development of practical Microbial Fuel Cells for electrical generation and biosensing applications” and the works presented in it are my own. I confirm that:

- The research for this thesis was completed while pursuing a degree at Ritsumeikan University.
- Any parts of this thesis previously submitted for a degree or qualification at any institution are noted.
- Any published work consulted is appropriately attributed.
- Any quotes from other sources are correctly cited. Aside from these quotes, the entire thesis is my work.
- All sources of help have been appropriately acknowledged.

Signed:_____

Date: _____

ACKNOWLEDGEMENTS

In this thesis, the research was conducted at the Biophotonics Laboratory at Ritsumeikan University from 2020 to 2023. Throughout my research journey, I would like to express my gratitude to everyone who supported me during this time, both in academic and non-academic ways.

I am incredibly grateful to my supervisor, Prof. Taguchi Kozo. I would like to express my deepest thanks for allowing me to join his lab as a doctoral student and work under his excellent guidance with continuous support throughout my study life. His invaluable advice, kind encouragement, and patience have motivated me to overcome the difficulties in pursuing my research. Working and studying under his supervision is my great honor and privilege.

I appreciate Assistant Prof. Dr. Nakamoto Trang for his treasured support that influenced my research direction. My study would not have been successful without his substantial help, encouragement, inspiration, and guidance.

I acknowledge Prof. Minemoto and Prof. Araki for their support in doing SEM and EDS tests during my research.

I am very grateful to Dr. Tran Quoc Thinh, who gave me valuable comments and advice for my research. I would like to thank my current and former lab mates for sharing their friendship and helpful assistance. They have helped me immensely in my research from the beginning of my doctoral program.

I also would like to convey my special thanks to Ritsumeikan University for exempting me from the academic tuition fee of my doctoral program.

I am deeply grateful to my loving family, especially my parents, who always comfort and cheer me up no matter my difficulties. Big thanks to my friends for their kindness and for helping me from the beginning of Japanese life.

Best of all, I want to express my thanks to my loving husband and children for their unwavering support and affection. They are the biggest motivation in my life.

Thank you all for making my research journey overflow with love and care.

Nakamoto Dung

December, 2022

Kusatsu, Shiga, Japan

LIST OF PUBLICATIONS

Journal Publications

1. Hoang-Uyen-Dung Nguyen, Dang-Trang Nguyen, Kozo Taguchi, "A Novel Design Portable Plugged-typed Soil Microbial Fuel Cell for Bioelectricity Generation", Energies, volume 14, issue 3, 553, January 2021.
2. Hoang-Uyen-Dung Nguyen, Dang-Trang Nguyen, Kozo Taguchi, "A portable soil microbial fuel cell for sensing soil water content", Measurement: Sensors, volume 18, 100231, December 2021.
3. Hoang-Uyen-Dung Nguyen, Dang-Trang Nguyen, Kozo Taguchi, "A compact, membrane-less, easy-to-use soil microbial fuel cell: Generating electricity from household rice washing wastewater", Biochemical Engineering Journal, volume 179, 108338, February 2022.
4. Hoang-Uyen-Dung Nguyen, Dang-Trang Nguyen, Kozo Taguchi, "Improving the Performance of Baker's Yeast-powered MFCs by Adding Dehydrogenase Enzymes to Oxidize Yeast-produced Ethanol", IOP Conference Series: Earth and Environmental Science, volume 994, 012004, June 2022.
5. Dang-Trang Nguyen, Hoang-Uyen-Dung Nguyen, Kozo Taguchi, "Portable Membrane-Less Soil Microbial Fuel Cell: Using Multiwalled CNT Paper Electrodes", Journal of Electronic Materials, July 2022.
6. Dang-Trang Nguyen, Hoang-Uyen-Dung Nguyen, Kozo Taguchi, "A biodegradable water-activated battery: using an activated carbon-based anode and a CNT-based air-cathode", IOP Conference Series: Earth and Environmental Science, volume 994, 012005, June 2022.

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ABBREVIATIONS

AC	Activated Carbon
ACP	Activated Carbon Paper
ACF	Activated Carbon Felt
ADHE	Alcohol and Aldehyde Dehydrogenase Enzymes
BES	Bio Electrochemical System
BKY	Baker's Yeast
BOD	Biochemical Oxygen Demand
CF	Carbon Fiber
CNT	Carbon Nanotube
COD	Chemical Oxygen Demand
DET	Direct Electron Transfer
EC	Electrical Conductivity
EDS	Energy Dispersive X-ray Spectroscopy
EET	Extracellular Electron Transfer
EFC	Enzymatic Fuel Cell
GRM	Growth Medium
LB	Luria-Bertani
MCNTP	Multiwalled Carbon Nanotube Paper
MET	Mediated Electron Transfer
MFC	Microbial Fuel Cell
MPD	Maximum Power Density
MWCNT	Multiwalled Carbon Nanotube
OCV	Open-Circuit Voltage
PEM	Proton Exchange Membrane
PLA	Polylactic Acid
PMFC	Plugged-type Microbial Fuel Cell

RVC	Reticulated Vitrified Carbon
RWW	Rice Washing Wastewater
SEM	Scanning Electron Microscopy
SMC	Soil Moisture Content
SMFC	Soil Microbial Fuel Cell
SSMFC	Stab-type membrane-less Soil Microbial Fuel Cell
SWHC	Soil Water Holding Capacity
TC	Total Carbon

CHAPTER 1 - INTRODUCTION

1.1 Overview

1.1.1 Introduction and the need for new clean energy

Nowadays, energy plays a critical role in human life as an essential light, heat source, and many other functions. Therefore, it may affect the entire national and global economy. However, the most consumed energy sources in the world, fossil fuels, are creating lots of environmental pollution and human health problems, particularly coal and petroleum, due to their releasing carbon dioxide into the atmosphere [1]. Furthermore, the rapid population growth, industrialization, and urbanization increase the global consumption of fossil fuels forcefully, consequently leading to a severe energy crisis. Since then, environmental protection and the energy crisis have been the two challenges that are receiving much research attention for the world's sustainable development in the future. Therefore, the demand for long-term availability, cheap and eco-friendly alternative energy sources with minimal hydrocarbon is critical to replace fossil fuels [2,9].

Unlike fossil resources depleting quickly, renewable energies, whose powers come from nature and can be regenerative, have attracted considerable interest and attention as an alternative resource that might contribute to the solution of environmental concerns, energy security, and economic impacts [4]. Due to the significant feature of plentiful supply, there has been a lot of development and research in renewable energy sources such as solar, wind, hydro, and biomass [5,6,7]. However, most renewable energy expenditures come from materials, facilities, and maintenance, which are more expensive than fossil fuels. Still, they could be comparable with conventional fuels when associated with environmental and social effects. Therefore, the renewable energy demand and supply are still increasing.

1.1.2 Microbial Fuel Cell technology

In recent years, Bio Electrochemical Systems (BES) have been considered promising green energy that can generate electricity for many different purposes, easy to implement at affordable prices. The most concerning type of BES is the Microbial Fuel Cell (MFC), which technology is described as the newest approach to producing electricity using microbial metabolism to convert chemical energy directly from organic substances such as sediment, soil, or wastewater [8,9]. In an MFC, the bacteria community is the core element for output performance. Presently, with the

advantages of effectiveness, simple operation, ease of maintenance, and low cost, MFCs have been proven that have great potential applications in (i) power generation; (ii) environmental treatment, including remediation and removal effect from a variety of pollutant environments such as contaminated soil, sediment, or wastewater; (iii) sensing and monitoring BOD, pollutant agents or toxicity in different conditions [10,11,12].

MFCs are constructed by using diversified materials and configurations. Many alternative innovative materials are being developed to address the disadvantage of expensive and toxic traditional materials such as platinum or palladium electrodes [13]. However, the goal of using MFCs for electricity generation is hindered by their low power output, which needs to be improved [14,15]. Furthermore, the setup mechanism of the conventional MFC in wetlands or soil is still complex that has been innovated with different new designs and techniques but still has difficulty in practical applications [16,17,18]. In terms of sensing and environmental treatment, the sensibility and stability of MFC are still a gap that has continuously been researched and optimized. The same problem as above, MFC-based sensors are not widely used because of their complicated setups, limited detection range, long response time, and low reproductivity [11,17].

In this thesis, a series of lab-scale studies with various new MFC designs and techniques accommodating different substrates evaluate the ability to enhance the electrical production of these MFCs. Furthermore, another utilization of MFC in this study is investigating the biosensing capability of new configurations, which is expected to promote agricultural applications, especially in the actual field. The results obtained from this thesis are expected to contribute to practical applications in the future.

1.2 Research aims, significances, and experimental conditions

1.2.1 Research aims

This research suggests a variety of novel, cost-effective, and convenient designs of new MFC that can produce electricity on demand and be utilized as a sensor for water sensing, contributing to clean and environmentally friendly technology. In this way, this research facilitates the practical application of MFC in many fields in the future. In detail, the specific objectives of this research are described below:

(1) A new method that combines MFC and EFC (Enzymatic Fuel Cell) technologies to improve the power production of Baker Yeast-powered MFCs has been introduced. This method allows for the full use of the ethanol produced by Baker Yeast in these systems.

(2) A new configuration of a portable plugged-type Soil Microbial Fuel Cell (SMFC) was designed and fabricated using low-cost materials was proposed to investigate electricity generation on demand.

(3) To investigate the utilization of household rice wastewater on the performance of the SMFC, another new plugged-type SMFC was proposed. This experiment also evaluated the effectiveness of designed SMFC on current production and compared the feasibility of SMFC when using different soil types.

(4) To investigate the effectiveness of a newly fabricated material - multiwalled carbon nanotube paper (MCNTP) in a stab-type membrane-less SMFC in current generation feasibility.

(5) A portable, compact, and easy-to-install SMFC was proposed for sensing soil moisture.

1.2.2 Research significances

On the scientific side, this study contributes to solving the limitations of current MFC technology by filling gaps regarding innovating the simple setup design, enhancing the output production, reducing design cost, and enhancing the function of MFC as a biosensor. Furthermore, this study identifies the basis of the association of two types of BES in both theoretical and experimental. Additionally, this study demonstrates an application of MFC as a biosensor in sensing the soil moisture content that contributes to improving agricultural productivity and simplifying water management. Using readily available waste materials, particularly rice wastewater, in MFC applications revealed the potential for high productivity and material savings. Therefore, it can provide essential information for designing and future MFC applications.

1.2.3 Research conditions

All experiments in this thesis were carried out at Ritsumeikan University's Biophotonics Laboratory in Kusatsu, Shiga Prefecture, Japan.

The soils utilized in all experiments, including muddy, sandy, and agricultural farm soils, were collected in Kusatsu, Shiga, Japan. The Biophotonics Laboratory of Ritsumeikan University supplied other substrates, chemicals, and equipment. The SEM test and EDS test were done at Optoelectronic Properties and Devices Laboratory and Photovoltaic Device Laboratory.

1.3 Thesis outlines

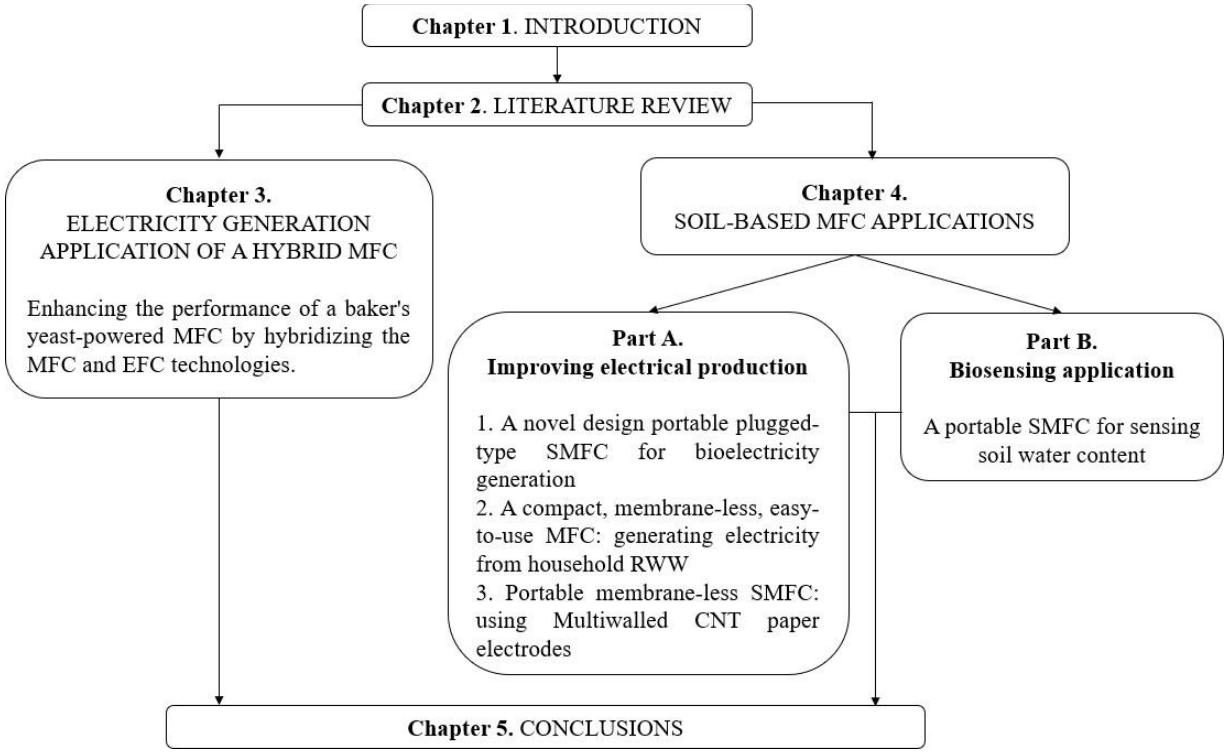

Figure 1.1. Thesis outlines

The thesis outlines with main contents are described in figure 1.1. This thesis is organized into five chapters, with the explicit contents of each chapter presented in the following way:

Chapter 1 gives an overview of the research's general background and defines objectives, scopes, main tasks, and the significance of this research.

Chapter 2 discusses the significance of MFC technology and its broad applicability in human life. This chapter also presents the Soil MFC (SMFC), which has garnered investigation for the last few decades. Finally, this chapter focuses on new approaches for improving electrical generation and how to employ MFC as an effective biosensor to detect target substrates.

Chapter 3 emphasizes new techniques equipped with innovative designs to improve the bioelectricity generation of an MFC. The theory and practice of integrating MFC and EFC technologies are explored. This hybridization can enhance the overall performance of MFC.

Chapter 4 demonstrates the applications of SMFC.

The first part discusses the efforts for the electricity generation application of SMFC. This section outlines the design of a small, inexpensive, and easy-to-assemble SMFC that can generate electricity on demand. Furthermore, the investigation of using household wastewater, especially

rice washing water, in SMFC operations to generate electricity is also presented. This waste material utilization is applied effectively to different types of soil. A new modified electrode technique is also examined for improving electricity production.

Another application of MFC is biosensing, which is recognized as environmentally friendly and effective in environmental remediation. This part provides the importance of soil moisture measurement and monitoring in human life, then proposes a convenient, portable, and low-cost MFC that can sense soil moisture which has potential in agricultural irrigation management applications.

Chapter 5 summarizes the main findings of this thesis. This chapter also highlights the limitations of the studies and suggests potential avenues for future research that build upon the thesis results.

CHAPTER 2 - LITERATURE REVIEW

2.1 Microbial Fuel Cell – an introduction and history of MFC evolution

Microbial fuel cell technology attracts tremendous interest among academic researchers. It represents the newest approach for generating electricity by using the inherent capability of microorganisms as catalysts to convert chemical energy from many different substrates to electrical power through the influences of microbial metabolism on biotransformation [19,20]. MFC technology was demonstrated with significant advantages such as directly converting substrates into the current, operating in low to high temperatures, and not needing input energy [21]. The earliest report about the concept of MFC was demonstrated more than 100 years ago by Potter when observing electrical generation at a low level by culturing microorganisms, including *Escherichia coli* bacteria and *Saccharomyces sp.*, using platinum electrodes [22]. After then, Barnet Cohen drew more attention to this area and used the stacked bacterial fuel cell system to confirm the activity of bacteria, which produced over 35 volts. However, this research did not gain much attention, and very few applications were implemented during the next few decades. Until the early 1980s, researchers learned about electron transportation in MFC, which facilitated the advancement to produce the basic design and still used to date. Significant efforts have been taken to develop MFC with the employment of mediators that enhance electricity generation and work continuously for a long time without maintenance. Mediators can be reduced by electrons captured from the membrane, moving to the anode to release the electrons, then oxidizing again in a cycle. This process facilitates the electron transfer hence improving the output product. However, mediators are usually toxic and expensive [14]. In 1999, by using natural electrochemical bacteria for the electron transportation chain, Korean researchers developed a mediator-less MFC to reduce the cost of mediator chemicals and eliminate their toxicity [23]. Since then, there has been a dramatic rise in the number of publications on MFC technology improvement, with many achievements that lead it to be considered a potential sustainable technology.

Describing more clearly, a basic MFC works as a sort of battery with three main parts consisting of (i) anode – which tends to give away electrons; (ii) cathode – which tends to receive electrons; and (iii) the electrolyte contains a conducted fluid with an optional proton exchange membrane (PEM) that allows protons flow from anode to cathode [24]. Besides, microorganisms

play an essential role that contributes to power generation. In an MFC, substrates are oxidized to carbon dioxide under the catalyzing of microbes. Electrons also are released in the anode and then transferred to the cathode [25]. This flow is continuously maintained to produce electricity. This direct conversion from substrates to electricity attained high efficiency. MFC does not need input energy to accelerate the operation and gas treatment after working [26]. This technology also shows the advantages of being cost-effective and environmentally friendly. In recent years, the potential of MFC has been well studied. Researchers continuously attempt to improve the MFC for effective current production, prolonged operation time, and environmental applications such as seawater desalination, wastewater treatment, soil remediation, and soil sensor [27]. However, thermodynamic limitations and low power output are the main challenges of MFC practical applications in the real world.

This chapter will review the working principle of MFC, configurations, applications, and the challenges of this technology. Primarily, Soil Microbial Fuel Cells (SMFC) will also be described.

2.2 MFC working principle and configurations

2.2.1 MFC working and energy harvesting principle

A classical MFC setup consists of two compartments: an anode chamber and a cathode chamber partitioned by a PEM or salt bridge and external resistance, which is used to harvest output power [28], as shown in figure 2.1. The anode electrode is located in the anodic chamber and works in anaerobic conditions. The bacteria communities in this space oxidize substrates such as glucose, ethanol, and wastewater (electron donor source) to generate electrons, extract protons and produce carbon dioxide. This process is called the oxidation reaction [29]. The biofilm transfers electrons to the anode, and then these electrons move to the cathode through an external resistance. At the same time, protons are transported from the anode to the cathode chamber through a PEM. Under aerobic conditions containing abundant oxygen from the air or bubbling water, oxygen in the cathode chamber plays as the primary electron acceptor to combine with electrons and protons to create water during the reduction reaction [28]. Electron acceptors that significantly influence MFC performance are diverse, such as ferricyanide, persulfate, nitrogen, and copper. However, the most commonly used is oxygen because it can oxidize other substances, is sustainable, and produces clean by-products [30]. After the overall reaction, water and carbon dioxide are by-products. These processes occur in a cycle and produce electricity [31].

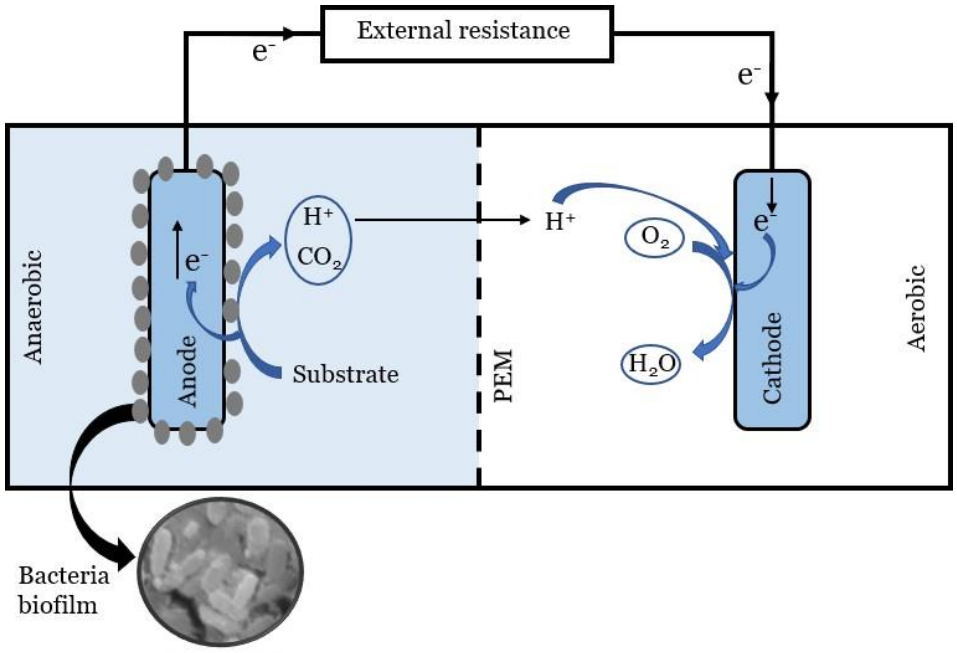

Figure 2.1. Schematic diagram of a basic design two-chambered MFC and working principle

Exoelectrogenic bacteria (microorganisms with electrons transferring missions) activities play a crucial role in MFC performance. Many species have this capability, such as *Shewanella putrefaciens* and *Geobacter sulfurreducens* [1,5].

Experimental energy is harvested by observing and calculating MFC's potential and power density. This potential varies depending on several factors, such as the reactor's design, the substances used to donate electrons, electron acceptors, pH, electrolyte strength, and bacteria communities in the MFC. With an external resistance R (Ω), the active surface area of anode electrode A (m^2) and MFC voltage V (Volt) that the data recorder can monitor, the current density I and power density P are calculated based on Ohm law as $I = V/(RA)$ and $P = VI$.

The amount of energy E (J) attained from this process through the time t (s) of operation is calculated as $E = Pt$.

Because biological reactions happen in the anode, the power output is often normalized based on the anode surface area. However, in some exceptional cases, such as when the anode is made of a material with an unknown surface area (like granular material), the power density can be calculated using the cathode surface area. MFC voltage output is inversely proportional to the current obtained. Each time the voltage output is high, the output current is low, and vice versa. The maximum power density point occurs when the external resistance matches the internal

resistance of the entire system. This maximum power density (MPD) is often used to evaluate the system's performance, as it represents the maximum output the system can attain [32]. The results from this thesis also observe and measure the current, output voltage, and MPD and their relationships. However, to date, electricity energy harvested from MFCs still insufficient to drive electronic devices and is limited in supporting other sensor devices or being used as a sensor itself because the highest power output recorded in an open circuit was only 750-800 mV. This voltage is much lower when measured in close circuit conditions due to potential loss or internal resistance.

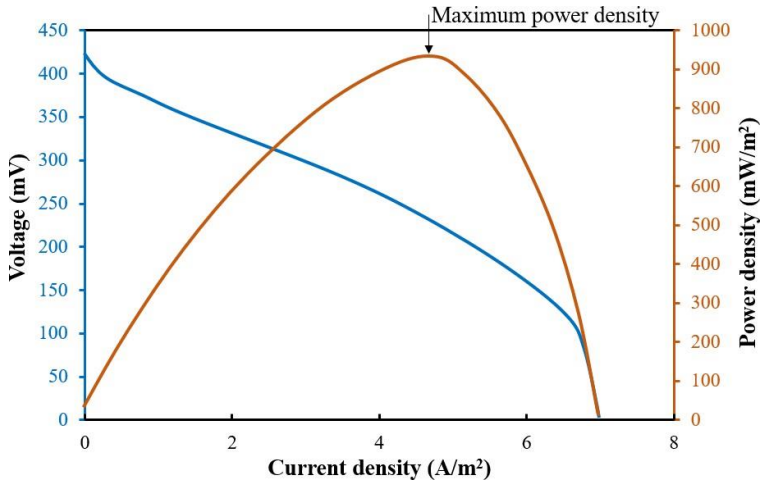

Figure 2.2. Typical polarization curves (blue color) and power density curves (red color) are achieved by an MFC

2.2.2 MFC reactor designs

In general, the performance of MFC is greatly influenced by the design and construction of the reactor, which are fundamental components of MFCs [30]. The designs vary in size, shape, materials, and configurations. Different configurations have been developed to optimize the overall output production but are typically divided into two main types: single-chambered and double-chambered MFC. Due to the critical feature of reactor design, there have been various research on combination designs to overcome the limitation of MFC and improve the demand output performance [29].

- ***Double-chambered MFC***

As mentioned above, a conventional double-chambered MFC comprises two separate chambers containing electrodes and requires a PEM as a separator. In general, these two chambers are often made of glass or plastic. The role of PEM in this design not only facilitates the protons

generated from the oxidation process in the anode to move to the cathode but also prevents the oxygen crossover to the anode [33]. The chambers can be in various shapes, and the most commonly used is H-type MFC. The main disadvantage of this type of MFC is its high internal resistance due to the long distance between its electrodes, which can reduce its overall performance [34]. Double-chambered MFCs can be operated in batch or continuous mode with the control of anaerobic conditions in the anode chamber and aerobic conditions in the cathode chamber. The possibility of controlling oxidation, reduction reactions at electrodes, flow rate, and restricting the connection between oxygen and anode helps enhance the performance of double-chambered MFCs and create broad applications, especially in wastewater treatment rather than electricity generation [35].

- ***Single-chambered MFC***

Single-chambered MFC has a simple design with only one chamber containing an anode and an air-cathode that exposes it to the air. The appearance of a PEM is optional, which helps the protons transfer from the anolyte to the air cathode [36]. One of the most significant advantages of this air cathode design comes from the unlimited oxygen source that enhances the obtained power output. In addition, reducing the space between the anode and cathode reduces the internal resistance, improves proton diffusion, and increases power density [35]. On the other hand, since single-chambered MFC has to face high evaporation possibility of water or liquid, an essential issue of this design is the management of water or substrate fluid to maintain stable operating conditions. In addition, with this design's simplicity and low-cost requirement, it is more appropriate for scaling up than double-chambered MFC, thus attracting intensive interest and research for practical applications. Due to the high cost of PEM, the single-chambered membrane-less has evolved to eliminate the need for a membrane [11]. However, in MFC configurations without PEM, the microbial contamination proportion between the cathode and anode is higher than one attached to a PEM [37]. Therefore, numerous research has been made on the adaptation configurations concentrating on enhancing membrane-less design while minimizing cross-contamination and high power output production.

- ***Other configurations***

Tremendous improvement has been made in reactor architecture for performance optimization and depends on the intended use.

A stacked MFC is a setup where multiple MFCs are connected in a series or parallel circuit to harvest higher overall power output compared to conventional MFC but not influence individual cells' Coulumbic efficiency in the system [35]. Voltage yield is higher when fuel cells are added in series, whereas the higher current is attained when fuel cells are linked in parallel. However, both types of construction have high efficiency in practical power production. Nevertheless, the efficiency of staked MFC is highly affected by many factors, including stack direction (horizontal or vertical type), the shape of the reactor, the type of electrode, and modulation.

Research reported that when a system connected six individual MFC units using horizontal stacked type, the current and MPD obtained from parallel connection were smaller than series connection when running at a similar flow rate [8]. A tubular air-cathode MFC was built horizontally for wastewater treatment with continuous plug flow [38]. Although horizontally stacked MFC configurations show the power generation increase, the difference in factors such as organic matter concentration and ion conductivity was proven to cause the different performance of each unit in the system [39]. Vertically stacked MFC does not require supplied pumps to adjust and provide substrates or air to the system, which are needed in horizontally stacked MFC. The use of these pumps requires extra energy. This configuration could also be used for wastewater treatment when the chemical oxygen demand (COD) eliminated level of over 95% was attained using artificial wastewater with the system of six MFC units under continuous mode [40]. However, in both types of stacked MFC, the imbalance of substrate loading during the operation could lead to the voltage reversal of MFC and adversely affect biofilm on the anode that needs voltage reversal control during the operation period [41].

An up-flow MFC is constructed with a continuous feeding mode by using a membrane-less cylinder shape reactor divided into two chambers by a layer of glass wool and glass beads. The anode is located at the bottom of the reactor, while the cathode is at the top. The fuel-feeding stream is supplied from the bottom of the anode, diffuses to the upper part, passes through the anode, and stays at the top. Even though up-flow MFC has the advantage of having a high-mass transfer rate and is easy to scale up but more expensive than the output it produces. Therefore, it is more suitable for wastewater treatment or other carbon sources than electricity harvesting.

Conversely, mini-size MFCs are attracting interest in research and application for their unique structural characteristics in a well-controlled environment with considerable potential for power generation. Miniature MFCs possess advantages, including flexible and easy-to-fabricate with

various material design, the small scale can vary from a few milliliters to microliters, small internal resistance that enhance the output, and short response time [43]. A 1.2 mL miniature MFC was reported, which achieved a high power density of 500 mW/m^3 using pure biofilm of *Shewanella oneidensis*. This miniature MFC provided a high ratio of surface area to volume in the chambers, improving the system's proton diffusion. Another improved air-breathing miniature MFC with a volume of 2.5 mL was demonstrated to generate high power densities of 627 W/m^3 in batch mode and 1010 W/m^3 when operated in a continuous mode [22]. Therefore, this design of MFC can be used as a sensor for long-term operation [44]. However, miniature MFCs also face many challenges, such as the biofilm formation or thickness of substrates.

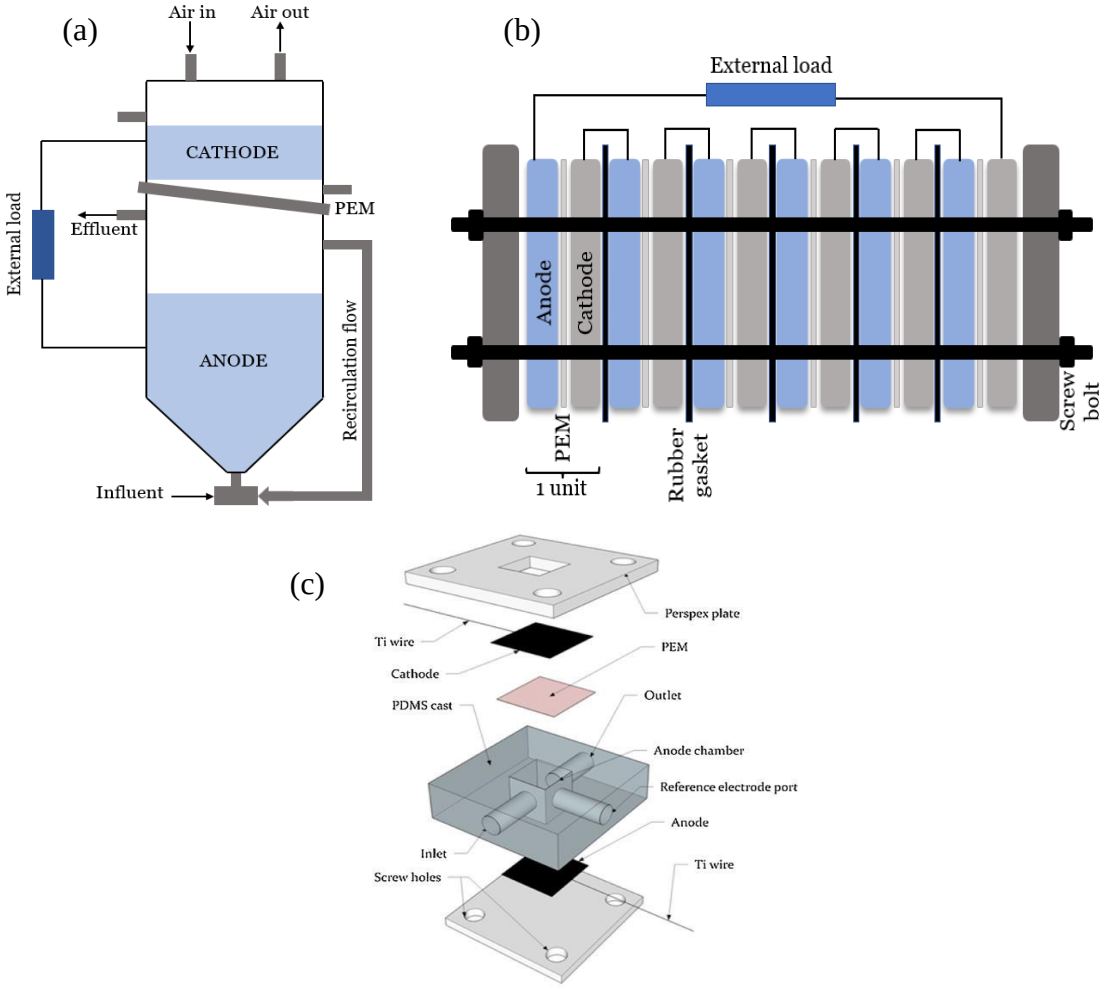

Figure 2.3. Other MFC configurations: (a) up-flow MFC, (b) stacked-MFC, (c) miniature MFC (the miniature MFC diagram reported by [42], which is allowed to be used again with permission from Elsevier's paper rights and content policy)

2.3 Electron transfer mechanism

The passage of electrons between microorganisms and the anode is a critical aspect of MFC operation that impacts MFC operation and promotes power generation capabilities. Direct and mediated electron transfer are the two primary pathways for the MFC's extracellular electron transfer (EET).

2.3.1 Direct electron transfer (DET) mechanism

According to one study, the use of a mediator in the electron transfer procedure can be eliminated. This process can occur from microorganisms to the electrode without a physical connection between the bacteria membrane and electrodes [45]. Alternatively, DET can be implemented via redox-active proteins (c-type cytochrome and multi-heme cytochrome), conductive pili, or proteinaceous filament appendages of bacteria known as microbial nanowires [46]. There is intensive research on EET-capable microbes recognized as exoelectrogens, which have the feature of transferring electrons by the oxidation process across the membrane themselves to the anode. Among them, the most extensively researched species are *Geobacter* and *Shewanella*. These bacteria feature particular genomes that contain an extracellular matrix network of membrane cytochromes engaged in electron transfer and increasing current density [18].

Another method of DET is via nanowires of conductive pili, which are formed in exoelectrogen and enable electrons to move to a rigid acceptor. This mechanism is suitable for long-range electron transport in multilayer biofilm due to the limitation of cells in electrode assessment. Possess unique characteristics, flagella and pili are homopolymers made of a single component, whereas microbial conductive nanowires comprise several cytochromes, periplasmic, and outer membrane proteins. It was found that more than 100 genes in *Geobacter sulfurreducens* have a crucial role in EET. The current density was reduced when the PilA gene was removed because of the reduction in nanowire production [47]. It was observed that the KN400 strain of *Geobacter sulfurreducens* in a 5-month operated MFC generated an 8-fold higher power density when more abundant conductive nanowires were obtained [48]. The applications of other exoelectrogenic bacteria species are also investigated [49]. These exoelectrogens contribute a significant role in reducing the use of mediators and producing more electricity.

2.3.2 Mediated electron transfer (MET) mechanism

Many microbes cannot transfer electrons to the anode directly because of possessing structure of the non-conductive membrane, peptidoglycans, and lip polysaccharides which impede the transferring of electrons activities [50]. Therefore, the mediated electron transfer mechanism is another effective way to accomplish the electron transfer mission. In such cases, mediators are helpful and usually act as electron shuttles to electron acceptors [46,51]. Property requirements for a good mediator include being non-toxic to bacteria, having the ability to go through the membrane, maintaining contact with the electrode surface, having low oxidation potential, and good solubility in anolyte [52]. There are two mediator types: exogenous mediator (artificial type) and endogenous mediator (microorganisms product type).

Since the first use of benzoquinone and potassium ferricyanide in 1930, various compounds have been reported as exogenous mediators, such as quinine, phenoxazine, phenothiazine, and phenazine [53]. Two of the disadvantages of the exogenous mediators are low current density production and instability. Furthermore, most of them are toxic chemicals that can cause human and environmental problems; hence their use in practical large-scale electricity generation in the natural environment becomes impossible [8]. Even though the use of exogenous mediators has been reduced because of their toxicity, this method is still standard in MFC applications for improving output production.

On the contrary, numerous research identified that several microorganism species and strains, such as *Shewanella sp.* and *Bacillus subtilis sp.*, can produce their mediators through the secondary metabolites known as endogenous mediators to promote the electron transfer process [54,55]. Products of these metabolite activities, such as flavins and quinones, act as electron shuttles that go to the cell in reduced form and interact with cytochrome [56]. They penetrate out of the cells in reduced form and contact with the electrode to be oxidized there to transfer electrons and then start the cycle again [51]. These electroactive bacteria help to eliminate the use of artificial mediators. They are non-toxic and have great potential for practical MFC applications without harm to humans and the environment [57].

Another indirect electron transfer refers to the interaction of anode and metabolite products. This mechanism needs an electrocatalytic anode to facilitate the oxidation of reduced products. However, this pathway is only accomplished in MFCs using fermentative organisms and yeast, which produce organic acids as fermentation products outside the cell, and electrons from this reaction will be transported to the anode [58]. Due to the requirement of electrocatalysts and slow-

reacted fermentation products, MFC efficiency is limited and unable to scale up for large-scale applications that might have more intensive investigation and exploration.

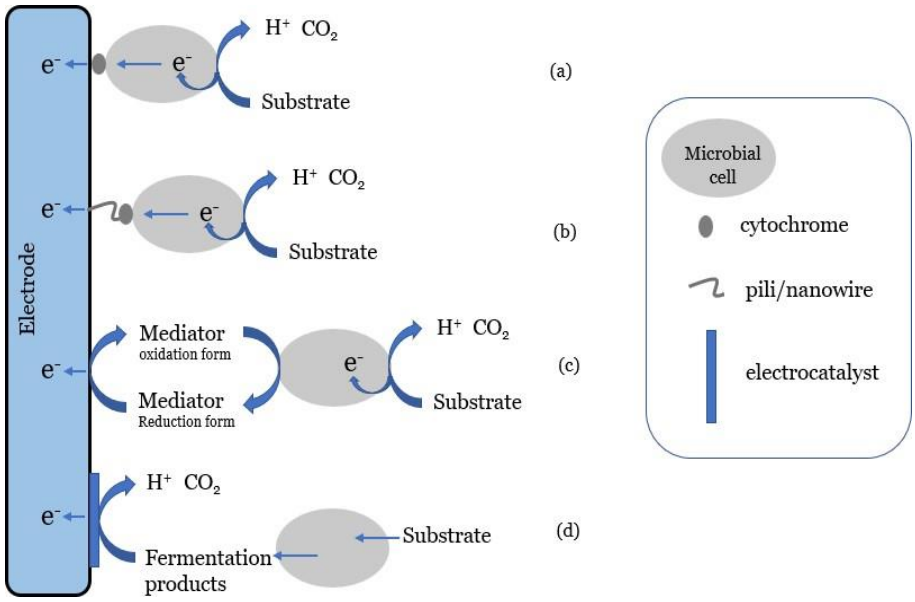

Figure 2.4. Simple illustration of electron transfer mechanisms: (a) DET via cytochrome, (b) DET via pili or nanowire, (c) MET via redox mediator (exogenous and endogenous mediators), (d) electron transfer via reduced metabolites

2.4 Factors affecting the MFC performance

So far, MFCs have often been accomplished in the laboratory rather than actual field and attained performance much lower than necessary for daily life. Furthermore, MFC performance varies by many factors, such as the design, electron donors, electron acceptors, electrolyte strength, pH, temperature, electrode material, and bacteria communities [59]. Therefore, innovation in optimizing such factors can improve MFC's overall performance.

2.4.1 Electrode materials

The electrode material is a critical element in MFC design which is not only having a substantial effect on the MFC output due to the different polarization losses but also can decide the cost of MFC [60]. Therefore, selecting the appropriate electrode material is crucial for MFC efficiency through enhancing microorganism adhesion and electron transfer. Even though having different criteria, both cathode and anode electrodes should be concerned with the maximized power density and minimized cost. Furthermore, the electrode materials should possess some

properties, including (i) specific surface area and porosity: high surface area enhances the kinetics of electrodes while porous electrode can limit moisture on the surface, reduce internal resistance, and enhance the microorganism immobilization, thus improving microorganism activities and encourage biofilm growth [61]; (ii) electrical conductivity: the suitable high electrical conductivity electrode offers low resistance to the substrate that facilitates electron flow rate [62]; (iii) chemical stability and durability affect to anode's swelling and longevity, the high surface roughness increases the durability but decreases the long-term performance of MFC [63].

Anode material significantly influences the electron transfer process and biofilm formation. A wide range of anode materials has been used, but there are two main types: carbon-based and metal-based materials. The most common carbon-based material is carbon felt because of its high surface area, high electrical conductivity, and being inexpensive but delicate [64]. Besides, carbon fiber felt [65], carbon fiber brush, activated carbon [66], carbon cloth [13], graphite rod [65], and graphite felt [67] are investigated simultaneously. Several studies reported that reticulated vitrified carbon (RVC) could be used as the anode electrode due to its porous characteristic [68]. However, the main disadvantage of RVC is its brittle texture, which limits its wide use. In addition, carbon nanotube (CNT) has been employed in many MFCs using modification electrodes. It is a promising material with unique properties, such as high electrical conductivity, mechanical stability, great cell adhesion, and can combine with many other active ingredients for output increment [69]. Based on the strong points of cost-effective, easy-to-use, carbon-based materials are promising for MFC large-scale applications.

On the other hand, metal-based materials are alternative materials chosen to be used as anode electrodes, such as stainless mesh and stainless-steel fiber felt [69] due to the advantages of low cost and corrosion resistance [70]. However, the application of metal-based materials still has a constraint because of their poor compatibility, lower interfacial impedance, and higher internal resistance than carbon-based materials [69]. Lately, with the primary purpose of increasing MFC efficiency, researchers have shifted to improve materials that can facilitate electron transfer, bacterial adhesive as well as bacterial activities by combining metal-based materials with a large specific surface, good conductivity, and biochemical stability carbon-based materials to take full advantages of carbon and metal materials on new electrodes. Therefore, the emergence of further anode modifications such as nanocomposite of metal oxides, conducting polymers, graphite/carbon electrode surface supported catalyst, etc., have been demonstrated in many

research and showed an improvement in MFC performance with rapid electron transfer and kinetics enhancement [71,72].

Cathode material properties significantly affect MFC performance. Generally, most materials used as anode electrodes can also be utilized as cathode electrodes [73] when good electrical conductivity, low corrosion, high porosity, high surface area, and high redox potential to capture protons are basic requirements [74]. In addition, cathode electrode materials should have other properties, including high mechanical strength and catalytic activity. Typically, oxygen is an important electron acceptor in the cathode chamber. Since carbon-based materials show slow oxygen-reduction reaction kinetics that affects the power efficiency, a different active catalyst is required to accelerate the reduction process and reduce oxygen reduction overpotential. In most cases, when utilizing oxygen as the electron acceptor, platinum (Pt) is the most often used catalyst since it is thought to minimize the cathode reaction activation energy and boost the reaction rate [75]. Even though Pt or Pt-coated cathodes produced higher current output than others that did not have any catalyst, the development of the PtO layer at the electrode reduced their activities. Furthermore, Pt's high cost and the unrecommended metal use in realistic practice limit the capability of large-scale applications. Due to Pt's sensitivity to pH and non-sustainability, alternative cheap and highly active catalysts have been developed to reduce the cost and still be competitive such as lead oxide, manganese dioxide, PANI/Cu hybrid. Furthermore, MFCs using aerobic bacteria as cathode catalysts are called biocathodes MFCs showed a potential technology with the development of biofilm on the cathode. They eliminated the need for expensive catalysts, but their efficiency still has controversial issues [76].

2.4.2 Proton exchange membrane

In most MFC configurations, the proton exchange membrane (PEM) is an essential part of MFC function and performance when acting as a chamber separator and allowing proton migration from anode to cathode to interact with oxygen [30]. PEM also influences internal resistance and polarization loss. The exception of not using PEM are MFCs with natural separation, such as sediment MFC and soil MFC, which use the available sediment or soil as the anodic and proton exchange layer [77], or special design single-chambered MFCs.

There are two main types of PEM, including porous and non-porous membranes, which are discriminated by their cross-sections [78]. PEMs should possess high ionic conductivity, chemical stability, low gas permeability, low swelling level, and dehydration resistance characteristics to

optimize the MFC applications [79]. On the other hand, these membranes transport the proton base on the humidification so that another membrane characteristic is well-hydrated to remain conductivity and resistant in different environments. In addition, PEM working surface area affects MFC power production and negatively correlates with the system's internal resistance [80]. Furthermore, PEM thickness also influences MFC performance.

Nafion has been the most often used membrane in MFCs since its inception in 1966, owing to its high proton permeability, equilibrates with the cations in anolyte and catholyte, and relatively low weight. However, it cannot work efficiently at temperatures above 80^{o}C due to thermal stress that makes it dry and damaged [36]. Ultrex CMI-7000 is another proper choice, which is more cost-effective than Nafion. Porcelain septum from kaolin was reported to take the place of Nafion in a single-chambered MFC. Nowadays, researchers are promoting fabricating new kinds of PEM using nanoparticles that have better performance than Nafion, such as Fe_3O_4 nanoparticles, which are environmentally friendly, conductive, and have better thermal resistance [81].

However, the use of PEM is facing many problems and disadvantages. These membranes may also be permeable to substances in the substrates, such as oxygen, ferricyanide, or organic materials, resulting in electron donor loss and decreased overall efficiency. Furthermore, the power output of MFCs with PEM was similar to those without PEM up to a specific period [82]. Moreover, PEM attends to be foul in wastewater fuel. Again, the high cost of PEM is another disadvantage that the elimination of PEM in MFC configuration is an alternative choice for large-scale MFC applications.

2.4.3 Microorganisms used in MFCs

The most critical component that decides the effectiveness of an MFC is the microorganism community having electron transfer ability, accomplishing the metabolism of organic or inorganic matter substrates, and converting for energy [83]. Thus, studying MFC microorganisms is an intense field that takes decades to reveal. Exoelectrogen or exoelectrogenic bacteria refer to microorganisms that can transfer electrons from substrates to the active anode [84]. Marine sediment, wastewater, activated sludge, and soil were the most common sources for exoelectrogenic bacteria isolation. The difference between typical catalysts and microorganisms is energy loss. While additional catalysts do not receive energy from their reactions, microorganisms are not real catalysts, so they need the energy for their growth during the operation. Thus, microorganisms consume a portion of the power generated by substrate oxidation.

Generally, bacteria that can reduce metal are exoelectrogenic due to their observation mostly coming from aquatic sediments with the muscular appearance of different metals. Three well-known representatives in this group are *Geobacter*, *Shewanella*, and *Rhodopseudomonas* [85]. Hundreds of exoelectrogens have been isolated and identified, with about 50 species belonging to three phyla *Proteobacteria, Firmicutes,* and *Acidobacteria*. Conversely, in self-sustained systems or when MFC uses complex substrates, electricity generation can be produced by nonelectrochemical microorganisms via synergistic cooperation processes [4]. Therefore, several efforts have investigated the relationship between MFC power generation and microorganisms' morphology, genetic characteristics, and metabolic capacity.

Geobacter species belong to dissimilatory metal-reducing microorganisms whose main oxidation activities occur under anaerobic conditions such as sediments or soil and generate useful biological energy. The electrons are then transported to the ultimate acceptors via direct interaction between metal-reducing bacteria and mineral oxides like Fe_2O_3 [85]. On the other hand, *Shewanella* uses a different mechanism without direct contact between microorganism cells and mineral oxides. In mediator-less MFCs, bacteria communities participate in anodic reactions mainly from *Shewanella*, *Geobacter*, and *Rhodoferax* families that work with the second mechanism due to the anode acting as the final acceptors [85].

Typically, MFC biofilms can be made in both pure and mixed cultures. Even though mixed cultures need a longer time to obtain the stable phase of current, the MFC performance produced from mixed cultures is significantly higher than pure cultures or co-cultures [86,87]. More efficiency of mixed cultures may come from taking advantage of different types of bacteria, which are more suitable for broad and complex substrates to generate electricity. In contrast, pure cultures require relatively strict operation conditions with only a few selective substrates [6]. Therefore, mixed cultures become favorable for MFC practical applications. Furthermore, the thickness of the biofilm strongly impacts the power generation effectiveness. The quantity of biocatalyst and bacterial viability on the anode biofilms improved by expanding the anode surface area and metabolic cells amount [37].

2.4.4 Operational conditions

- ***Effect of pH***

MFC operation is based on the protons production fundamental, which is directly affected by pH in both cathode and anode chambers. Because of the membrane's sluggish and selective proton flow, the concentration of protons increases in the anolyte after a long operation. Then it changes the anode chamber pH to become more acidic [88]. In contrast, the proton quantity is reduced in the cathode chamber due to the reduction reaction, making the cathode chamber more alkaline [76]. Therefore, there is a difference in pH between the two chambers. Several studies claimed that high pH could reduce the current output, while others reported that maximum output was achieved at pH 7 to 9 [89]. Generally, the best pH for microorganism growth is neutral, and they react to the pH changes by regulating their activity [3]. In contrast, the low anodic pH can decrease bacterial activity and biofilm formation [10]. In addition, variations in pH can affect other factors like ion concentration and membrane potential [90]. Therefore, pH maintenance at two chambers is critical for improving MFC performance which is easier with two-chambered MFC and more complicated with air-cathode MFC.

- ***Effect of temperature***

It has been found that temperature is an essential factor that affects MFC performance through the impact on system kinetics, mass transfer, and thermodynamics [91]. The temperatures usually used to conduct MFC experiments are around 20 - 35^{o}C (room temperature), whereas MFCs working in the range of 4 - 30^{o}C showed a longer startup time and decreased reproducible capability. Higher temperatures were determined to increase ionic conductivity, steady biofilm, and high performance [91]. The microbial communities have different favorable temperatures, and the ideal temperatures for bio-electrocatalytic activities are 30 - 45^{o}C [92]. It was shown that MFCs could not produce power at temperatures lower than 15^{o}C, while the highest power density achieved at 40^{o}C was two times greater than that obtained at 30^{o}C and decreased four times when the temperature was raised to 50^{o}C [2].

- ***Effect of substrates***

In MFC, substrates have an important position in power generation in terms of power density and Coulombic efficiency. They also significantly impact the bacterial population in the anode biofilm [4]. Substrates are diverse, from pure molecules such as glucose, acetate, and cellulose to complex organic materials such as wastewater and domestic sewage that have been applied in MFCs [29, 93]. Among these substrates, wastewater has different types from different sources

containing sustainable rich organic matters that could be abundant fuel for powering MFCs. In addition, sediment and soil are alternative sources containing vast amounts of complex organic matter that can generate electricity [94]. Substrate concentration is another factor that affects MFC performance. A study claimed that the MFC output increased from 0.2 to 1.2 W/m^2 when substrate concentration rose from 100 to 850 mg/L, but the power output did not change at high concentrations of 1000-1500 mg/L [95].

2.5 Applications of MFCs

In several decades since the first introduction, extensive research on MFC has been studied in laboratories and shown to be useful in various applications, including power generation, wastewater treatment, bioremediate, and biosensing, that obtained massive helpful results.

2.5.1 Electricity production

One thing for sure is that most studies of MFC are performed for electricity production, which becomes the spearhead target application of MFC technology. To date, because of the limitation of low power production, MFCs are most appropriate to be used in small telemetry systems and wireless sensors due to small power supply needs [96]. The electric power from MFCs still cannot be economical due to the limited output from a few dozen to a few hundred mW/m^2. Little research obtained results over a thousand mW/m^2 that constrain the utilization of MFCs in practice. The electrical output of MFC depends on many factors, as discussed above. Therefore, many approaches have been made to optimize the effectiveness to increase MFC's overall output.

Table 1.1 Performance of some MFCs for electricity generation

MFC type	Substrate	Maximum power density	References
Single-chambered MFC	Glucose	68 mW/m^2	[97]
	Wastewater	114 mW/m^2	[98]
Double-chambered MFC	Cellulose	188 mW/m^2	[99]
	Glucose	855 mW/m^2	[12]
	Wastewater	1777 mW/m^2	[100]

The innovations to enhance MFC performance focus on improving MFC designs, attempting to reduce the system’s internal resistance, modifying electrode materials with high conductivity

and electrical storage capacity by using specific materials or treatment methods and reducing the price by using cheap or without PEM. On the other hand, improving easy setup and scale-up is another approach for large-scale applications. Different modifications of the basic design of MFC have been ameliorated, thus providing new ideas and constructions for power generation development.

2.5.2 Wastewater treatment

MFC technology is considered to be a long-term solution for treating wastewater and generating power simultaneously while oxidizing organic and inorganic compounds in wastewater. Since the early 1990s, MFCs have been used in wastewater treatment, and this has been a vital study field for MFC application [101]. MFCs have been reported to have high potential in treating different wastewater from industrial, urban, and domestic sources, which contain a considerable amount of compounds that can operate MFCs working [26]. Substrates from wastewater, such as carbohydrates, proteins, lipids, minerals, etc., facilitate microbial metabolism and be good inoculum sources [102]. In addition to being able to use free substrates, the excess sludge formed from the process decreases by 50 - 90%, which is profitable in reducing disposal costs [103]. Although wastewater treatment using MFCs can generate power, the efficiencies of these processes are still limited to lower than 12%, indicating that enhancing these systems' power generation may provide a more affordable cost for offset wastewater treatment [26]. The effectiveness of MFCs in treating wastewater can be determined by comparing measurements of key water quality indicators such as BOD, COD, and nitrogen levels before and after the operation [104]. Even though MFCs cannot treat completely highly toxic wastewater, the COD after treatment can meet the requirement of discharge regulation. The COD treatment capability fluctuates in the range of 60 - 99% depending on MFC designs, substrate sources, and other parameters [104].

2.5.3 Bioremediation

The refractory of pesticides, fertilizers, and other toxic compounds, such as heavy metals that exceed the limit regulation, seriously impacts human health and the environment. Many remedial techniques have been developed to remove harmful elements in soil, sediment, or wastewater, but these methods are expensive and may create some problems [105]. Ample research has proven that MFC is a viable method for organics removal by using soil MFC, sediment MFC, or plant

MFC [106]. Generally, bioremediation success depends on the amount of electron acceptor or donor to promote biodegradation. In MFC, when the cathode is at a negative potential, bacteria communities may send electrons to the anode and absorb electrons from it. The study found that the movement of electrons during the electron transfer process can improve organic pollutants' metabolic processes, thus improving the removal and power output simultaneously [107]. Furthermore, with simple configuration, low energy consumption, and less damage to soil structure, MFC is considered the most appropriate technology for future bioremediation applications.

2.5.4 Biosensor development

In recent years, MFC technology gained much interest and has been applied for environmental monitoring by sensing and analyzing pollutants as well as components in soil, sediment, and wastewater due to their portable and working onsite feasibilities [108]. In addition, the MFC-based biosensors used for in situ procedure monitoring are widely examined (Chang et al., 2005) and are considered to be a potentially valuable application of MFC technology. MFC-based biosensors still possess common problems of basic MFCs, such as low stability, signal reproducibility, and long-term operation. However, biosensors' working focuses on the relationship between current response during the operation period corresponding with the change of pollutants or components that need to be monitored other than power output [16]. Using MFC-based biosensors, numerous studies measured parameters such as BOD, COD, dissolved oxygen (DO), toxicants, and microbial activities.

Table 2.2 MFC-based biosensor performance in analyzing some parameters

	Parameter	Detection range	Response time	Reference
BOD		32-1200 mg/L	200-1200 mins	[111]
COD		100-500 mg/L	31-825 mins	[110]
Toxicants	Heavy metals	1-4 mg/L	Not mentioned	[112]
	Antibiotics	1-75 µg/mL	120-240 mins	[113]
	Organic toxicants	10-50 mg/L	27 mins	[9]
	Acidic toxicants	pH 2-6	3.5-22 mins	[114]

In an MFC-based biosensor, the biofilm acts as the biological sensor that responds to the changes which affect the electron transfer and thus converts to measurable signals [71]. Many

parameters, such as pH, temperature, and conductivity, influence the performance of MFC-based biosensors [109]. As a result, MFC-based biosensors are more cost-effective than other detected techniques, but the sensitivity is still limited. Furthermore, response time is another critical parameter of biosensor evaluation which is commonly calculated as the time necessary to achieve the steady state after the operation or the next new steady phase during the whole working time [110]. Therefore, many initiatives have been taken to improve sensitivity (lowering the lower limit and raising the upper limit) to meet the regulation requirements and shorten response time.

2.6 Challenges and prospects

In the last few decades, MFC technology has shown massive potential in many applications, with considerable research have been done to improve performance and efficiency, focusing on environmental friendliness and electricity production and achieving many positive results. Efforts for optimal MFC performance have been considered in different aspects, such as substrates, electrode or PEM materials, electrode spacing, internal and external resistance, and solution ionic strength. Other factors of working conditions are also investigated, such as operation mode [115], culture time [116], gravity, and geometric flow [117]. Although significant progress has been made, MFC technology is still not commercialized because many problems haven't been solved yet. Hence most of the MFCs were conducted on laboratory scale than real-world scale. The main drawback of MFCs is the output production is still under the requirements for commercial use, which often necessitate a few volts of continuous power supply for the regular functioning of low-power electronic components [118].

MFC performance is often not steady for long-term operation, even in continuous mode, due to the dependence of MFCs on the biofilms to transfer electrons. Anodophilic microorganisms were proven to increase the electron transfer process, hence enhancing the power density [119]. Various microbial strains have been applied in MFCs for their ability to self-mediate the transfer of electrons. Although *Geobacter* species were reported could increase MFC current ten thousand times if they transfer electrons at the same rate as its ferric ion acceptor, in fact, microbes are slow electron transformers that cannot attain that rate [103]. In addition, there are some reduction periods in which microorganisms have electrochemical activity stress and swelling [118]. The formation of a layer of dead microbial cells on the electrode surface, known as biofouling, can reduce biofilm effectiveness and result in a drop in potential and current production [46]. Furthermore, some studies reported different power densities obtained when using the same

substrates and microbial culture [120]. The operation of MFC depends on microbial activities, so temperature becomes a limitation when the microbe's reaction decrease at temperatures lower than 20^{o}C [96].

Another restriction of MFC technology is scalability on large and commercial scales. Scaling up MFCs is a priority solution to increase electricity output significantly compared with individual units. Even though high power output and power density were achieved when scale-up MFCs, this increment is disproportional with the corresponding increased anodic chamber volume [121]. In larger volume chamber MFC, the substrate diffusion rate is insufficient for an acceptable current level and cell potential leading to a decrease in diffusion efficiency [122]. Furthermore, when scaling up the models to large quantities of ten folds or hundred folds, the system has to deal with controlling many components such as operational complexity, utility supplies, and logistic supports, which lead to several voltage losses to maintain a similar efficiency. Another factor that needs to be considered for scaling up MFCs is minimizing the cost by choosing cost-effective materials and configurations.

Finally, even though facing many obstacles have not been solved yet to launch in real-world applications, MFC technology still has excellent potential for various applications as a sustainable source of green energy.

2.7 Soil MFC and thesis targets

Soil is the fundamental resource of life for all living things on earth. SMFC uses the abundant exoelectrogenic bacteria in the soil to convert organic matter into a current signal. It also uses soil organic matter as the feeding source for electrogenic bacteria and nutrient medium for SMFC operation. In recent years, SMFC has attracted considerable attention as green technology and shows lots of potential results in renewable electricity generation, soil remediation, and biosensor [123]. Microorganisms that live in the soil are important for electricity production, and the physical and chemical properties of the soil influence their activities. Many previous studies have found that various soil types, including sand, forest, and paddy soil, contain diverse electrochemically active microorganisms such as *Pseudomonas, Geobacter,* and *Clostridium* [124,125]. Since the first SMFC was found, most of the tested soils were in saturated conditions such as sediments, muddy soil, and paddy soil [94,126]. The saturated conditions facilitate the operation of SMFC and the activities of microorganisms. However, in actual field application, most soils are not saturated, so the development of non-saturated SMFC becomes essential. Furthermore, SMFC still

has some drawbacks, such as low power production and large ohmic losses, which should be resolved.

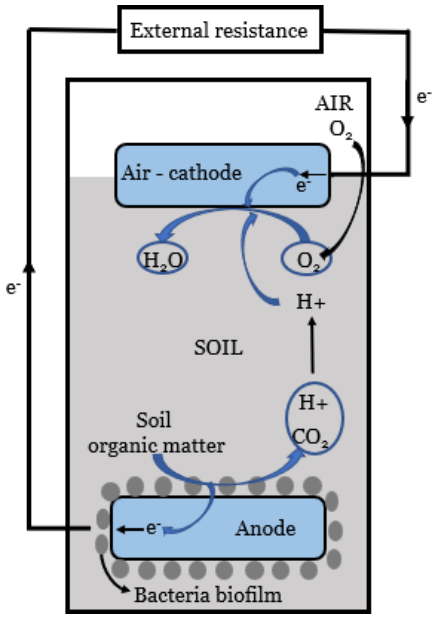

Figure 2.5. Schematic of a conventional SMFC

The setup of an SMFC considerably influences its performance. Figure 2.5 shows the construction of a traditional SMFC, which includes an air cathode hanging on the soil surface and an anode buried deep into the soil. It is difficult to install, has limited mobility, and is unsuitable for practical field deployment. Therefore, various efforts to optimize SMFC configuration with different sizes, structures, materials, and cost-effective purposes simultaneously have been made, but not much concentration is on portable SMFC construction [36]. Therefore, this thesis will introduce a series of low-cost portable SMFCs that are convenient, high-performance, and suitable for field application.

Many factors impacted SMFC performance, including electrode materials, measured soil internal resistance, temperature, and organic substrates [127,128]. Several electrode materials were examined, from carbon-based materials to capacitor materials or modified electrodes [128,129,130]. The results reported that carbon-based materials had the advantage in cost but did not show significant output production. In contrast, although capacitor materials may improve electrical performance, they are costly and require intricate fabrication procedures. Based on this literature, this thesis will examine the effectiveness of new SMFCs using both carbon-based and newly simple modified electrodes.

Numerous studies investigating the relationship between the performance of SMFC and soil properties reported that the soil type in the anodic chamber strongly impacted SMFC performance. Furthermore, SMFCs have been successfully operated using different kinds of organic wastewater [131,132]. Taking advantage of the rich organic content of rice washing wastewater, this thesis will investigate the performance of the new design SMFC when using home wastewater to generate electricity in different soils with different sources of organic matter.

The new emerging application of SMFC is biosensing. There are some advantages of SMFC-based biosensors, including (i) SMFC sensor employs available exoelectrogenic bacteria in soil/sediment to generate baseline voltage then limiting the inoculation of external microorganisms; (ii) exoelectrogenic bacteria could be protected from being inhibited by heavy metals ions owing to heavy metal absorbent of soil/sediments thus the sensor could normally work in the long term even after a shock; (iii) the sensor components are integrated and rigid [133]. However, the fundamental drawback of SMFC sensors is their limited sensitivity and long response time. A lot of research has been reported on using SMFCs as biosensors [123], but none of them have tried to sense soil water content. This thesis will propose a portable SMFC that can monitor soil water content to benefit agriculture management in the future.

CHAPTER 3 - ELECTRICITY GENERATION APPLICATION OF A HYBRID MFC

Enhancing the performance of a Baker's Yeast-powered MFC by hybridizing the MFC and EFC (enzymatic fuel cells) technologies.

3.1 Background

S. cerevisiae (Baker's yeast) is commonly used in the bread-making and beer-production industry. This specie of microorganism was used as a biocatalyst in MFCs due to its low cost and ability to metabolize a range of substrates, easy to handle, fast, mass cultivation, and it's also easy to preserve in a dried state [134]. Using the biofilm anode method can improve the performance of these types of MFCs by increasing the rate of electron transfer from yeast cells to the anode surface [135].

The primary enzymes responsible for breaking down ethanol in the human body are alcohol and aldehyde dehydrogenase enzymes (ADHE). These enzymes have also been used as biocatalysts in enzymatic fuel cells [136]. In addition, certain microorganisms, such as BKY, produce ethanol as a byproduct during fermentation [137]. This property can be used in microbial fuel cells (MFCs) to utilize the ethanol produced by BKY efficiently.

This study introduces a method to take advantage of the ethanol produced by baker's yeast during fermentation by adding commercially available alcohol and aldehyde dehydrogenase enzymes to the anode chamber. This allows the ethanol to be oxidized to acetic acid, contributing more power output to the MFC. The BKY biofilm anode formed in porous 3D-activated carbon paper was used in combination with ADHE to improve the performance of the MFC. This resulted in an increase of about 19% in the maximum power density compared to the MFC without ADHE. Also, aerobic and anaerobic culture conditions were investigated. The result showed that under the anaerobic culture condition, the MFC generated about 22% higher maximum power density than that of the aerobic condition. These results imply that the addition of ADHE can take full advantage of BKY-produced ethanol to boost overall BKY-powered MFC performance.

3.2 Materials and methods

3.2.1 Materials

Dry BKY was purchased from Nissin Food Products Co., Ltd. ADHE-based product composed of alcohol dehydrogenase and aldehyde dehydrogenase enzymes, acetic acid bacteria extract, vegetable oil, gelatin, and emulsifier (~ US$2 g-1, purchased from Kewpie Corp.). This product is a commercial supplement food product used for catalyzing the transforming reaction of alcohol to acetic acid inside the digestive system of people who often drink alcohol. Nitrogen, phosphoric, and potassium sources are included in a product called Hanakojo. Potassium ferricyanide, glucose, ethanol, and deionized water were also employed.

Electrodes were made of carbon-based materials, including AC paper C3P1AC1-60 (activated carbon 60 wt%, carbon fiber 20 wt%, cellulose fiber 20 wt%) and carbon paper C1-P2 (carbon fiber 50 wt%, cellulose fiber 50 wt%). Nafion PEM was used to separate the chambers.

3.2.2 Two-Chamber MFC configuration

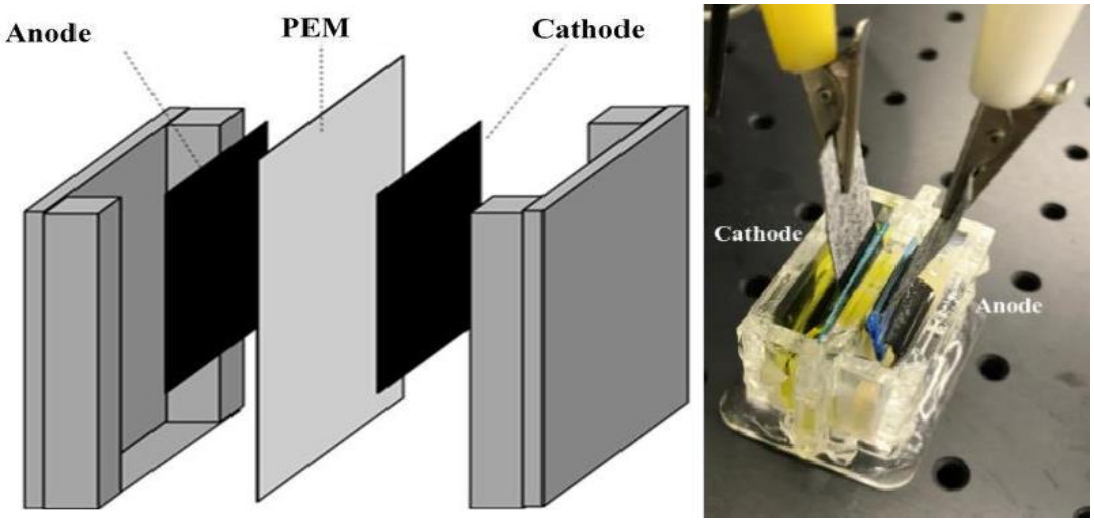

Figure 3.1. The BKY-powered MFC's setup (a) and picture image (b).

Figure 3.1 illustrates the setup of the two-chamber microbial fuel cell (MFC) used in this study. The MFC case was constructed with acrylic board and had an anode and cathode chamber with a volume of 5 cm^3 each. The Nafion PEM had a working area of 2.25 cm^2. The anode and cathode were made of C3P1AC1-60 and C1-P2, respectively, and each had an active surface area of 1 cm^2 (the immersed area in anolyte and catholyte).

In this study, the pre-established biofilm of BKY on the anode of the MFC reduces the start-up time and enhances the MFC's power density [135]. The growth medium consisted of 1 g of

glucose (2% (w/v)), 10 ml NPK, and 40 ml deionized water. The biofilm anode was created by placing C3P1AC1-60 with a size of 1 cm × 2 cm in microtubes containing 2 ml of GRM and 0.1% (w/v) dry BKY for 48 h at 30°C. After this incubation period, the biofilm anode and BKY culture solution were used in MFC experiments under aerobic and anaerobic conditions. A hydrophobic carbon paper C1E-1 was attached to the top of the electrodes to prevent oxidation of the metal clips.

3.2.3 Experimental methods and measurement

This study is the first research using a commercial alcohol dehydrogenase enzyme (ADHE) for electricity generation. Two experiments were conducted to test its performance. The first experiment, called Ex.1, involved the pH changing of ethanol solution using ADHE, while the second experiment, called Ex.2, involved using ADHE to produce electricity from an ethanol solution. In Ex.1, 3.5% (w/v) ADHE was mixed with commercial ethanol (96%), and the pH of the solution was measured at three different times (0 h, 0.5 h, and 24 h). In Ex.2, the anode chamber of the EFC was filled with 3 ml of commercial ethanol containing 3.5% (w/v) ADHE, and the concentration of ethanol was varied (1%, 5%, and 20%) for testing. In these experiments, the cathode chamber was filled with a solution of 30 mM potassium ferricyanide, and the C3P1AC1-60 anodes were used without a biofilm.

Finally, an experiment called Ex.3 used both BKY and ADHE. The anode chamber was filled with 1 ml of BKY culture solution, 2 ml of GRM, and the BKY-preloaded biofilm anode was placed in the chamber. The goal of the experiment was to generate electricity using the BKY and ADHE. In two experiments, the effects of a 3.5% concentration of ADHE on biofilm anodes were studied. The first experiment was conducted in aerobic conditions, while the second experiment was conducted in anaerobic conditions. In both experiments, the cathode chamber was filled with a solution of 30 mM potassium ferricyanide.

The output voltage was regularly checked using a data acquisition system and a computer system. Different loads were used to measure the power density from 100 to 0.2 kΩ. The MFC was measured under the conditions of open-air and room temperature. Each experiment was repeated at least twice, and the average results were used in the study.

3.2.4 Analysis

Current density (I) and power density (P) were calculated as mentioned in part 2.2.1, with the active surface area of the biofilm anodes measured in cm^2.

In the present work, 2% (w/v) glucose was used as a substrate and carbon source for growing BKY. When the concentration of glucose is above 0.01% (w/v), BKY will produce ethanol through primary fermentation under both aerobic and anaerobic conditions, as shown in equation (1) [138].

Under the catalytic activity of ADHE, ethanol transforms to acetaldehyde under the effect of the alcohol dehydrogenase enzyme (equation (2)) and then to acetic acid through the action of the aldehyde dehydrogenase enzyme (equation (3)). This two-step process allows for the utilization of both the ethanol electro-oxidation and acetaldehyde electro-oxidation reactions to generate electricity [136].

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (1)$$

$$2C_2H_5OH + 2O_2 \rightarrow 2CH_3CHO + 2H_2O \quad (2)$$

$$2CH_3CHO + 2O_2 \rightarrow 2CH_3COOH \quad (3)$$

In the preparation procedure for the SEM test, the anodes were lightly washed with water to eliminate any leftover dirt on their surface. It was then immersed in a sterilizing solution at 4°C for 24 hours to destroy bacterial cells. Next, they were continually dehydrated with 20%, 50%, 70%, and 99% ethanol solutions. Finally, they were dried for 24 hours at ambient temperature.

3.3 Results and discussion

3.3.1 The preliminary experiments of ADHE

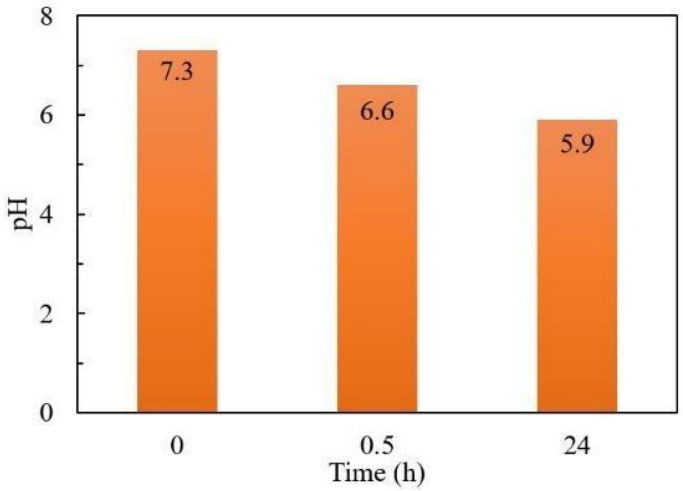

Figure 3.2. The change in the acidity level of ethanol when influenced by ADHE

Ex.1 was carried out first as a preliminary experiment to confirm the performance of the commercial ADHE. The outcome of this experiment is depicted in figure 3.2. The ethanol solution had an initial pH of 7.3. It dropped to 6.6 and 5.9 after 0.5 and 24 hours, respectively. The rise in acidity validates ADHE's catalytic activity in aiding the conversion of ethanol to acetic acid.

The second experiment Ex.2 was done to test the ability of ADHE to generate electricity in different concentrations of ethanol. Figure 3.3 shows the power density produced by the EFC with 1%, 5%, and 20% ethanol concentrations. The results showed that the EFC generated the most power when the ethanol concentration was 5%, suggesting that ADHE positively affects electricity generation from ethanol.

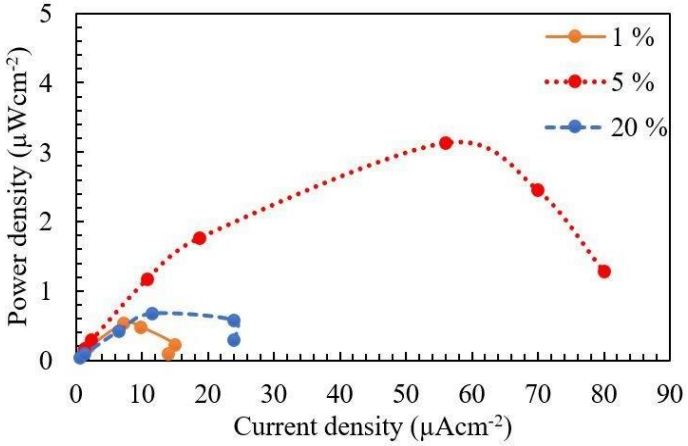

Figure 3.3. The power density of the EFC in Ex.2 (produces from ethanol through ADHE)

3.3.2 MFC performance

In Ex.3 experiment, the benefits of combining MFC and EFC technologies were tested. The biofilm anode and BKY culture solution were employed under aerobic culture conditions. Figure 3.4 shows the power density of the MFC with and without using ADHE in the anode chamber. In this case, the MFC with ADHE produced 19% more power density than the MFC without it, at 18.05 μWcm^{-2} compared to 15.19 μWcm^{-2}.

According to the experimental results, the application of ADHE in the anode chamber of the MFC improved the maximum power density (MPD) by 19 - 20%. These findings show that the inclusion of ADHE can fully use BKY-produced ethanol to improve overall BKY-powered MFC performance. Furthermore, under the anaerobic culture condition, the MFC with ADHE generated about 22% higher maximum power density than that in the aerobic culture condition. This result

confirms the effectiveness of anaerobic fermentation on the performance of the biofilm anode and potentially higher the concentration of produced ethanol.

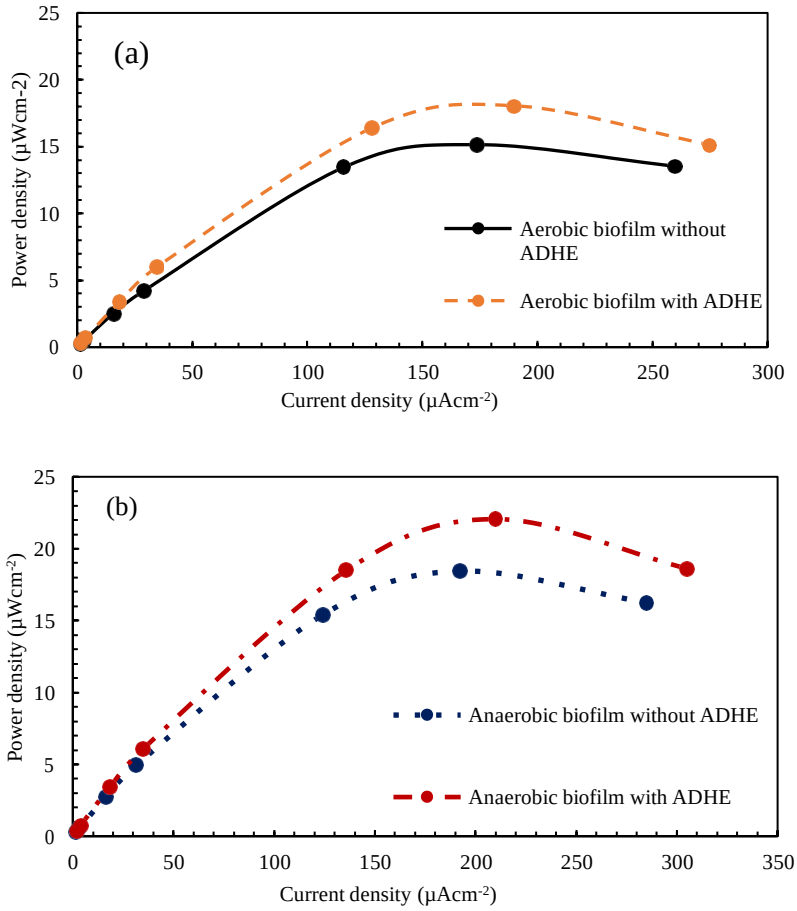

Figure 3.4. The power density in two circumstances, with and without the addition of ADHE to the MFC, utilizing (a) aerobic and (b) anaerobic conditions to produce biofilm anode and BKY culture solution containing BKY-produced ethanol

3.3.3 SEM image of the BKY biofilm

Figure 3.5 depicts SEM pictures of the BKY biofilm on the surface of the aerobic biofilm anode. It can be seen that BKY cells are attached not only on the surface but also deep inside the anode due to the porous 3D structure of the activated carbon paper. The direct contact between BKY cells and the conductive anode substrate facilitates a direct electron transfer mechanism [139]. As a result, utilizing the biofilm anode can considerably increase the overall performance of the mediator-less MFC [135].

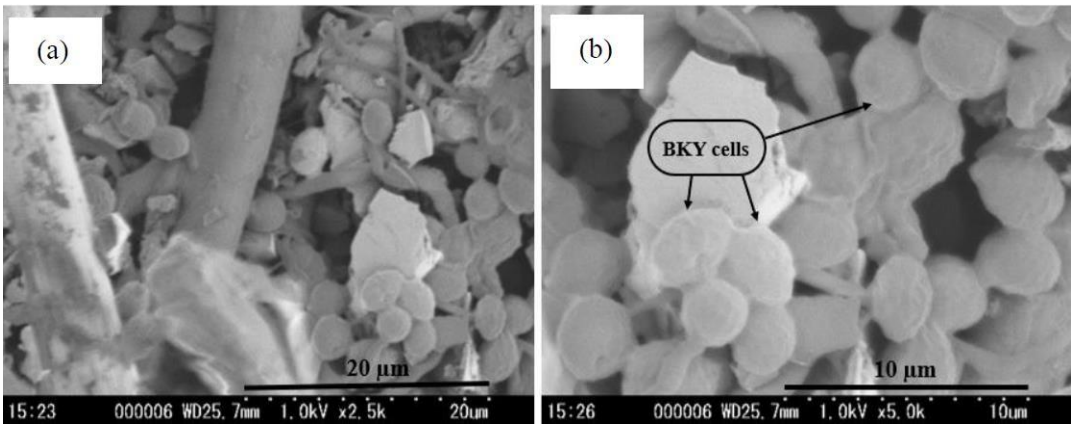

Figure 3.5. BKY biofilm SEM images on the anode surface at two magnification levels: (a) 2.5 k and (b) 5.0 k

3.4 Conclusions

The combination MFC enabled about 19% improvement in the maximum power density compared with the MFC without ADHE. The maximum power density of MFC under anaerobic conditions was 22% higher than that of the aerobic condition. This study has successfully demonstrated theoretically and experimentally the idea of hybridizing the MFC and EFC technologies in the BKY-powered MFC to take full advantage of the intrinsic property of the system and improve the overall power density. The commercial ADHE used in this study has some characteristics, such as cost-effectiveness, long preservation time, and safe to handle. Further studies should pay attention to the optimization and long-term stability of the system.

CHAPTER 4 - SOIL-BASED MFC APPLICATIONS

PART A. IMPROVING ELECTRICITY PRODUCTION

A1. A novel design portable plugged-type SMFC for bioelectricity generation

4.A1.1 Background

The success of SMFCs relies heavily on their design and structure. Previous SMFC designs have been difficult to set up and large in size. Additionally, the air cathode placed on the soil and covered by water has limited oxygen exposure, determining the effectiveness of SMFC. Most current research has been on improving SMFC designs, including single-chambered MFCs with or without membranes, column-type MFCs, and double-chambered MFCs. However, there has been little research on creating portable SMFCs.

In this work, a low-cost portable plugged-type SMFC (PSMFC) for on-demand micropower production was designed and built. The cathode of the PSMFC was placed inside a chamber to avoid contact with the soil, while the anode was left exposed to the soil and activated simply by inserting it into naturally moist soil, which is easily accessible. The PSMFC used carbon-based electrodes to save on cost. After starting, it began producing energy after 1 hour and reaching a power density of 7.3 mW/m^2 after 48 hours. The PSMFC has potential applications for generating electricity for remote sensors and soil sensing devices.

4.A1.2 Materials and methods

4.A1.2.1 Materials and soil sampling

ACF and Multi-walled carbon nanotube (MWCNT) dispersion coating liquid N7006L containing 6.1 wt% MWCNT were used to fabricate the electrodes. Acrylic board (2 mm thickness) and stainless mesh (0.1 mm thickness) were used to make the PSMFC.

The muddy soil used in this study was obtained between 10 and 30 cm deep from drainage near rice paddies in Shiga Province, Japan. The physicochemical parameters of the tested soil were as follows: pH 4.5, EC 1.36 dS/m, and TC 85,200 mg/kg.

4.A1.2.2 PSMFC configuration and operation

Figure 4.1 (a) illustrates the proposed PSMFC structure. The reactors were constructed using acrylic boards with the following exterior dimensions: 7.5 cm height, 4.0 cm width, and 2.5 cm thickness. A Nafion PEM was chosen to separate the cathode and anode chambers. The cathode chamber was sealed to prevent soil water from entering. A small hole was made at the top of the cathode chamber (above the soil) to allow air to reach the cathode. Both electrodes were made of carbon-based materials. Each anode electrode was made of activated carbon felt with a surface area of 4 cm^2 and a thickness of 0.5 mm. The anode was dipped in an LB medium solution, which helped promote the formation of the biofilm on the anode to shorten the startup time and allow it to dry naturally. The anode was placed directly in contact with the soil.

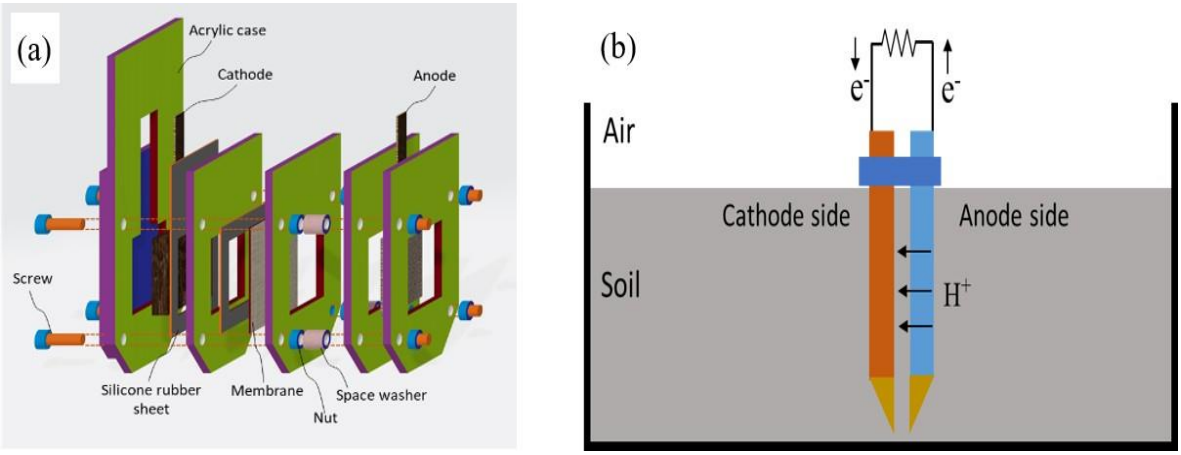

Figure 4.1. PSMFC structure schematic (a) and experimental setup (b)

In order to assess the effectiveness of the cathode electrode, this study examined two different cathode patterns. The first cathode, labeled PSMFC 1, was created by adding 2 mL of a mixture containing 1 g CF, 1 g AC powder, 5 mL CNT, and 2 mL Nafion solution to a sponge of dimensions 2 cm height x 2 cm width x 0.3 cm thickness. The sponge was then dried at 40°C for 24 hours. The second cathode, labeled PSMFC 2, was made in the same manner but with a mixture containing 0.2 g CF, 0.2 g AC powder, 3 mL CNT, 1.5 mL Nafion solution, and a sponge of dimensions 2 cm height x 2 cm width x 1 cm thickness. Both cathodes were placed inside the cathode chamber for testing.

No foreign microorganisms were required to function PSMFC since many electrochemically active bacteria were already present in the soil.

Figure 4.2 shows images of the PSMFC and its experimental setup in saturated condition with stainless mesh connecting to electrodes to collect current through a 2 kΩ external resistor at 30 - 33°C. All tests were carried out in triplicate; the data was gathered and displayed as average results.

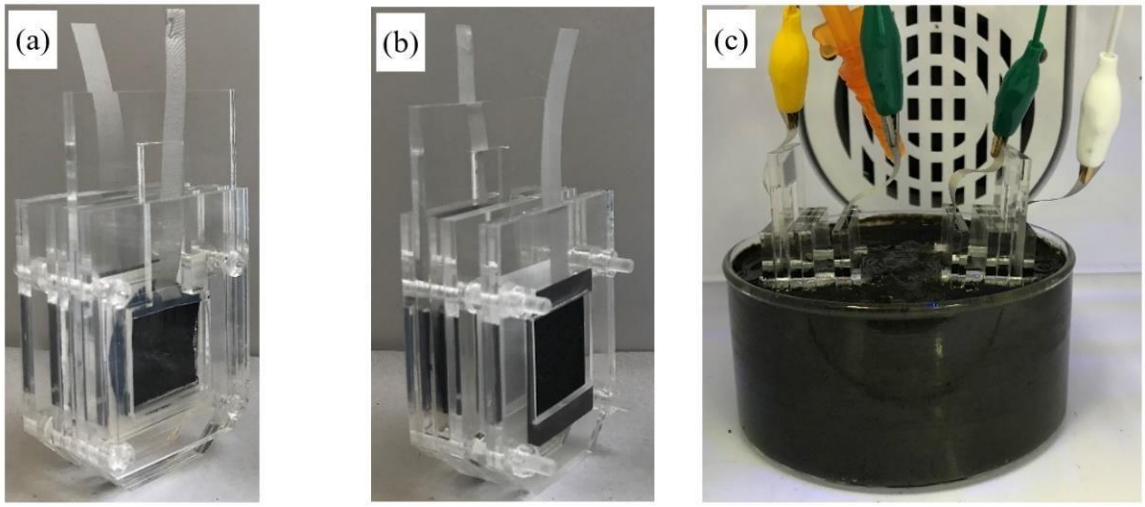

Figure 4.2. Photo images of the fabricated PSMFC: cathode side (a), anode side (b), and experimental setup with two PSMFCs (c)

4.A1.2.3 Analysis and calculation

The surface morphology of the anode was examined using the SEM test with the preparation procedure mentioned in section 3.2.4.

The output voltage across the external resistor was measured every 20 minutes for 7 days. The current density (I) and power density (P) were calculated using the method described in section 2.2.1. Polarization and power density curves were obtained by varying the external resistance between 0.3 and 10 kΩ.

4.A1.3 Results and discussion

4.A1.3.1 Characterization of anode surface morphology and biofilm formation

SEM examined the surface morphology of the surface of the anode electrode. As shown in figure 4.3 (a), the anodic electrode's surface was porous with layers of clustered particles of activated carbon attached to carbon fibers and cellulose fibers. Although the preparation process of the samples for SEM measurement may significantly remove many cells attached to the surface of the anode, the SEM images at high magnification clearly showed biofilms of bacteria on the

surface (figure 4.3 (b) and (c)). Previous research found that a greater microporous surface improved biofilm growth on the anode, resulting in increased current production and power density [140].

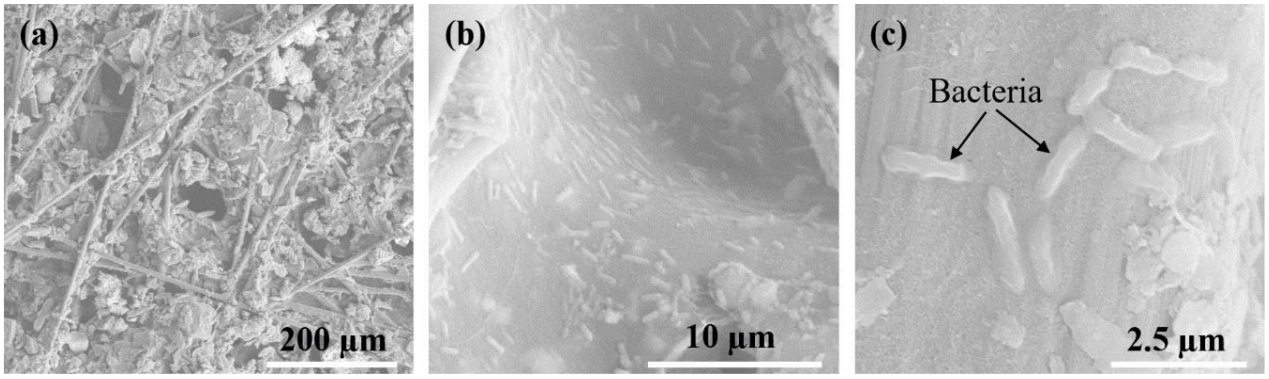

Figure 4.3. The surface of the anode electrode at different magnifications by using SEM: (a) 150, (b) 4000, and (c) 12,000

Bacterial populations in the soil employed in this investigation were not identified because the necessary tools for characterizing them were unavailable. However, according to specific reported literature, paddy soil has a high amount of exoelectrogenic bacteria such as *Shewanella sp., Geobacter sp., and Pseudomonas spp*. [141,142]. Therefore, the muddy soil employed in this investigation is likely to include the same species.

4.A1.3.2 PSMFC performance and the effect of different cathodes

During the testing period, the output voltage of the PSMFC 1 and PSMFC 2 was recorded across a 2 kΩ external load to assess their performance. Figure 4.4 (a) shows the computed current densities against time. It can be seen that the current densities increased rapidly in the first 5 h and reached the peaks after about 35 h. The maximum current density of the PSMFC 1 (100.12 mA/m^2) was higher than that of the PSMFC 2 (81.48 mA/m^2). This result is considered a short startup time, which may be attributed to the LB medium absorbed in the anode that facilitates the biofilm formation and microorganism activities.

After reaching their maximum, the current densities of PSMFC 1 and PSMFC 2 steadily dropped. The current densities of PSMFC 1 and PSMFC 2 were similar after 168 hours (41.85 mA/m^2 and 39.52 mA/m^2, respectively). These values represent a 58% and 51% decrease from their peaks. This decline in current density may be due to a reduction in the amount of organic

matter in the soil, which can lead to reduced bacterial activity. Both PSMFCs showed similar behavior patterns, but PSMFC 1 had a higher output in the first 72 hours.

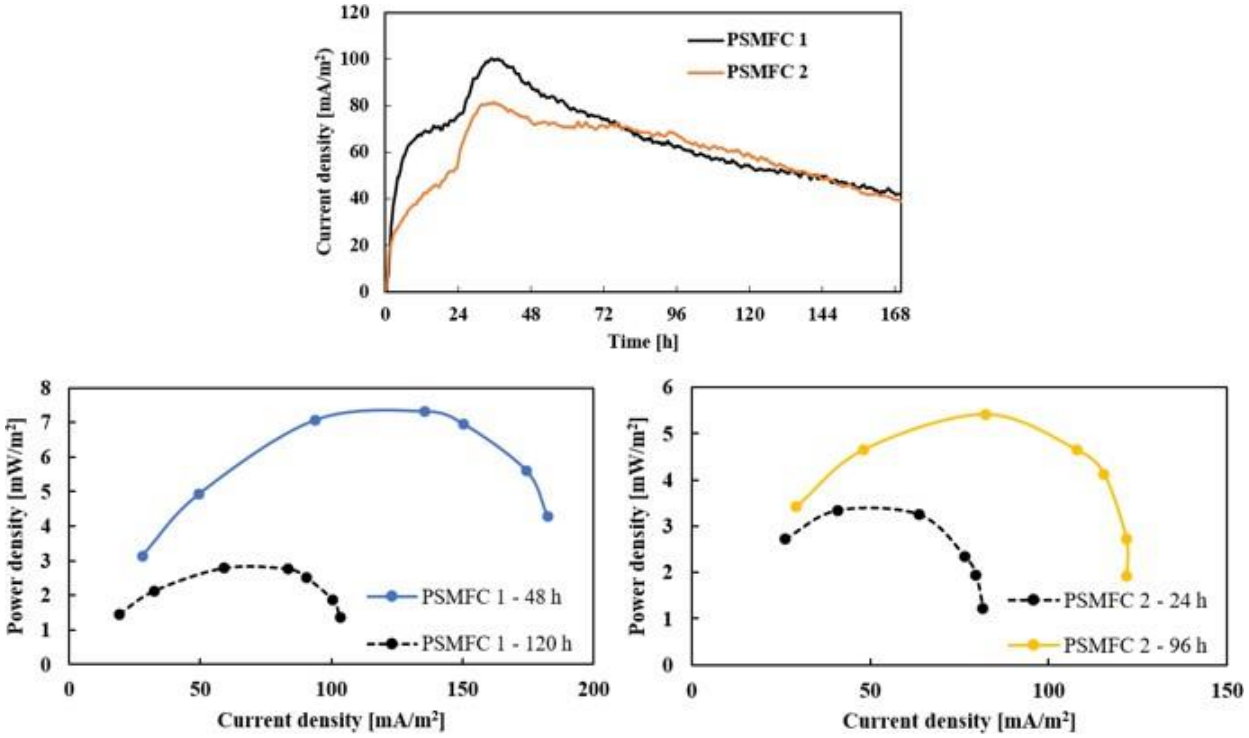

Figure 4.4. Electricity production attributes: (a) polarization curve of two PSMFCs, (b) PSMFC 1 power densities at 48 h and 120 h, and (c) PSMFC 2 power densities at 24 h and 96 h

The testing duration in this study was set at 7 days. However, as shown in figure 4.4 (a), the PSMFCs might continue to create output for a relatively more extended period. The deterioration of the production is determined by the soil's nutrients and other complicated variables. Therefore, the PSMFCs' end-of-life determination might take considerably longer.

Additionally, the power densities of PSMFC 1 and PSMFC 2 were recorded and displayed in figures 4.4 (b) and (c), respectively. The findings showed that the power density of PSMFC 1 at 48 was 7.3 mW/m^2, whereas that of PSMFC 2 at 24 h was 5.4 mW/m^2. These results match the polarization curves displayed in figure 4.4 (a).

4.A1.4 Conclusions

The PSMFC utilized in this investigation was designed and built with low-cost materials. By simply putting the PSMFC into naturally damp soil, it began producing power after 1 hour and continued to increase to the maximum after 24 - 48 hours. Experimental results showed that the

PSMFC could produce a maximum power density of 5 - 7 mW/m^2. Also, it can be expected that more power can be obtained if the cathodic quality is improved further. The compact and simple construction of the proposed PSMFC makes it a potential power source for remote sensors and soil quality detecting devices.

A2. A compact, membrane-less, easy-to-use SMFC: Generating electricity from household rice washing wastewater

4.A2.1 Background

The fundamental disadvantages of SMFCs are their low power output and high operating cost, making them impractical for real-world applications. Furthermore, the cost of PEM often accounts for more than half of the overall material cost of MFCs [143]. As a result, membrane-less structures are preferred to decrease overall costs, particularly in SMFC applications where scalability is critical. This work presents a small, membrane-less, user-friendly SMFC manufactured with a 3D printer and low-cost carbon-based electrode materials to overcome these issues. The SMFC has double anodes and double air cathodes composed of ACF-coated MWCNT to enhance the electrodes' working surface and achieve better power output. The anodes and cathodes of the SMFC were placed in the case, and the case could be readily inserted into the soil to activate.

Furthermore, the significance of organic compounds has been revealed in several studies. The rich organic content of rice washing wastewater (RWW), including starch, minerals, and vitamins, makes it a good candidate for use as a low-cost and abundant biofuel. Because rice is the most widely consumed food in Asia and RWW is readily available in almost every household in the region, it was chosen as a potential biofuel for powering the SMFC.

Muddy and sandy soil were also employed in this study to test the effectiveness of a proposed soil microbial fuel cell (SMFC) in different soil conditions. A control experiment, in which just water was injected into the soil to maintain humidity, was carried out for comparison. 15 ml of RWW or water was added to the SMFCs every 48 hours. The result showed that when muddy soil was used, the MPD of the SMFC with RWW supplied was 485.2 mW/m^2, which was 2.4 times higher than the controlled SMFC (202.9 mW/m^2). When sandy soil was used, the SMFC with RWW supplied generated the MPD y of 112 mW/m^2, while that of the controlled SMFC produced nearly none. These results imply that the proposed SMFC can operate in different types of soil and effectively generate bioelectricity from RWW.

4.A2.2 Materials and methods

4.A2.2.1 Materials and soil sampling

ACF, 6.1 wt% MWCNT are the main components to make electrodes.

RWW from Shiga white medium-grain rice was prepared by soaking and washing 100 g white medium-grain rice in 100 mL tap water for 1 minute and then collecting the rinsing water. The concentration of the organic content in 100 mL of RWW was 1 g $\pm$ 3%.

All soil samples were taken between 10 and 30 cm below the surface.

Table 4.1 Soil sampling and measurement conditions

		Muddy soil	**Sandy soil**	**Test standards/devices**
Soil properties	pH	4.5	6.6	Soil:water = 1 : 2.5
	EC (dS/m)	1.36	0.01	Soil:water = 1 : 5
	TC (mg/kg)	85200	1970	Elemental analyzer
Collected place		drainage near rice paddies in Shiga, Japan	playground at a park in Shiga, Japan	

4.A2.2.2 SMFC design

In this work, a membrane-less SMFC with double anodes and double cathodes was constructed and implemented, as illustrated in figures 4.5 (a), (b), and (c). The SMFC case was created using a 3D printer with PLA filament. The casing measured 7.0 cm in height, 3.0 cm in width, and 3.0 cm in thickness. The case's bottom was built with tapered forms for easy insertion into the soil. The cathodes were air cathodes that were placed in the cathode holder, which was then fixed on the case. The anodes were placed in the anode holder slits at the case's bottom.

When the SMFC was inserted into the soil, the cathodes were on the soil surface, and the soil encircled the anodes. The tubulous chamber was used to provide RWW and water to the anode. Two small sticks were fixed inside the tubulous chamber to monitor the maximum and minimum water levels inside the case. These sticks help maintain the optimum soil humidity for the operation of the SMFC because dry soil does not favor bacteria activity and proton exchange, but too much water causes low air-cathode activity.

The anodes and cathodes in this system were made by combining two layers of ACF with a stainless mesh current collector. The layers were held together by coating them with a dispersion of MWCNT and then drying them at 40^{o}C for 24 hours. Next, the SMFC was plugged into a plastic bottle filled with 600 g water-saturated soil, as shown in figure 4.5 (d).

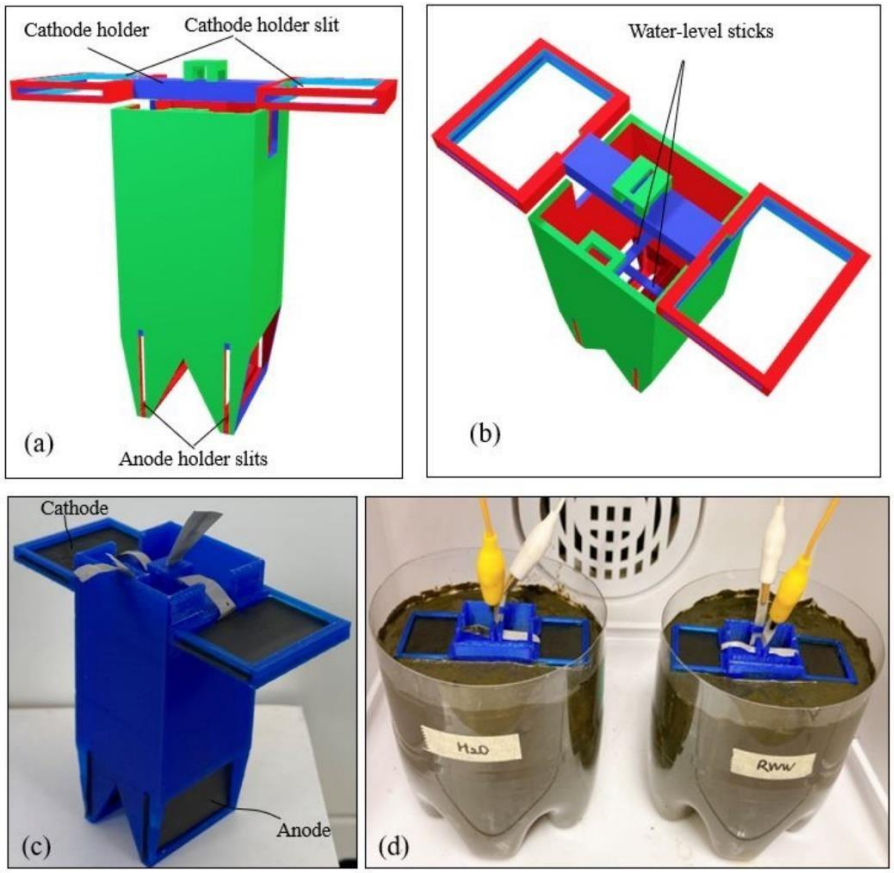

Figure 4.5. The SMFC design: SMFC case's schematic structures (a, b), photo image of the constructed SMFC (c), and experimental setup in muddy soil's image (d)

4.A2.2.3 Experimental setup

This study examined the impact of RWW on the performance of SMFC in different soil types. In the first experiment, called Ex.1, two identical SMFCs were placed in muddy soil, with one being fed tap water and the other being provided RWW (SMFC 1 and SMFC 2, respectively). The second experiment (Ex.2) used the same setup but with sandy soil instead of muddy soil (SMFC 3 and SMFC 4, respectively). For both experiments, tap water and RWW were added to the SMFC chambers after activating for 24 h.

The soil moisture level in the experiments gradually decreased over time due to natural evaporation. Therefore, tap water and RWW were added every 48 h to fill the chambers of the SMFCs up to the max water level stick (about 15 ml each time). All the tests were carried out in an incubator at 30 $\pm$ 2°C. Each experiment was performed at least two times, and the average results were presented.

4.A2.2.4 Analysis

The concentration of chemical oxygen demand (COD) in the samples was determined using conventional procedures with a DR3900 spectrophotometer and TNT 822 reagents (made by Hach Company). As a control, deionized water was utilized. The RWW samples were diluted with deionized water to create a 0.1 dilution before being measured for COD.

The SEM test is also carried out in this work.

The examined period was 20 days. The current density (I) and power density (P) were calculated using the method described in section 2.2.1. The power density curves were measured by varying the external resistance in the range of 10 – 0.15 kΩ.

4.A2.3 Results and discussion

4.A2.3.1 COD analysis

The COD concentration in the sample of RWW was determined to be 4,350 ± 100 mg/L, which is much higher than the concentration of organic matter in tap water (around 2 mg/L). This result confirms that RWW contains a significant amount of organic matter, mainly in the form of starch, protein, and vitamins derived from the rice grains themselves [144].

4.A2.3.2 SMFC bioelectricity production in muddy soil

Figure 4.6 presents the results of the Ex.1 experiment. SMFC 1 and SMFC 2 were both self-activated and had the same response over the first 24 hours (figure 4.6 (a)). After 24 hours, SMFC 1 and SMFC 2 had an open-circuit voltage (OCV) of 0.31 V (figure 4.6 (a)) and power densities of 65.3 mW/m^2 and 67.8 mW/m^2 (figure 4.6 (b)), respectively. This result may be due to the presence of exoelectrogenic bacteria in the muddy soil, which can contribute to high fuel cell performance.

The first turn of adding water and RWW to SMFC 1 and SMFC 2 was accomplished. After RWW was added to SMFC 2, its OCV and MPD quickly increased and peaked at 142 hours. After reaching the peak, the output of SMFC 2 slowly decreased until it reached a stable state. Meanwhile, the OCV and MPD of SMFC 1 increased slowly and got a nearly stable condition. As shown in figure 4.6 (b), the MPD of SMFC 2 was significantly higher than that of SMFC 1 after RWW was added.

After 142 h, the OCV and MPD of SMFC 2 were 0.84 V and 485.2 mW/m^2, respectively, which were 1.53 and 2.39 times greater than those of SMFC 1 (0.55 V and 202.9 mW/m^2).

Following the peak, the SMFC 2 output voltage progressively declined until 256 h and remained steady until the experiment was completed. SMFC 2 had an average MPD of 371.1 mW/m^2 during Ex.1. Contrary to SMFC 2, OCV of SMFC 1 continued to increase until 256 h and kept the same steady stage as SMFC 2 with an average maximum power density of 236.8 mW/m^2. This result might be related to the abundant organic compounds already present in muddy soil, as evidenced by its high TC of 85,200 mg/kg.

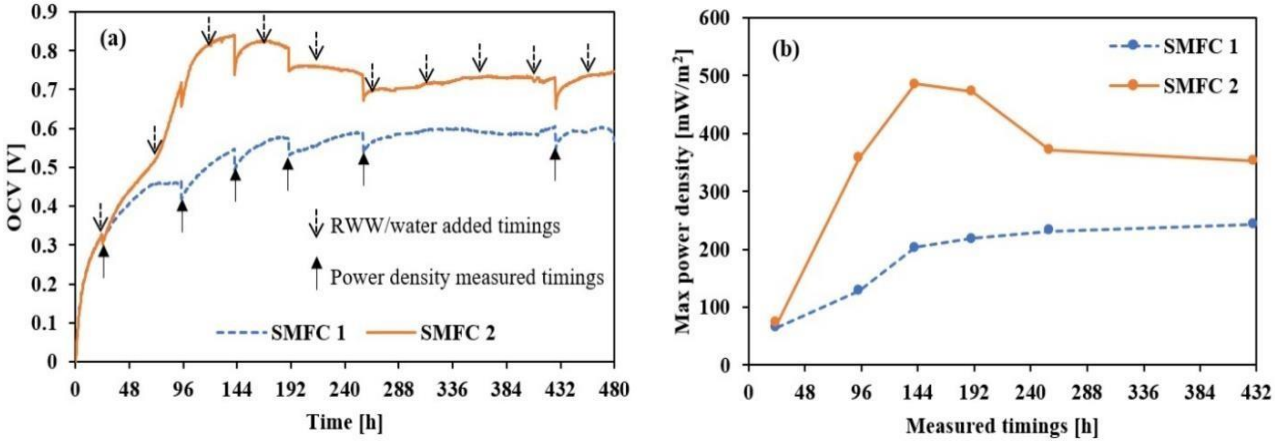

Figure 4.6. SMFC 1 and SMFC 2 responses generated from muddy soil: (a) OCV and (b) MPD

The higher power output of SMFC 2 compared with SMFC 1 could be attributed to the addition of RWW. This result proved that RWW was a good nutrition source for soil microorganisms. RWW supplies organic matter to microorganisms to proliferate on the anode electrode and generate electricity through SMFC operation.

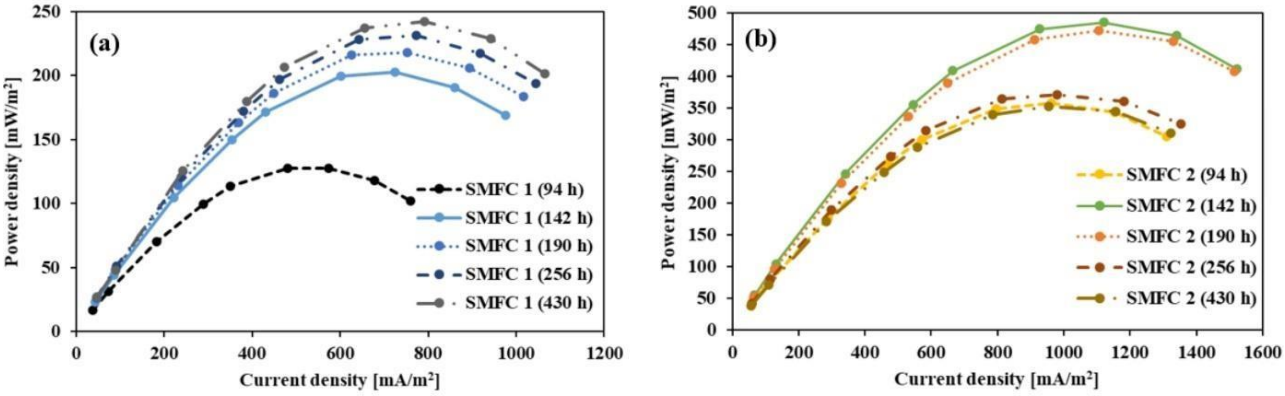

Figure 4.7. SMFC 1 (a) and SMFC 2 (b) polarization curves at various measurement times.

Moreover, the polarization curves of SMFC 1 and SMFC 2 are exhibited in figure 4.7. It can be seen that the power densities measured at different timings of SMFC 2 were significantly higher

than that of SMFC 1. These results reaffirm the positive impact of RWW on improving the electrical generation of the SMFC.

4.A2.3.3 SMFC bioelectricity production in sandy soil

The effectiveness of RWW as a feedstock for SMFC might be validated further by employing sandy soil, which possesses a particularly low TC of 1,970 mg/kg. SMFC 3 and SMFC 4 generated nearly no voltage in sandy soil over the first 24 hours without adding water or RWW (figure 4.8). The low bacterial activities and the poor levels of organic matter in sandy soil might explain this behavior.

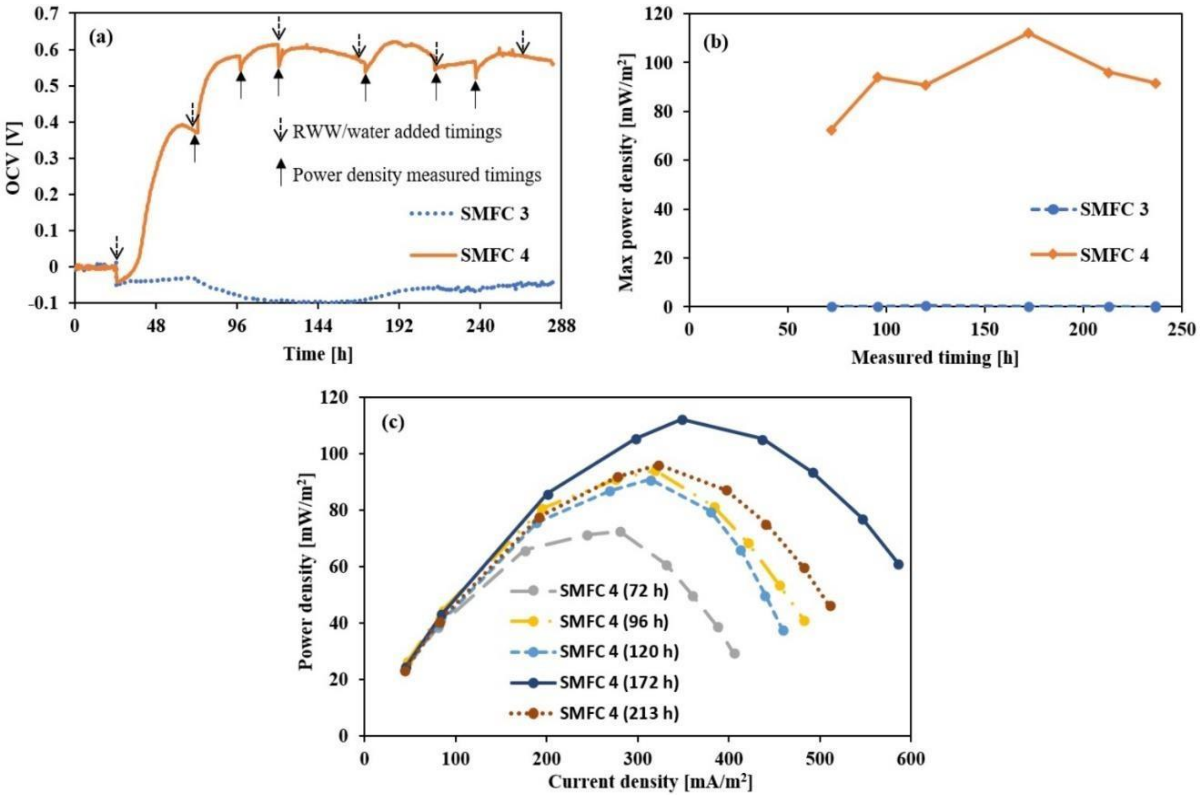

Figure 4.8. SMFC 3 and SMFC 4 responses generated from sandy soil: (a) OCV and (b) MPD, and (c) polarization curves of SMFC 4 at various measurement times.

When RWW was added to SMFC 4 at 24 hours, its OCV increased quickly and peaked at 72 h with an MPD of 24.05 mW/m^2 (as shown in figure 4.8(b)). Simultaneously, SMFC 3, which was supplied with water only, was still unable to produce any output. Furthermore, the voltage generated by SMFC 4 continued to increase and get a stable stage after 96 h with an average output voltage of 0.6 V. The MPD of SMFC 4 peaked at 112.0 mW/m^2 after 172 h. Figure 4.8 (c) shows the evolution of the polarization curves of SMFC 4 measured at different timings. The output of

SMFC 4 showed a downtrend after reaching the peak. These results once again confirmed the key role of RWW in enhancing the operation of the SMFC.

4.A2.3.4 SEM images of the anodes

Biofilms of rod-shaped bacterial cells were seen on the anodes of SMFC 1 and SMFC 2 (figure 4.9 (a) and (b)), which were operated in muddy soil. The anode of SMFC 2 had denser biofilms than SMFC 1 anode, indicating that better bacteria cells developed when RWW was supplied. Consequently, in terms of power generation, SMFC 2 outperformed SMFC 1.

In contrast, operating in sandy soil, almost no bacterial cells were observed on the anode of SMFC 3, whereas dense biofilms were seen in the anode of SMFC 4 (figure 4.9 (c) and (d)), which resulted in SMFC 3 producing almost no power output compared to the higher production of SMFC 4. In addition, the similar behavior of biofilms on the anodes of SMFC 2 and SMFC 4 could also be seen in figure 4.9 (b) and (d) when RWW was used.

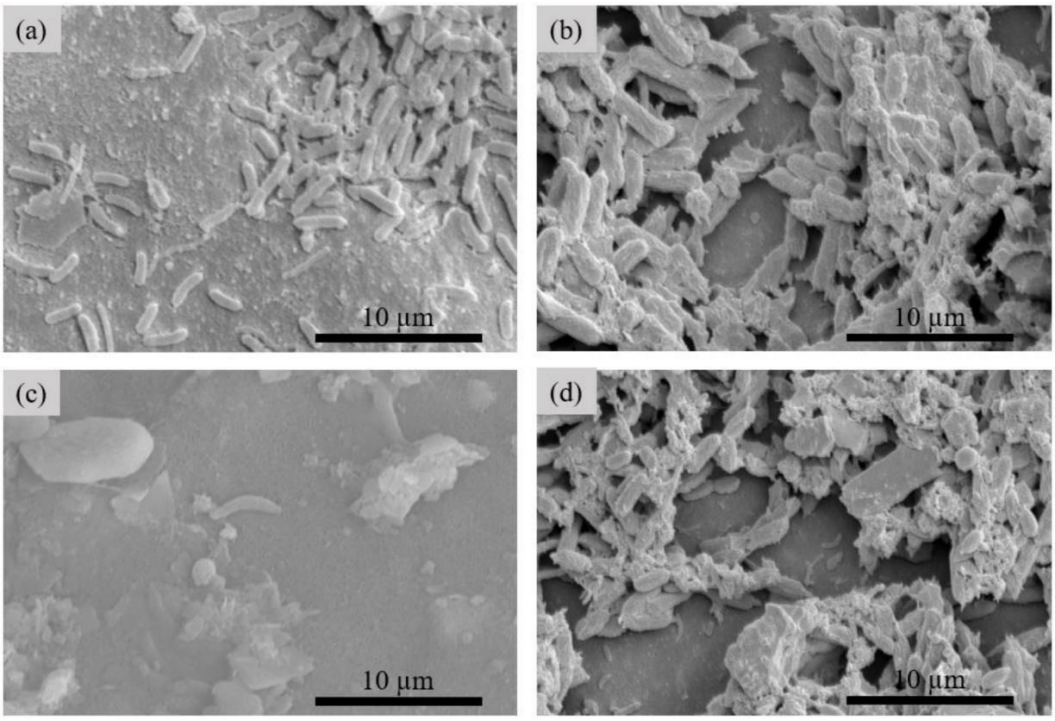

Figure 4.9. The anode biofilms of (a) SMFC 1, (b) SMFC 2, (c) SMFC 3, and (d) SMFC 4 by using SEM

4.A2.3.5 Discussion

The experiment involved monitoring the pH of liquid inside four SMFCs with the same initial pH of 4.5. Over the first 144 hours, the pH of SMFC 1 and SMFC 2 rose to 6 and 5.5, respectively.

From then to the end of the experiment, the pH within SMFC 1 was steady at 6, while the pH inside SMFC 2 declined and stabilized at about 4. Furthermore, the pH inside SMFC 3 and SMFC 4 behaved similarly. The pH reduction in SMFC 2 and SMFC 4 might be attributed to the fermentation process of RWW starch to acetic acid, as indicated in 3.2.4, which affects the surface charge and interface potential characteristics, resulting in SMFC 2 and SMFC 4 power reduction.

Another issue that might impact the endurance and stability of SMFCs is electrode deterioration [145].

According to the testing results, without RWW, the greatest MPD generated by SMFC 1 in muddy soil without RWW at a stable phase was 236.8 mW/m^2, while SMFC 3 created tiny production when operating in sandy soil. Besides, the MPD of SMFC 2 in muddy soil was about 4–fold higher than that of SMFC 4 in sandy soil in the case of feeding RWW. These findings might be explained by the difference in exoelectrogenic strains found in the two soil types. Even though this argument could not be proven empirically in the current investigation by detecting soil bacterial characteristics, it might be supported partly by the SEM pictures.

Furthermore, previous research has reported that the EC of employed soils influences SMFC performance, with a higher EC leading to a better output [146]. In the current investigation, the sandy soil EC was much smaller than muddy soil (0.01 dS/m and 1.36 dS/m, respectively). As a result, it is understandable that SMFCs operated in sandy soil produced significantly less power than those working in muddy soil.

The performance of this SMFC design is higher than some previously reported conventional SMFCs utilize carbon-based materials of 16.4~28.6 mW/m^2 [84], 17.3 mW/m^2 [146], and 49 - 61.5 mW/m^2 [27].

4.A2.4 Conclusions

A low-cost membrane-less SMFC with double air cathodes and double anodes made of carbon-based materials was developed. RWW has a substantial influence on improving the efficiency of the SMFC in various soil types. The small, simple-to-install construction SMFC enabled it to yield high and consistent output in muddy soil, with an MPD of 485.2 mW/m^2 when fed by RWW and 202.9 mW/m^2 with tap water. Even operating in low EC and a poor nutrient environment of sandy soil, the SMFC achieved an MPD of 112 mW/m^2 when supplied by RWW. The investigated SMFC may enable new possible applications due to its high electrical production and adaptability in diverse soil types.

A3. Portable membrane-less SMFC: Using multiwalled CNT paper electrodes

4.A3.1 Background

Because of their outstanding features and strong conductivity, carbon-based materials are the most often utilized electrode materials in SMFCs. Recently, the combination of AC and conductive materials such as carbon fibers, graphene, and CNT has been a good choice for making electrodes [147,148]. However, SMFC electrodes employ AC sheets composed of AC, CF, and cellulose without a robust binder, resulting in low durability. An appropriate binder for this purpose should have high conductivity and water-resistant properties.

In this research, the electrodes were fabricated using a low-cost MWCNT paint as a powerful binder to bind AC powder, CF, and cellulose fiber to create a paper-like material (MCNTP). After drying, the MWCNT paint is highly conductive and water-resistant, giving the MCNTP characteristics of high conductive, flexible, and durable. Furthermore, a stab-type membrane-less SMFC (SSMFC) was developed and manufactured using 3D printing.

To tackle the common air exposure problem of the typical SMFC design, the SSMFC was developed as a module fixed with three anodes in series and a floating air cathode. When the SSMFC is placed in wet soil, it can be quickly activated and generates an MPD of 60 - 70 mW/m^2 for three independent trial cycles. The floating cathode outperformed the non-floating one. In addition, it was also used to power a clock to demonstrate its practical application.

4.A3.2 Methods

4.A3.2.1 MCNTP electrodes fabrication

Materials needed for this procedure include MCNT paint, CF, AC powder, cellulose fiber, and deionized water.

The steps involved in producing MCNTP are illustrated in figure 4.10. The process begins by mixing 6 ml of MWCNT paint, 0.5 g CF, 0.5 g AC powder, and 0.5 g cellulose fiber in 50 ml of deionized water. Next, the solution is stirred for 20 hours at 600 rpm using a magnetic stirrer, then put into a flat acrylic container with dimensions of 10 cm width x 11 cm length x 2.5 cm height and left to dry at 40^{o}C for 48 h. The final product, MCNTP, is shown in figure 4.10 (c).

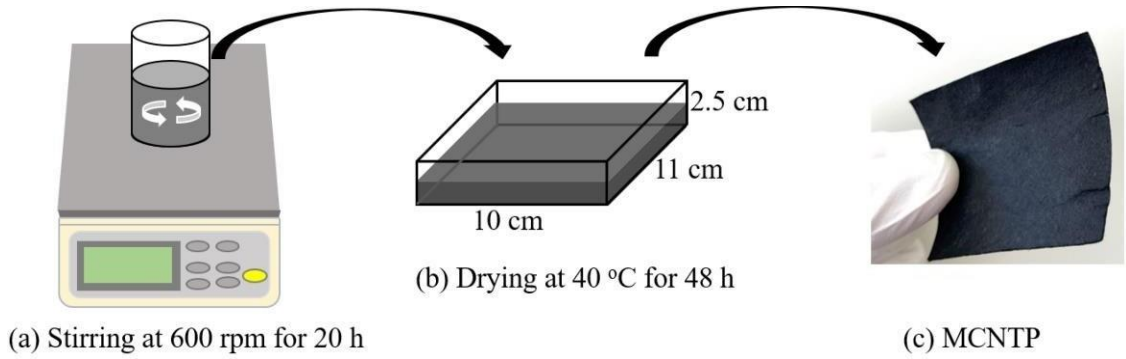

Figure 4.10. MCNTP fabrication process: (a) mixing materials, (b) drying, and (c) photo image of the fabricated MCNTP

Following the above simple fabrication technique, the fabricated MCNTP resembles a black paper sheet but is durable and flexible due to the binder in the multi-walled CNT paint links and blends the carbon-based materials to create that particular structure. This structure allows the MCNTP to sustain in wet soil for an extended period without decomposing.

4.A3.2.2 SSMFC configuration

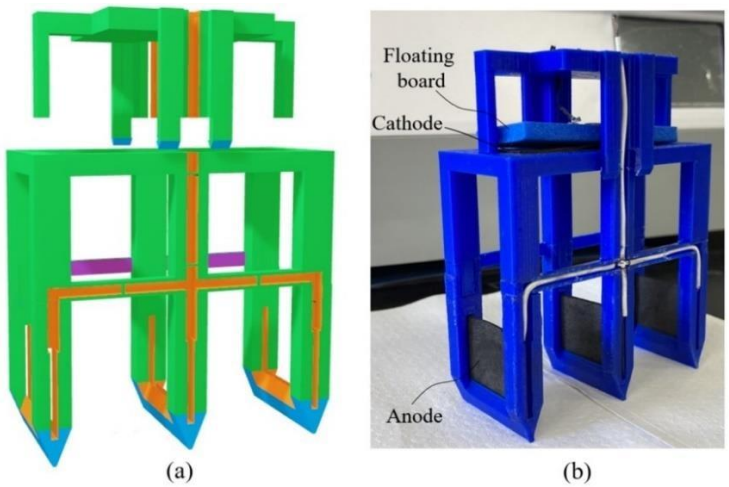

Figure 4.11. The SSMFC case architecture (a) and a picture image of constructed SSMFC (b)

Figure 4.11 (a) exhibits the SSMFC case's construction which included three legs with wedge-like edges for easy stabbing into the soil. An anode holder was located on the lower portion of each leg. The gap between the legs measured 2.5 cm. The top section was used to support the floating cathode and was able to be disconnected from the bottom section. Figure 4.11 (b) is a picture of the installed SSMFC module. Three anodes were set vertically and connected in series

by copper wires. The cathode was attached horizontally to the bottom of a floating board made of styrene foam.

The anode was fabricated by attaching two layers of the fabricated MCNTP with 0.5 ml multiwalled CNT paint, followed by a drying process at 40°C for 10 h. The size of each anode was 2 × 3 cm (6 cm^2). The air-cathode was fabricated by attaching three layers of MCNTP by 1.5 ml of the mixture of multiwalled CNT paint and Nafion 10 wt% with a ratio of 2:1, followed by a drying process at 40°C for 10 h. The size of the cathode was 2 × 6 cm. Stainless mesh (60 µm thickness) was used as the current collector for the cathode. The cathode was fastened to the floating board by the stainless mesh.

4.A3.2.3 Experiment and measurement

The utilized soil in this work was taken from drainage near rice paddies up to a depth of 15 cm Kusatsu, Shiga, Japan. The soil was kept at room temperature.

The MCNTP resistance was measured using the 4-point probes technique and an NPS Resistivity Processor (Model Sigma-5+). The materials' elemental composition was determined using energy-dispersive X-ray spectroscopy in conjunction with a scanning electron microscope system (SEM/EDS). SEM was used to examine the surface of the MCNTP (both cathode and anode) before and after the experiment.

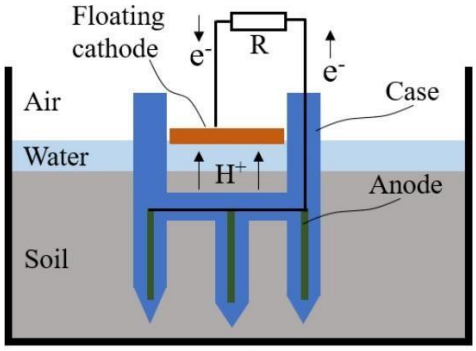

Figure 4.12. The SSMFC construction is set up in the soil.

When plugging into the soil, the anode was 5 cm below the soil surface, while the cathode remained floating on the water, as shown in figure 4.12. An experiment was carried out with two SSMFCs coupled with floating and non-floating cathodes to examine the floating cathode's effectiveness. The non-floating cathode was similar to the floating cathode but did not have the

associated floating board. As a result, the non-floating cathode sunk to the soil's surface, but the floating cathode floated on the water's surface.

The SSMFC was operated in an incubator for 10 days at a temperature of 30 - 33°C. The soil was maintained saturated with a 1 to 1.5 cm water layer above the soil. During the investigations, tap water was provided regularly to stabilize the setup. The electrode stability with mature biofilm was investigated by reusing the same SSMFC without replacing the electrodes but changing the soil. OCV and discharge voltage over a 1 kΩ resistance were tested regularly. The power density was measured multiple times during the experiment at 24, 96, 168, and 216 hours, using external resistances in the 10 - 0.2 kΩ range. The power density was then calculated using Ohm's law with the surface area of the anodes of 18 cm^2.

4.A3.3 Results and discussion

4.A3.3.1 MCNTP characteristics

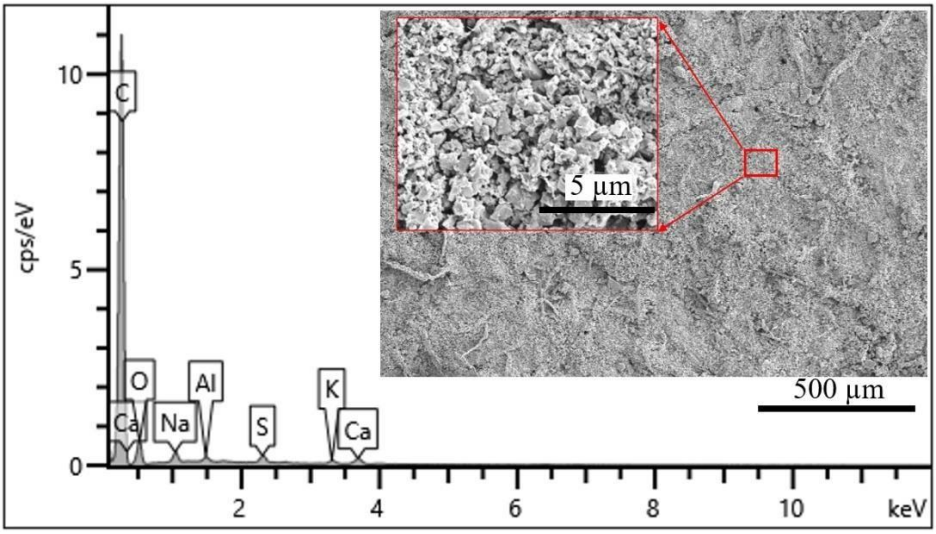

Figure 4.13. EDS spectrum and SEM image (the inset) of the MCNTP

The thickness of the manufactured MCNTP was 0.2 mm, the density was 23 mg/cm^2, and the sheet resistance was 2.2 Ω/sq. (Ohm per square unit). The MCNTP demonstrated three times lower sheet resistance than the electrodes fabricated of ACF as described in [140], but conductivity was three times greater.

Figure 4.13 shows the elemental composition and morphology of the MCNTP surface. The EDS spectrum shows diffraction peaks corresponding to carbon (C) (76.5 wt%), oxygen (O) (19.7 wt%), sodium (Na) (1.4 wt%), aluminum (Al) (0.4 wt%), sulfur (S) (0.7 wt%), potassium (K) (0.5

wt %), and calcium (Ca) (0.8 wt%). As shown in the SEM image, the MCNTP was formed by the combination of cellulose fibers, carbon fibers, MWCNT, and AC powders. The mixture of carbon fibers and MWCNT in the MCNTP enabled high conductivity. As a result, a microporous surface could be observed at higher magnification, as shown in the inset. The binder in the MWCNT paint helped to support this microporous structure. The microporous anode surface facilitates biofilm formation, leading to a higher direct electron transfer rate and power density [149].

4.A3.3.2 Soil parameters

Table 4.1 shows the soil parameters, with the TC being 85,200 mg/kg. Compared to the TC of 795 soil samples obtained from agricultural areas in Japan published by Araki [150], the TC of the soil utilized in this study falls into the high TC soil group. This result may be due to the soil collected from drainage near rice paddies, where there might be a significant accumulation of organic matter over time. High TC implies rich organic conditions and diversified bacterial species.

4.A3.3.3 The effectiveness of the floating cathode

Figure 4.14 shows that the floating cathode performed better than the non-floating cathode. This experiment demonstrates the importance of air in the performance of the air cathode. The non-floating cathode sinks to the topsoil water, preventing the reduction process from oxygen and resulting in insignificant electrical generation. On the other hand, the floating cathode demonstrated a more stable performance.

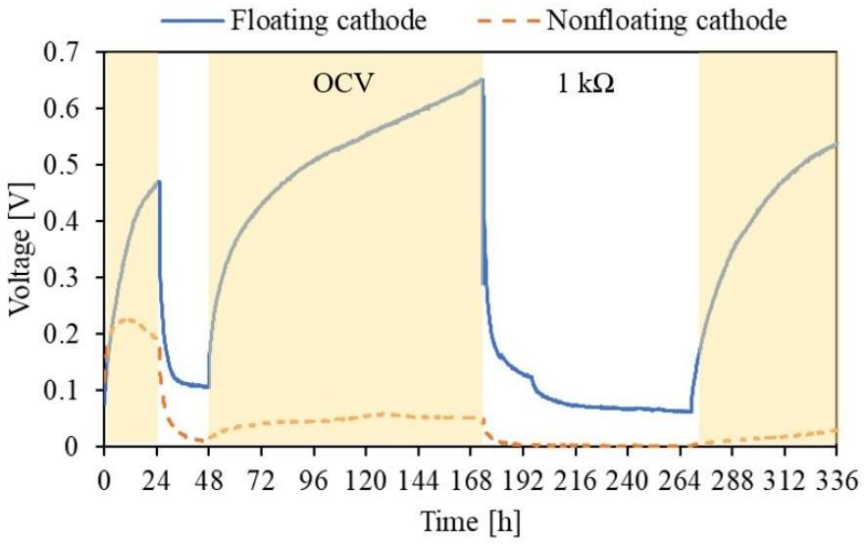

Figure 4.14. SSMFC output voltage with floating and non-floating cathodes (the highlighted and non-highlighted intervals indicate OCV and output power recorded over 1 kΩ, respectively)

In this experiment, the cathode potentials were not measured separately using a reference electrode. However, the anodes of the two SMFCs were set up in identical conditions. Thus, it is logical to expect that the anode potentials of the two SMFCs were the same. As a result, the output voltage (sum of the anode and cathode potentials) difference between the two SMFCs came from the cathode potential difference. This is consistent with the experimental results shown in figure 4.14.

4.A3.3.4 SSMFC performance

Figure 4.15 presents the output voltage curves of SSMFCs operating in three cycles (R0, R1, and R2). The outputs of the three cycles behaved similarly. The OCV steadily grew, whereas the output voltage via the 1 kΩ declined rapidly at first (for some hours) and then decreased more slowly over time. Because no organic matter was supplied throughout the testing period, the SSMFC functioning tends to decline after it reaches its maximum level.

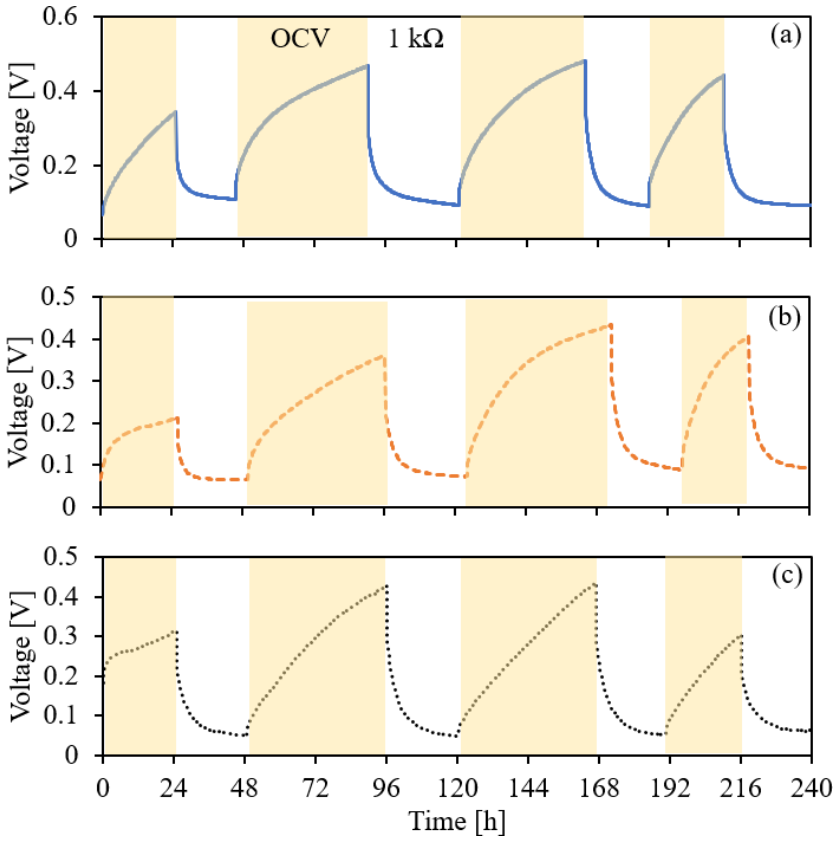

Figure 4.15. The OCV (highlighted period) and 1 kΩ discharging voltage (unhighlighted period) generated by the SSMFCs during three cycles (a) R0, (b) R1, and (c) R2

The deterioration of the SSMFC might take a long time (weeks or months), depending on the amount of organic matter in the soil. As a result, determining the SSMFC's end of life would require considerable time. Therefore, in the current investigation, the experimental period for each cycle was set at 10 consecutive days.

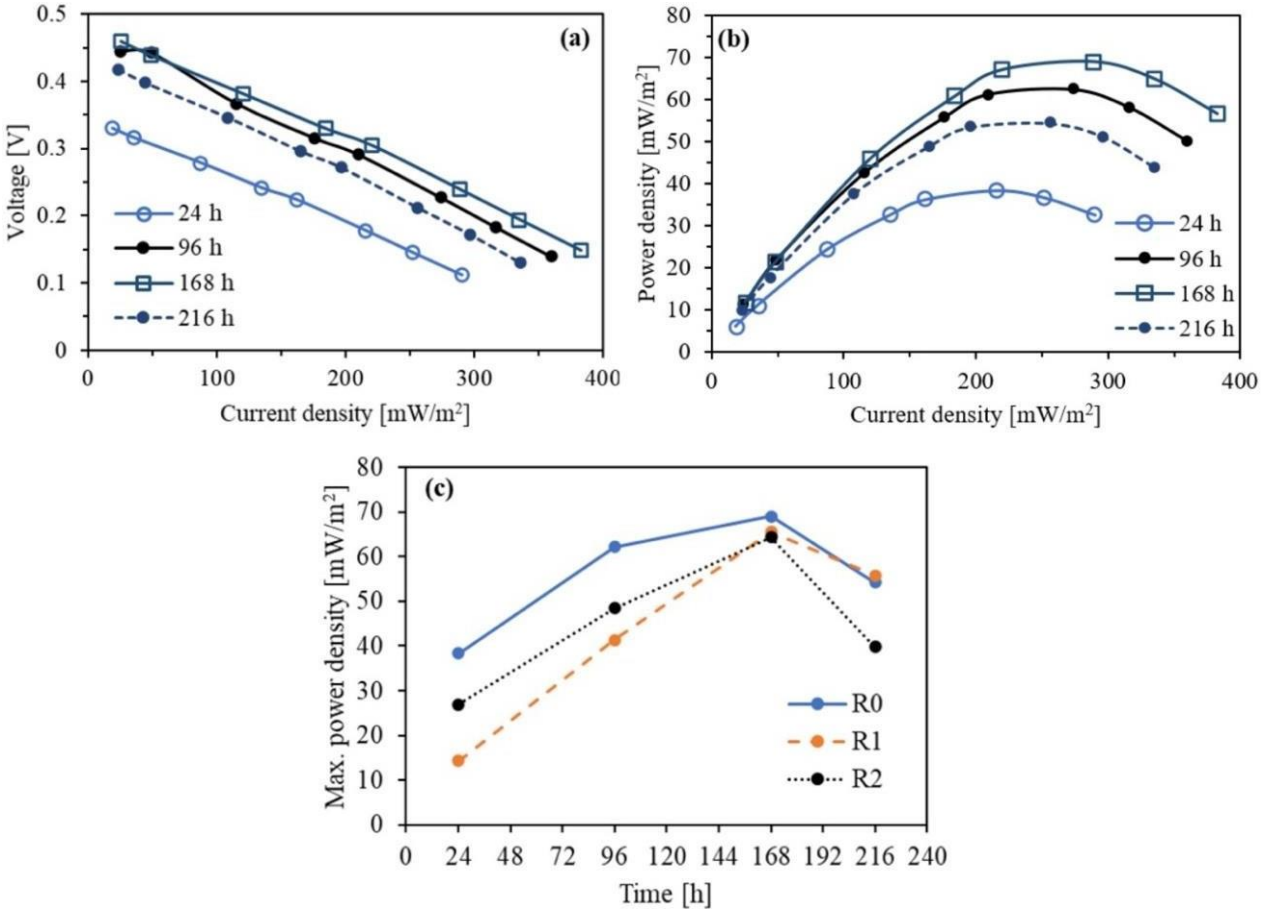

Figure 4.16. SSMFCs performance characteristics: polarization curves (a), R0 power densities measured at 24, 96, 168, and 216 h (b), MPD over time of SSMFCs over 3 cycles (c)

The power density was tested at several points throughout the experiment. The resulting polarization and power density curves of cycle R0 are shown in figure 4.16 (a) and (b), respectively. The power density continuously increased until it reached 168 h. This result agreed well with the result presented in figure 4.15. MPDs at various times throughout three cycles of the experiment are shown in figure 4.16 (c). All three cycles had the same tendency with the MPF rising gradually in the first 7 days, reaching their maximum values on the seventh day, and then declining. This result demonstrates that the SSMFC had a fast-starting phase, with the MPD of cycle R0 of 38

mW/m^2 at 24 h and the highest performance with the MPD peak of 69 mW/m^2. Even though R1 and R2 output voltage decreased significantly in the first 96 hours, their MPD were 65 mW/m^2 and 64 mW/m^2, respectively, nearly the same as cycle R0. This research proved the MCNTP electrodes' stability with the developed biofilm.

Figure 4.17 also shows the picture images of the electrodes after the experiment. A light brown layer was visible on the cathode's backside, which may result in decreased air exposure and proton exchange rate, reducing R1 and R2 performance. This layer might be caused by mineral ion accumulation in the soil, such as calcium and iron.

Regarding comparisons, the SSMFC presented in this work produced greater MPD than certain regular SMFCs employing other types of carbon-based electrodes. For instance, SMFC made of carbon felt anode and the cathode made of platinized carbon paper obtained MPD of 16.4 - 28.6 mW/m^2 [84]. On the other hand, the MPD of the SMFC made of graphite rod anode and ACF cathode was 17.3 mW/m^2 [146]. Furthermore, compared with other types of MFCs, such as microalgae microbial fuel cell (MMFC) [151] with MPD of 18 mW/m^2, floating MFC (FMFC) [152] with MPD of 48 mW/m^2, the SSMFC proposed in this study generated a comparatively high MPD.

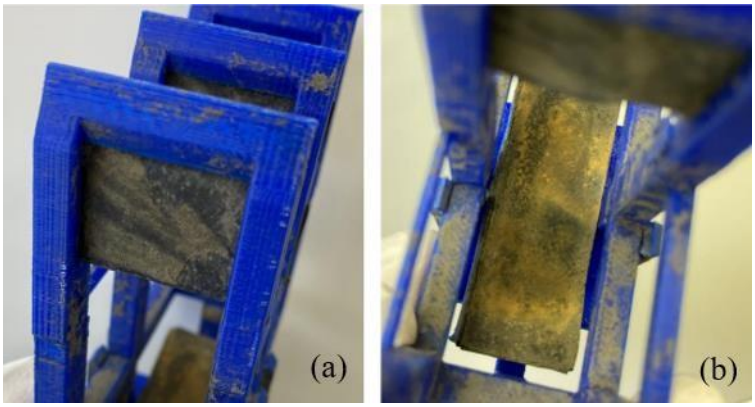

Figure 4.17. Anode (a) and cathode (b) of the SSMFC after the experiment

The biofilm on the anode was observed using a scanning electron microscope, as shown in figure 4.18 (a). The clusters of bacterial cells were visible on the surface, even though a considerable number of cells may have been washed away during the preparation process for the SEM analysis. The anode surface's porous 3D structure contributed to the growth of biofilms.

Additionally, the SEM examination of the cathode's surface revealed a trace of bacterial biofilm, as shown in figure 4.18 (b). Generally, the biofilm on the air cathode reduced the cathodic

potential, which resulted in decreased MFC performance [153]. This problem was unavoidable since biofilm might adhere to any solid surface and contacted with water over time.

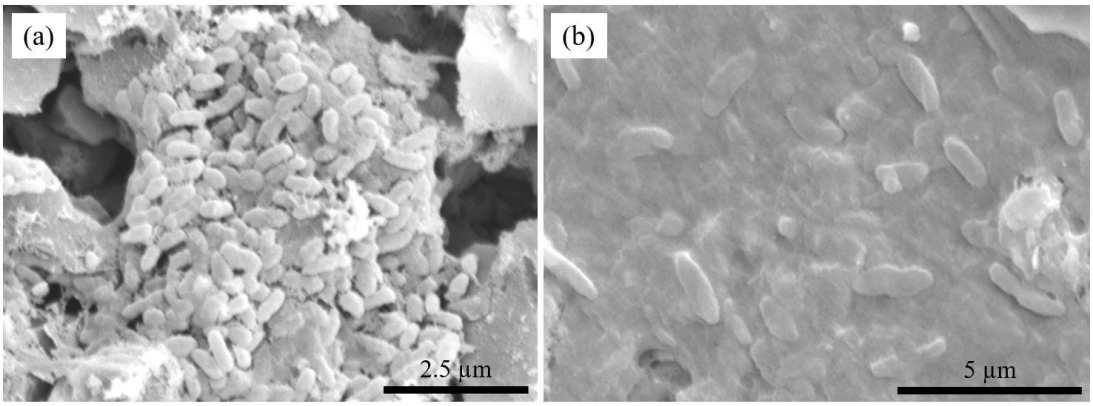

Figure 4.18. SEM images of the (a) anode's surface and (b) cathode's surface after experiment

4.A3.3.5 Practical application demonstration

Even if the suggested SSMFC could sustain the MPD of 60 mW/m^2 equivalent to 6 µW/cm^2 was used for calculation ease, the SSMFC with an anodic surface area of 18 cm^2 could produce at least a steady 108 µW output (= 6 ×18). As a result, the SSMFC is suitable for practical applications requiring a microwatt power supply.

A single SSMFC was used to power a clock through an energy control and storage circuit to demonstrate its capabilities. The clock needs a stable power source of at least 20 µW to work properly. The investigation was set up in figure 4.19. The clock was connected to an SSMFC 24 hours after the operation and was fully functioning by the produced bioelectricity from the SSMFC.

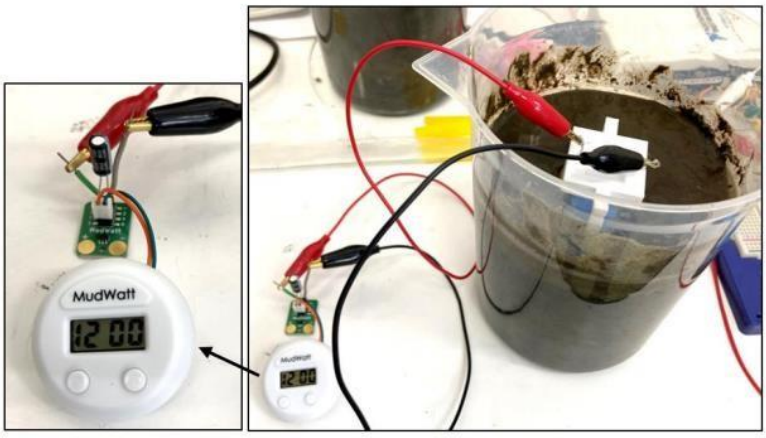

Figure 4.19. SSMFC is used to power a micropower clock

4.A3.4 Conclusions

A porous and highly conductive paper-like MCNTP was created utilizing a simple procedure that employed MWCNT paint as an efficient binder for bounding AC powder, CF, and cellulose fibers, which was used to make electrodes for the SSMFC. Additionally, the SSMFC was developed and manufactured to be portable and simple to install and operate by simply inserting it into wet soil and powered by soil microbes and nutrients. Three cycles were used to examine the effectiveness of the MCNTP electrodes in the SSMFC, with the MPD achieved in the 60 - 70 mW/m^2 range. This finding suggests that the MCNTP electrodes have a relatively high performance.

PART B. BIOSENSING APPLICATION

A portable SMFC for sensing soil water content

4.B.1 Background

Soil moisture content is a soil characteristic that influences plant development, especially in agriculture, as a nutrient itself or regulates soil temperature. Sufficient soil water levels in different growth stages are essential for high crop yields. As a result, water deficiency or redundancy in the soil causes water stress on plants, leading to plant harm and production decline. Accurate soil moisture prediction and estimation enable effective controlling irrigation, nutrients, pesticide, and other soil input that affects soil and crop plants [154].

Sensing soil moisture has been researched and practiced for decades, but it is still challenging and somewhat limited because of time-consuming, hard equip-installation and short durability. SMFC is a new approach as a sustainable technology that can be used for sensing. However, the practical applications of conventional SMFCs are also limited because of their complicated installation, which is unsuitable for actual field setup and operation.

Furthermore, for most SMFCs and SMFC-based sensors, the durability and stability of SMFCs are affected by the biofilm growth on the anode and electrode degradation during the operation time [155]. Usually, typically tested soils are in a water-saturated condition, such as sediment or muddy soil, since water reduces the internal resistance and creates favorable conditions for ionic mobility between electrodes of the system [156]. However, the water-saturated condition is rare in the actual field, making it difficult for practical application. Therefore, few studies use SMFCs in non-water-saturated soil, especially pursuing portable purposes and simultaneously sensing soil water.

Therefore, to overcome these challenges, this study proposed an easy, convenient portable plugged-type SMFC based on low-cost materials for sensing SMC without using external microorganisms for acclimation and forming biofilm to reduce the preparation time. In this SMFC design, low-lost carbon-based materials were chosen to fabricate the cathode and anode electrodes. A dry cellulose sponge soaked in LB media was attached to the anode electrode. As the cathode electrode, a particular combination was utilized, which was placed within an enclosed chamber, and a PEM was used to separate it from the anode. The cathode electrode was not exposed to the

soil, but the anode was meant to make direct contact with the soil. This design allows the SMFCs to be easily plugged into the soil.

The major goal of the proposed SMFC was to explore how the power output of the SMFC is affected by changes in soil moisture and rely on that to examine the ability of SMFC as a soil moisture sensor, which will profit agricultural management. The relationship between electricity production and soil moisture was investigated simultaneously. The tested soil was regular agricultural soil with different moisture levels from very low to saturated conditions at four levels of moisture 40%, 60%, 80%, and 100% soil water holding capacity (SWHC), corresponding to 24%, 36%, 48%, and 60% soil moisture content. The experiment was run for 25 days. The SMFC was most sensitive to humidity within 60-80% SWHC. Below 60% SWHC, the soil moisture was insufficient to support the SMFC's function. The SEM test also examined the biofilms of different SWHC-level anode surfaces. The results imply that the proposed SMFC can be potentially used as a soil moisture sensor in the range of 60 - 80% SWHC, which is normally good for the growth of a significant number of plants. Besides, the recycling possibility of the proposed SMFC design was also tested. This SMFC is expected to be used as a low-cost and convenient sensor that can facilitate practical SMFC applications in the future.

4.B.2 Materials and methods

4.B.2.1 Materials

ACP, CF, MWCNT, AC powder, Nafion solution (10% Nafion), Nafion PEM, and stainless mesh were used to make electrodes. Tripton, yeast extract, and Sodium hydroxide (NaOH) were used to make LB medium.

4.B.2.2 Soil collection and properties of soil samples

The soil used in this research was collected at an agricultural field in Kusatsu city, Shiga province, Japan. This field has a history of planting varieties of vegetables, such as peas, lettuce, cabbage, and tomato, with an average root depth of 30 - 40 cm. The soil was collected from the top 40 cm of the ground and let dry for a week, then sieved with 2 mm holes to remove any larger particles and mixed the remaining soil to ensure it was evenly distributed.

After this period, measurement of the physicochemical properties of the soil sample was carried out with results as follows: pH 7.3, EC: 0.33 dS/m, total carbon TC: 33,420 mg/kg, original

soil moisture content (SMC) 23%, and SWHC was 60%. Soil pH, EC, and TC measurement was mentioned in table 4.1. Such soil sample was stored in a box with a lid at room temperature

4.B.2.3 SMFC design

The proposed portable SMFC was constructed as shown in figure 4.20. The SMFC has dimensions of 10 cm height × 4 cm width × 2 cm depth with three parts, including a cathode chamber, anode chamber, and bottom case.

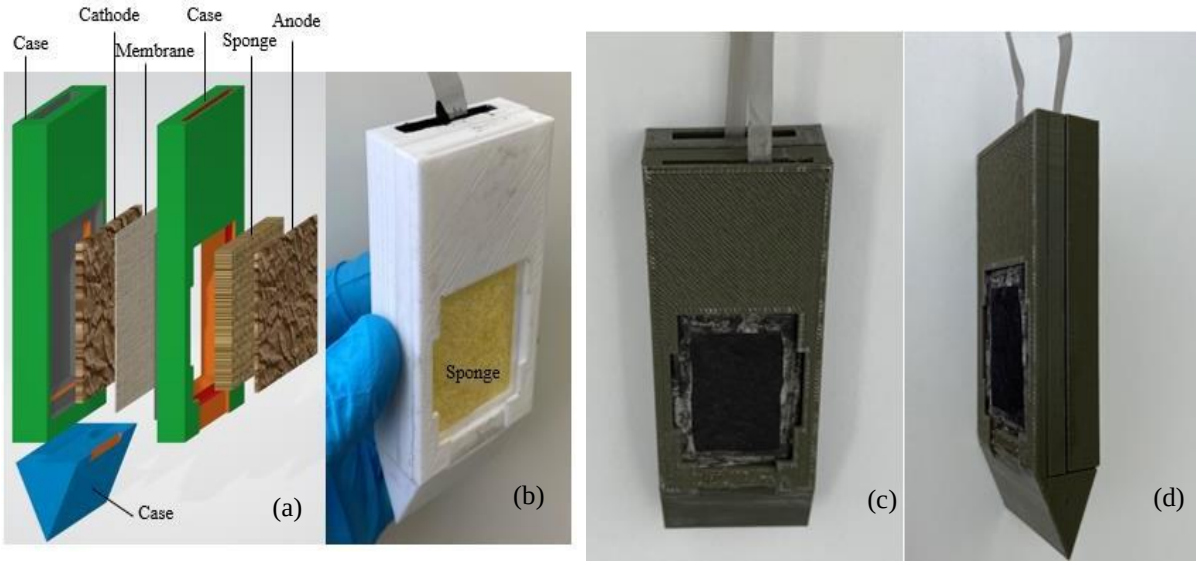

Figure 4.20. Schematic structure diagram of proposed SMFC case: (a) photo images SMFC architecture and (b, c, d) fully work out SMFC using 3D printer

A liquid combination of 9 mL MWCNT, 3 mL Nafion solution, 0.5 g CF, and 0.5 g AC powder was mixed to fabricate the cathode electrode. This mixture was stirred for 24 hours at a speed of 700 rpm, then placed in the cathode chamber, which consisted of a case and membrane. The Nafion proton exchange membrane was used to separate the two chambers and was attached to the cathode side. The cathode electrode was then left to dry at 40^{o}C for 72 hours. Two layers of activated carbon sheet of 4 cm length × 3 cm width × 0.2 mm thickness were used as the anode electrode connected with stainless mesh covered around to collect current. In addition, one cellulose sponge with dimensions of 3.8 cm × 2.8 cm × 0.7 cm was soaked in LB medium and dried at 40^{o}C for 48 hours before being attached to the anode chamber and anode electrode. The LB medium was prepared and adjusted using NaOH.

The cathode chamber and anode chamber were joined together and attached to the bottom case to create a functional SMFC contact with the soil more effectively. Figure 4.20 (b), (c), and (d) show the photo images of the fabricated SMFC.

Four plastic bottles with a capacity of 800 mL were filled with 600 g of treated soil and four levels of moisture 40%, 60%, 80%, and 100% SWHC, respectively. Then four SMFCs were plugged respectively into those four bottles.

4.B.3 Experimental design, analysis, and calculation

4.B.3.1 Experimental design

As mentioned above, the original soil moisture content (soil humidity) of treated samples was 23% and SWHC was 60%. In this research, to investigate how soil moisture affects the performance of an SMFC and its ability to sense water content, experiments with different levels of soil moisture were established. SWHC was used to determine the four moisture levels, as SWHC represents the maximum amount of water that the soil can hold. The observed soil moistures were in order of 40%, 60%, 80%, and 100% SWHC, corresponding to 24%, 36%, 48%, and 60% water in the soil.

Figure 4.21. Experimental setup photos of the SMFCs in four soil moisture contents

Soil moisture is a key parameter in measuring soil health, and plants need different water amounts to grow depending on plant type and soil type. However, the water content level present in the soil that most plants need is between 30% and 60%. Therefore, the experiment was designed

with the range of SMC from 24% to 60% (saturated condition). The SMFCs were plugged into the soil, as shown in figure 4.21.

Even though most of the electrochemical active microorganism strains were isolated from wastewater or sediments, there are studies reported about the presence of exoelectrogenic bacteria in soil that can activate SMFC. Muddy soil is suitable for activating SMFC without adding external bacteria. Therefore, with the goal of convenience and shortening preparation time by taking advantage of available microorganisms, no need for added microorganisms before the experiment for biofilm formation on the anode.

In this research, the trials were carried out in an incubator at a temperature of 30 ± 2°C for a period of 25 days. Each SMFC was connected with a 10 kΩ load for discharging. Discharge voltage and soil moisture content changing during the experiment were monitored over time.

Due to the natural evaporation process, the water content in the soil decreases over time. Therefore, the evaporation amount was determined by a scale frequently, and the lost moisture amount was compensated by adding water. For the first 48 h, to improve the electrical generation of the SMFCs, water was compensated for every 24 h. After that, to assess the relationship between power production and SMC and the stability of the SMFCs, we set different water compensation time schedules at 93 h, 118 h, 165 h, 194 h, 239 h, 285 h, 312 h, 333 h, 356 h, 383 h, 449 h, 499 h, 547 h, and 580 h.

After 25 days of the experiment, all SMFCs were taken off the soil. After two days, the SMFCs were plugged again into a new set of soil with the same conditions as above for seven days to examine the recycling ability of the SMFC design. Finally, the SEM test was performed to investigate the biofilm on the surface of the various anode electrodes.

4.B.3.2 Analysis and calculation

Soil moisture content was calculated using the following equation:

% Soil moisture content (SMC) = (m-d)/d (1)

m: moist soil weight (g)

d: dry soil weight (g)

The weight of dry soil was measured after drying the soil at 105^{o}C for 24 hours in the incubator.

The quantity of water evaporated over time was estimated using the equation below:

$Ew\ (g) = m_o - m_t$ (2)

m_o: original bottle weight (g)

m_t: bottle weight at measuring point (g)

Each original bottle contained the total weight of soil, SMFC, additional water, and the bottle itself. After the experiments, the SEM of the anodes was tested.

4.B.4 Results and discussion

4.B.4.1 Bioelectricity generation of SMFCs

In this study, along with observing the electricity generation of SMFCs, the relationship between output production and soil moisture content changing in terms of the evaporated water amount was also investigated.

As shown in figure 4.22, all four SMFCs have quick startup times, with the voltage output raised rapidly in the first 7 hours and then continued increasing and reaching their first peaks at different times due to their different level of moisture (73, 30, 15, and 105 hours for SMFC 1, SMFC 2, SMFC 3, and SMFC 4, respectively). Although no external microorganism was added, the startup time of the SMFCs is still fast. The LB medium-loaded sponge in the anode chamber may have contributed to the short startup time by providing nutrients to the microorganisms in the soil, which facilitated their growth and the biofilm formation.

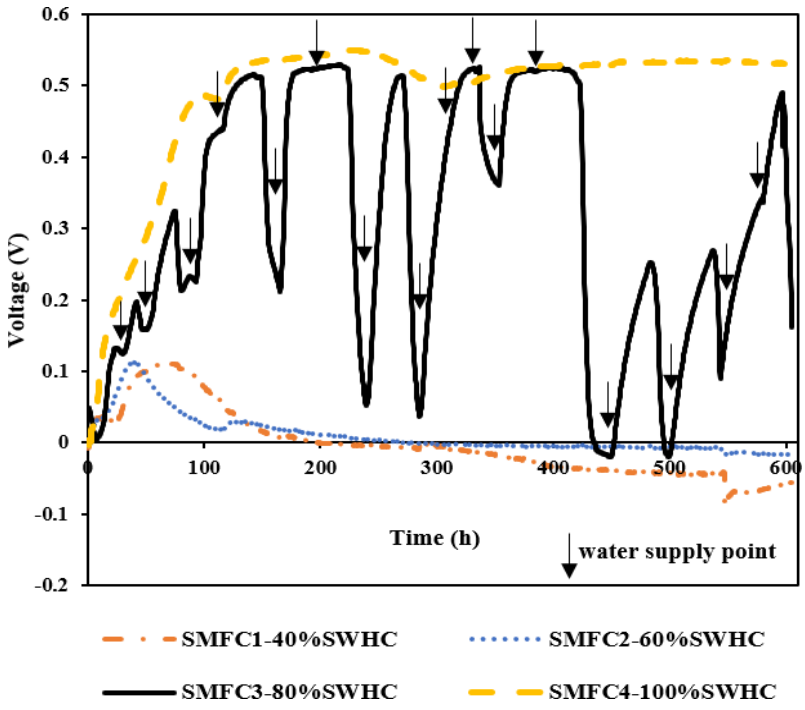

Figure 4.22. The relationship between the SMFC voltage and the fluctuation in water content varies over periodical water supply times

The water supply times were established at different time frames to examine the sensing ability of SMFCs. At first, the water was supplied after every 24 hours, then changed to a long time, about 42 hours, and repeated. Afterward, a water stroke was made for 66 hours, and finally, shorter supply times were changed to observe the recoverability of the SMFCs. Figure 4.23 shows the amount of water evaporated at various periods as scheduled, which were equivalent to voltage signals attained in figure 4.22.

SMFC 1 and SMFC 2 showed a similar tendency when after peaking at similar maximum values of 0.13 V and 0.11 V, the voltage output of SMFC 1 and SMFC 2 promptly decreased to 0 V and continued to fall to the negative side even though having water supply periodically. However, after 300 h, there was a difference between them. While SMFC 1 experienced a decline in its output over time, further into negative territory, SMFC 2 output kept the output value around 0 V until the water stroke. This result may be a cause of soil water deficit.

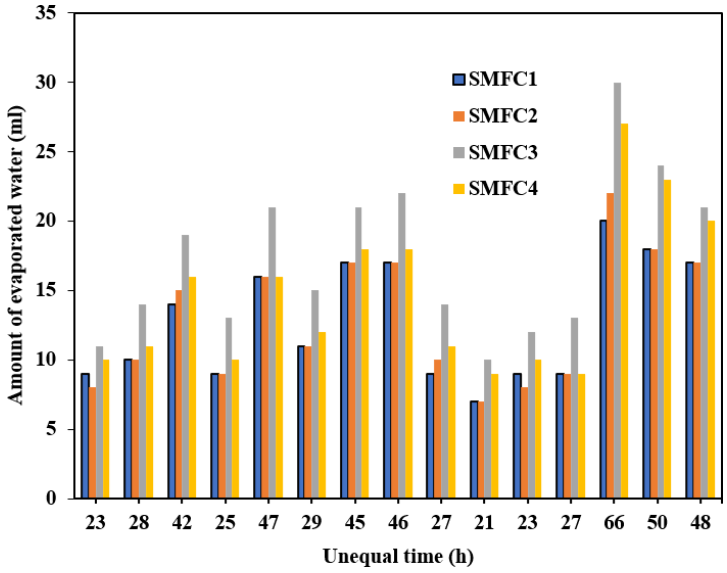

Figure 4.23. The amount of water evaporates during various supply periods of different soil moisture levels

In recent years, most research on SMFC has been conducted in water-saturated conditions or sediment because the excess water is favorable to SMFC operation and its durability. With 40% SWHC and 60% SWHC corresponding to 24% - 36% soil moisture content, this moisture level is too low to facilitate the bacteria communities in the soil and not enough to maintain the SMFC output effectively. This result indicates that at a level lower than 60% SWHC (< 36% SMC), the

SMFC does not respond to the water variation in the soil, which also means the SMFC has no sensitivity to the changes in water at this level.

Contrarily, SMFC 3 output voltage exhibited a chain of specific signals that varied through time, corresponding with changes in soil moisture. In terms of evaporated water, figure 4.24 shows the relation between the output production and changes in humidity throughout every rehydration time. When the water was supplied daily, the SMFC 3 voltage output increased gradually and reached its peak of 0.51 V after 144 h. As shown in figure 4.24, when water adding times lasted about 48 h, the moisture decreased to lower than 70% SWHC, and the output signal decreased rapidly. However, after adding water, the output could recover to the stable phase quickly. Especially when the water stroke was made by expanding the water compensating time to 66 h (around 385 - 449 h), the output signal dropped off deeper than before. Despite this sudden decline, the output could still recover to stable value, but it would take longer than before. These results indicate that this SMFC can sense water in the range of 60 - 80% SWHC.

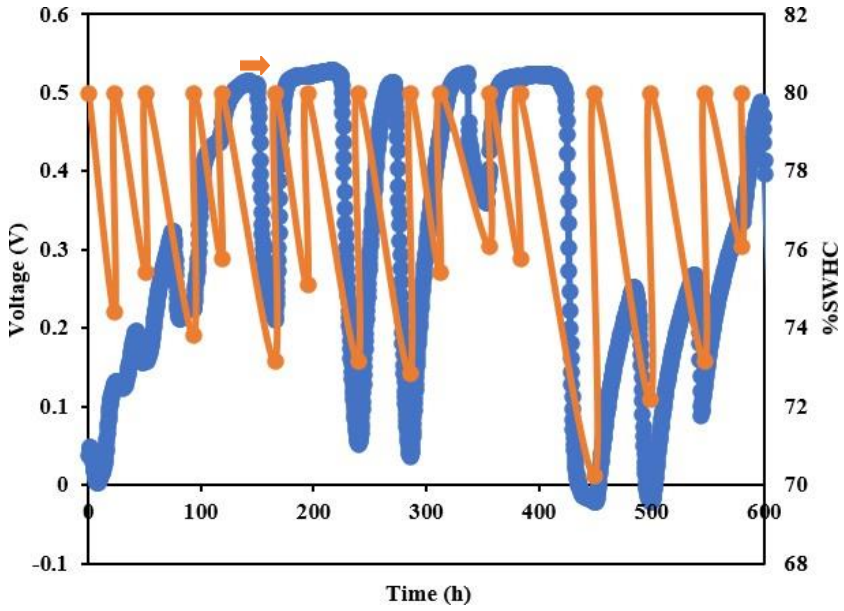

Figure 4.24. The correlation between voltage output and soil moisture changing over water supply times of SMFC 3

On the other hand, from the beginning, the SMFC 4 voltage increased through the water supply times to the maximum value of 0.53 V and then steadily maintained at this level during the whole experiment without any notable changes in voltage signal due to the difference of evaporated water. Furthermore, the output performance remained stable and did not show any significant signals

even after a stroke with a continuous not supply of water for 66 hours, where the soil moisture was still at a level higher than 80% SWHC. This outcome might be due to an abundance of soil water suitable for activating and maintaining SMFC. Consequently, the result implies that soil with high moisture at 80 - 100% SWHC (60% SMC) can enable SMFC to attain a highly stable output of more than 0.5 V, but the proposed SMFC cannot sense SMC at this high moisture.

Research on crop plants indicated that appropriate conditions for disease development included low pH and high moisture content of about 80%. In comparison, the conditions of higher pH and lower moisture content of about 40 - 60% showed significant reductions in diseases [157]. Therefore, the obtained results imply that this SMFC can be an SMC sensor in SWHC above 60%, especially 60 - 80% SWHC corresponding to 36 - 48% SMC in the tested agricultural soil, in which moisture is the favor for the development of plants and restrict the growth of diseases. Therefore, even though SMFC cannot sense moisture at a low level, this result suggests that the system has the potential to be improved and could be applied in the actual field.

4.B.4.2 SEM images of the anodes from different moisture

In this experiment, taking advantage of the existing microorganism community in the soil, there was no inoculation process before activating the SMFC. The SEM test provided information about the biofilm's morphology on the anodes' surfaces, as shown in figure 4.25.

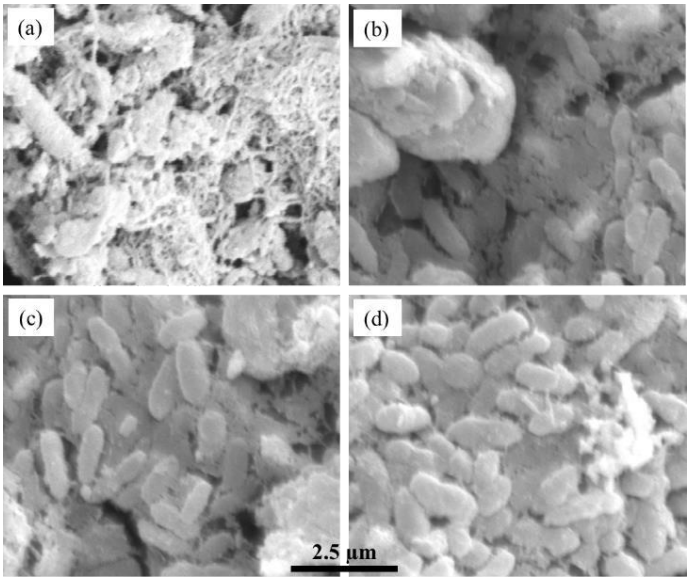

Figure 4.25. The anode images from the SEM test of SMFC 1 (a), SMFC 2 (b), SMFC 3 (c), and SMFC 4 (d) after experiment

The anodic electrodes had a porous surface with activated carbon particles bonded together on carbon felts. SMFC 1 and SMFC 2 anodes with low soil moisture still had the presence of rod shapes bacteria cells biofilms but in very low densities (figure 4.25 (a) and (b)). On the other hand, SMFC 3 and SMFC 4 anodes, which were in higher moisture conditions, showed thicker biofilm (figure 4.25 (c) and (d)), which means the proliferation of soil microorganisms in high moisture levels is better. The densest observed biofilm belonged to SMFC4 with 100% SWHC, while the opposite result belonged to SMFC1 with only 40% SWHC. At the same time, the biofilm growth affected the power production of SMFC 3 and SMFC 4 were also much higher than SMFC 1 and SMFC 2. This result again confirms water's importance in the operation of the SMFC system.

4.B.4.3 Recycling ability test

In order to examine the recycling possibility of the proposed SMFC design, after 25 days of the main experiment, all SMFCs were taken off from the soil for two days and then plugged in a new set of soil with the same conditions as before for seven days. The result of this experiment is shown in figure 4.26.

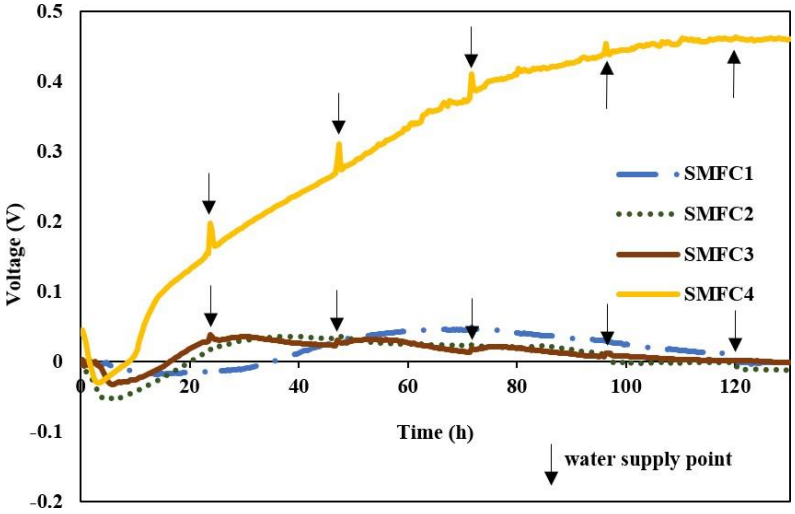

Figure 4.26. The voltage output over time with five water supply times of recycled SMFCs

From the beginning, the output voltage of all SMFCs dropped as usual and began to increase after about 5 h; only SMFC 1 needed 35 h to reach a positive voltage output due to the low moisture content in the soil. However, after 40 hours, the output voltage of SMFC 1, SMFC 2, and SMFC 3 showed the same level, gradually decreased through time, and did not show unique signals during five times of water supplied (24, 48, 72, 96, and 120 hours). The result may be caused by the

degradation of cathode electrodes during the previous experiment and the death of biofilm on the anode electrodes. After being pulled out and dried for two days before the recycling experiment, the biofilm on the anodes was injured, and when plugged into the soil again, the anode electrodes were damaged further. Along with the lack of moisture in the soil samples, the biofilms had difficulties recovering.

For SMFC 3, which showed clear signals in the previous experiment, the output voltage also gained small peaks when having a water supply but was not clear due to the need for more water from the SMFC. 80% SWHC was insufficient to recover all the damage to electrodes and bacteria.

With 100% SWHC, SMFC 4 output voltage increased rapidly after the first 5 h. When having water supply in the recovery stage, the output voltage showed precise peaks through 5 adding times. Even though having 100% SWHC, the soil water still evaporates daily. The damages from the anode electrode and biofilm gradually recovered when supplied water, which gave SMFC 4 a longer time to increase the output and depicted more precise signals than in the first experiment. Through time, when the recovery was gradually complete, the magnitude of signals also decreased until stable (after 100 h). At the stable phase, no more special signal or peak appeared the same as in the first experiment because of the excess water in the soil.

In short, even though this SMFC design can be used as an SMC sensor, it could not be recycled for the reasons of cathode electrode degradation and anode electrode injuries that need to be resolved in the future.

4.B.5 Conclusions

This study proposed and fabricated a portable soil moisture content sensor using SMFC-based technology. This portable SMFC was designed using low-cost materials and is easy to set up by plugging in the natural agricultural soil. With the compact design, this one-time sensor began generating electricity after one hour and gradually increased until it reached its maximum output after 15 - 105 hours, depending on the difference in soil moisture. The findings showed that when the soil moisture was low (below 60% SWHC), the SMFC did not effectively detect changes in the soil's water content. The proposed SMFC could produce a maximum voltage of 0.53 V and steady along with the soil moisture higher than 80% SWHC to saturated. The SMFC can detect SMC within a range of 60 - 80% SWHC, which corresponds to 36 - 48% SMC, which is favorable moisture for growing various plants. The proposed SMFC has the advantages of being compact

and portable and sensing the appropriate soil moisture levels for various crops, making it a potential tool for quickly and inexpensively managing irrigation in agriculture.

CHAPTER 5 - CONCLUSIONS AND FUTURE RECOMMENDATIONS

5.1 Summary and conclusions

In this thesis, I worked on MFC developing technologies for electricity generation and sensing applications. For this purpose, a hybrid MFC and four SMFCs were designed to enhance the current output and biosensor application.

First, I have combined MFC and EFC technology with baker yeast to enhance the performance of the hybrid MFC system. This issue focuses on both theoretical and experimental purposes. During the metabolic process of Baker yeast, ethanol was oxidized to acetic acid by commercial alcohol and aldehyde dehydrogenase enzymes (ADHE). As a result, these processes enhanced the power production of the proposed MFC. The 3D-activated carbon paper anode was good material for BKY biofilm formation and growth. Using this biofilm anode together with ADHE increases the maximum power density of MFC by approximately 19% compared to using the biofilm anode alone. Anaerobic culture condition is more suitable for MFC operation than aerobic condition with 22% higher maximum power density. Supplying ADHE shows a positive impact on BKY-powered MFC performance.

Second, I conducted a series of experiments on different configurations of SMFC to be compact, easy to use, and cost-effective. These SMFCs were used to collect power generation and biosensing targets. My experiments did not use external microorganisms for the operation, which is often required in other research.

For the purpose of enhancing electricity generation, there are three different SMFCs have been proposed.

The first portable plugged-type double-chambered SMFC was made using inexpensive materials and an LB dipped-anode in order to help form a biofilm by inserting it into wet soil. After one hour, the SMFC began generating electricity, with a maximum power density of 5–7 mW/m^2. This design is the base for my later designs.

The second compact, membrane-less SMFC was used to investigate the electricity generation ability from household rice washing wastewater (RWW) in different soil types. When SMFC working in muddy soil with much higher TC and EC than sandy soil, SMFC with RWW generated

a maximum power density of 485.2 mW/m^2, which was 2.4 times greater than the SMFC that was only supplied with water (202.9 mW/m^2). When placed in sandy soil, the SMFC with RWW generated a maximum power density of 112 mW/m^2, while the control SMFC generated almost no power. This suggests that RWW is a good source of carbon for the SMFC and that the SMFC is effective at generating bioelectricity from RWW in different types of soil.

The third portable membrane-less SMFC used to harvest electricity is made of modified electrodes using multiwalled carbon nanotube paper (MCNTP). The high conductivity, flexible, and durable MCNTP was fabricated by bounding AC powder, CF, and cellulose fibers with MCNT paint. The SMFC was designed with three anodes in series, and a floating air cathode could be activated by poking it into wet soil. The floating cathode outperformed a non-floating cathode in tests. When the SMFC was inserted into wet soil, it generated electricity quickly and obtained the maximum power density of 60 - 70 mW/m^2 over three separate cycles. The SMFC was also used to power a clock, showcasing its practical use, and the clock was fully functioning.

Biosensing application of SMFC

A new design of low-cost SMFC was used as a biosensor for sensing soil moisture content. This SMFC contains a dry cellulose sponge dip-loaded LB medium anode electrode and a specific cathode mixture. SMFC sensing ability was investigated at four levels of moisture 40, 60, 80, and 100% SWHC for 25 days. In the range of 60 - 80% SWHC, the sensitivity of SMFC was the highest, whereas humidity less than 60% is not suitable for activating and sustaining SMFC.

With the SWHC of 60 - 80% corresponding to 24 - 36% soil moisture which is good moisture for lots of plants, the proposed SMFC can be potentially used as a sensor for agriculture applications.

5.2 Future research recommendations

Some challenges in this thesis need to be solved and ameliorated.

To achieve better performance, scaling up is one of the most critical ways that need to be solved. In addition, the source of RWW should also be considered in terms of uniformity in quality within large quantities.

Besides, because the necessary equipment was unavailable, the soil bacterial communities could not be characterized. Therefore, future research needs to complete this job for information and a holistic view of the microbial communities in the utilized soil. This can determine on a deeper level how microorganisms affect SMFC for further research.

Nevertheless, enhancing the sensitivity of SMFC by lowering the lower limit with better signals is another crucial mission for future research. Other applications of SMFC in biosensing, such as investigating the sensibility of SMFC on other factors such as microbial activities in the soil, soil nutrients…

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