國立臺灣大學工學院環境工程學研究所

博士論文

Graduate Institute of Environmental Engineering
College of Engineering
National Taiwan University

Doctoral Dissertation

農業濕地植物微生物燃料電池的產電研究

Electricity Generation from Agronomic Wetland Plant Microbial Fuel Cells

東納太

Natagarn Tongphanpharn

指導教授：于昌平

Advisor: Chang-Ping Yu, Ph.D.

中華民國111年8月

August, 2022

doi:10.6342/NTU202203744

國立臺灣大學碩(博)士學位論文
口試委員會審定書

Certificate of Thesis/Dissertation Approval from the Oral Defense Committee
National Taiwan University

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Agronomic Wetland Plant Microbial Fuel Cells

本論文係東納太君(學號 D04541015) 在國立臺灣大學環境工程學研究所完成之博士學位論文，於民國 111 年 08 月 04 日承下列考試委員審查通過及口試及格，特此證明

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doi:10.6342/NTU202203744

Acknowledgement

The success of this dissertation would not have been possible without many individuals' enthusiastic support and full cooperation. I would like to take this opportunity to express my appreciation to the following people for their contributions.

I would like to express my sincere gratitude and my deepest appreciation to my advisor, Professor Chang-Ping Yu, for his time, guidance, supervision, and encouragement through my study in Taiwan.

I am really grateful to the committee members, Professor Hwong-Wen Ma, who advised me to study Ph.D. at the Graduate Institute of Environmental Engineering, NTU, Professor Hsin-Hsin Tung, Professor Pei-Te Chiueh, and Professor Chia-Hung Hou for their valuable advice and constructive comments, which improved the quality of the dissertation. I also especially appreciate Professor Chi-Te Liu, Professor Yo-Jin Shiau, Professor Shiue-Lin Li, and Professor Chung-Yu Guan for their valuable suggestions, supervision, and comments for my final dissertation defense.

My special thanks are contributed to Yi-Ching Cheng and Chao-Chin Chang for their help, encouragement, and wonderful friendship in Taiwan. My thanks are also given to my labmates for their discussion and help as well as the GIEE staffs. Not forgotten to thanks all friends and colleagues in Thailand for their support.

Most of all, I deeply appreciated my lovely family for their infinite love, understanding, constant encouragement, and support. I really couldn’t have achieved graduation without them. Thank you.

Natagarn Tongphanpharn

doi:10.6342/NTU202203744

摘要

植物微生物燃料電池(PMFC)是一種新型的整合了植物光合作用的微生物電化學系統，能通過植物根際的微生物發電。為了瞭解影響 PMFC 運行的關鍵因子所扮演的角色，本論文於第一部份研究深度探討了不同植物和土壤改良劑對 PMFC 性能的影響，實驗於受照明與溫度(27°C)及濕度(75%)控制的培養箱中進行為期 200 天的試驗。植物方面，在 PMFC 系統中應用了兩種耐澇農業植物，水稻(*Oryza sativa*)和茭白筍(*Zizania latifolia*)；土壤改良劑方面，選擇了由食物廢棄物製成的堆肥和由廢棄木材生物質製成的生物炭作為土壤改良劑。結果顯示，不同的 PMFC 系統在運行期間觀察到不同的發電量。其中，帶有堆肥的水稻 PMFC 表現出相對更穩定的發電量(15.57 ± 8.15 mW/m^2)和顯著更高電壓的產生，在所有 PMFC 中達到最高輸出電壓 894.39 ± 53.44 mV (34.78 mW/m^2)。此外，具有植物的 PMFC 的輸出電壓顯著高於不具植物的土壤 MFC，且水稻 PMFC 的輸出電壓顯著高於茭白筍 PMFC，這意味著不同植物根系的根際沉積可能對發電性能具有重要意義。另一方面，具有生物炭的水稻 PMFC 的電壓產生明顯低於沒有生物炭的樣本，這可能是由於廢棄木材生物質製成的生物炭的抑製作用。陽極微生物群落的分類鑑定表明，變形桿菌門是最豐富的門，而伽瑪變形桿菌門和三角變形桿菌門是微生物群落中最主要的類別。進一步分析表明，具有生物炭的水稻 PMFC 具有最明顯的陽極微生物群落結構，以 *Gallionellaceae* 為主，而不是其他 PMFC 中的 *Geobacteraceae*。*Geobacter* 是所有樣品中微生物種群的主要屬，並且在帶有堆肥的水稻 PMFC 中顯示出最高的相對豐度，這表示該菌屬是 PMFC 系統中參與發電的主要貢獻者。本研究結果顯示，PMFC 系統的功率輸出會受到不同農業植物和由廢棄生物質製成的土壤改良劑的影響，建議未

doi:10.6342/NTU202203744

來進一步了解陽極微生物群落、不同植物根系的根圈與電化學機制的關聯以邁向進一步的放大應用。

此外，提高農作物或濕地 PMFC 的發電量仍然受到關注。為了填補稻田等農業領域產電研究的空白，本論文於第二部份研究中，專注於提高稻田 PMFC 性能，探討水稻植物 (*Oryza sativa*) 在 PMFC 系統中使用化學肥料和不同 PMFC 串接放大方式長達 150 天的性能表現。結果顯示添加化肥的水稻 PMFC 最高輸出電壓為 0.776 ± 0.33 V，而孔隙水的 COD 濃度為 123.2 ± 11.3 mg/L。這表明在稻田中使用的化學施肥可能會影響陽極的電活性菌的活性並增加發電量。此外，串聯 PMFC 的平均電壓最高，為 0.48 ± 0.17 V，其次是並聯水稻 PMFC，為 0.40 ± 0.15 V。總體來看，具植物的 PMFC 的電壓輸出不僅明顯高於不具植物的土壤 MFC，而且串聯也比並聯有更高的功率輸出 ($P < 0.05$)。來自系統的溫室氣體通量顯示，串聯的 PMFC 的甲烷排放通量達到最高值，為 3.82 ± 0.92 mg m^{-2} h^{-1}，而並聯的土壤 MFC-的氧化亞氮平均排放通量最高，為 106.48 ± 80.58 μg m^{-2} h^{-1}。 16S rRNA 基因高通量測序表明，變形菌門是陽極微生物組中最豐富的門。*Geobacter* 也是 PMFC 中產電相關菌群中的最豐富的菌屬。總體而言，這項研究探討了如何透過添加不同土壤肥料、串並聯方式來減少水稻 PMFC 溫室氣體的排放與產電量的增加。

關鍵字：植物微生物燃料電池、農業植物、廚餘、生物炭、生物電、溫室氣體

doi:10.6342/NTU202203744

Abstract

Plant microbial fuel cell (PMFC) is a novel bioelectrochemical system that integrates the photosynthetic reaction from the living plants to generate electricity via microorganisms at the rhizosphere of the plant roots. To elucidate factors that are critical for PMFCs operation, this study investigated the effects of different plants and soil conditioners on PMFCs performance. The experiment was done in a controlled lighting incubator at 27 °C and 75% of humidity for 200 days. Two waterlogged agricultural plants, paddy (*Oryza sativa*) and water bamboo (*Zizania latifolia*), were applied in PMFC systems; besides, the compost made from food waste and biochar made from waste wood biomass were selected as soil conditioners. Results showed that varied electricity generation during the operation was observed for different PMFC systems, but the Paddy-PMFC with compost (PC-PMFC) demonstrated relatively more stable electricity generation for 200 days (15.57±8.15 mW/m^2) and significantly higher voltage production, reaching the highest output voltage of 894.39±53.44 mV (34.78 mW/m^2) among all PMFCs. It was observed that the output voltage of PMFCs was significantly higher than soil-MFC, and the output voltage of P-PMFC was significantly higher than water bamboo-PMFC, implying rhizodeposition of different plant roots could be important for the performance of electricity production in PMFCs. However, Paddy-PMFC with biochar (PB-PMFC) demonstrated significantly lower voltage production than those without biochar, likely due to the inhibitory effect of biochar made by waste wood biomass. The taxonomic identification of the microbial community at the anode showed that Proteobacteria was the most abundant phylum, and *Gammaproteobacteria* and *Deltaproteobacteria* were the most dominant classes of the microbial communities. Further analysis showed that the PB-PMFC had the most distinct anode microbial community structure, with the predominant family of *Gallionellaceae*, instead of *Geobacteraceae* as in other PMFCs. *Geobacter* was the major genus of the microbial population in all samples and

doi:10.6342/NTU202203744

showed the highest relative abundance in PC-PMFC, suggesting that it was the main exoelectrogen involved in electricity generation in our PMFC systems.

To fulfill the gaps in bioelectrochemical systems, paddy PMFCs adding chemical fertilizer and the different paddy PMFC connections were investigated the performance through 150 days. Paddy PMFC adding chemical fertilizer (F-PMFC) showed the highest output voltage of 775.96 ± 230.21 mV while the COD concentration of pore water was 123.2 ± 11.3 mg/L. It indicated that chemical fertilization likely influenced the EAB acitivity at the anode and increased the power production. Besides, PMFC connected in series presented the highest average voltage of 474.97 ± 164.84 mV, followed by paddy PMFC-parallel at 396.89 ± 149.91 mV. By comparison, the voltage output of PMFCs was not only significantly higher than soil MFCs, but the series connection also has a higher power output than the parallel connection (P<0.05). The greenhouse gas (GHG) flux from the systems revealed that CH_4 flux of PMFC-series reached the highest values at 3.82 ± 0.92 mg m^{-2} h^{-1} while soil MFC-in parallel showed the highest average N_2O emission flux of 106.48 ± 80.58 μg m^{-2} h^{-1}. The 16S rRNA gene high throughput sequencing showed that Proteobacteria were the most abundant phyla of the anodic microbiome. *Geobacter* is also the most abundant group that is associated with the bioelectricity generation in PMFCs. Overall, this study demonstrated the enhancement of the bioelectricity production from the paddy PMFCs with various soil additions, and electricity production and GHG emission from the serial and parallel connections of PMFCs, which will be beneficial for the future scale-up application of PMFCs.

Keywords: Plant Microbial Fuel Cell, Agricultural plants, Compost, Biochar, Bioelelctricity, Greenhouse gaeses.

doi:10.6342/NTU202203744

List of Acronyms and Abbreviations

BES	Bioelectrochemical Systems
CW-MFC	Constructed wetland with Microbial fuel cell
CV	Cyclic Voltage
DET	Direct Electron Transfer
EAB	Electroactive Bacteria
EIS	Electrochemical Impedance Spectroscopy
F-PMFC	Paddy plant microbial fuel cell adding chemical fertilizer
GHG	Greenhouse gases
LMW	Low molecular compounds
MET	Mediated Electron Transfer
MFC	Microbial Fuel Cells
OCV	Open circuit voltage
ORR	Oxygen Reduction Reaction
PB-PMFC	Paddy plant microbial fuel cell with biochar
PC-PMFC	Paddy plant microbial fuel cell with compost
P-PMFC	Paddy plant microbial fuel cell
PMFC-parallel	Plant microbial fuel cell with the series connection
PMFC-series	Plant microbial fuel cell with the series connection
Soil-MFC	Soil microbial fuel cell
Soil MFC-series	Soil microbial fuel cell with the series connection
Soil MFC-parallel	Soil microbial fuel cell with the parallel connection
W-PMFC	Water bamboo plant microbial fuel cell

doi:10.6342/NTU202203744

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Chapter I Introduction

1.1 Background of the research

Nowadays, the demand for renewable energy or sustainable energy sources has been increasing. Bioenergy is considered as one of the renewable energy sources. Plant microbial fuel cell (PMFC) is a novel biotechnology which converts solar energy to electrical energy via plants and microorganisms. In the PMFC, the photosynthetic reaction from a living plant is integrated to generate the electricity via microorganisms at the rhizosphere of the plant roots (Guan et al., 2019a). During the photosynthesis process, a wide variety of organic compounds or rhizodeposition such as root exudates, secretions, lysates, and gases can be released to the rhizosphere (Gregory, 2008). The rhizodeposition from plant photosynthesis could function as the self-sustained organic compounds, and the oxidation of the rhizodeposition at the plant roots via electrochemically active bacteria plays a key role in PMFC systems to generate electricity (Timmers et al., 2013a). The electricity can be collected through electrodes with an external circuit.

Wetland plants or the waterlogged plants are suitable to use in PMFCs (Guan et al., 2019b) because the soil subsurface remains anaerobic when the soil is submerged in water, and the community of anaerobic microorganisms (comprised of sulfate-reducing bacteria, iron-reducing bacteria, fermentative bacteria, and methanogenic archaea, etc.) will be established (Chin et al., 1999). Several wetland plants have been used to generate bioelectricity in PMFCs, for instance, reed mannagrass (*Glyceria maxima*) (Strik et al., 2008), cattail (*Typha latifolia*) (Oon et al., 2015), Chinese pennisetum (*Pennisetum alopecuroides*), and common reeds (*Phragmites communis*) (Guan et al., 2019a). To date, the maximum power output achieved was 679 mW/m^2 in PMFCs with *S. anglica*, and more

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efforts are still underway by different researchers to achieve a higher power output (Santos et al., 2018). It is suggested that the high root biomass (Timmers et al., 2013b) and the plant growth medium or nutrients could improve the power output of PMFCs (Helder et al., 2013). PMFCs also show the potential to integrate with agricultural plants. The rice plant is one of the most important crops, particularly in Asian countries. Rice plants are typically cultivated in flooded land in which the soil can be under different redox zones, including oxic zone, anoxic or anaerobic bulk soil, and rhizosphere. These redox zones could cause microscale chemical gradients and a heterogeneous spatial distribution of microbial communities (Liesack et al., 2006), which could be observed also in the PMFCs (Guan et al., 2019b).

According to the concept of PMFCs, that is the power sources of bioelectricity generation which based on plant rhizodeposition, photosynthesis and waterlogged environment (Helder et al., 2010). Wetland and flooded agricultural lands such as the rice fields are the ideal for the integration of PMFCs. Due to the water plays an important role in maintaining the anoxic condition at the anode, that is the prerequiste condition for power production in PMFC systems (Chiranjeevi et al., 2013; Tapia et al., 2017). The paddy PMFC has been demonstrated (Kaku et al., 2008), but the voltage generation was relatively small and faced the limitation for the growth of the roots by the electrode materials. Moqsud et al. (2015) studied factors which influence the power output of rice paddy PMFCs with paddy field soils. They found that the highest electricity production from rice paddy PMFCs was around 700 mV when rice paddy soil was mixed with additional compost. Although PMFCs have been developed for a decade, it is still difficult to conclude the factors which are critical for PMFCs operation, since most of the study reported highly varied electricity generation during operation. The dual-chamber PMFCs and the tubular design with anode were applied

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to PMFC with crops and wetlands (Timmer et al., 2013; Nitisoravut and Remi, 2017). Furthermore, the various materials of electrodes have also been intensively researched in PMFC configurations. As a result, the gaps in the improvement of bioelectricity production from flooded agricultural lands require further investigation, such as mechanisms and processes, low and inconsistent current, smart farm land application, and greenhouse gas flux reduction.

1.2 Research objectives

This dissertation aims to investigate the critical factors of PMFCs and integrate the PMFC systems with the soil remediation technology, including the followed objectives.

(1) The first objective of this research is to systematically investigate the long-term performance of different agricultural plant, rice paddy (*Oryza sativa*) and water bamboo (*Zizania latifolia*), on the electricity generation and microbial community of PMFCs in the controlled lighting incubator.

(2) The second objective is to evaluate the effect of soil substrates on the long-term electricity generation of paddy (*Oryza sativa*) PMFC in the controlled incubator. The soil substrates in this research are compost that made from municipal waste and biochar which was converted from the wood biomass.

(3) The third objective is to evaluate the long-term electricity generation in the greenhouse using paddy (*Oryza sativa*) PMFCs with serial and parallel connections, as well as to investigate the greenhouse gas emissions from bioelectrochemical systems.

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1.3 Research framework

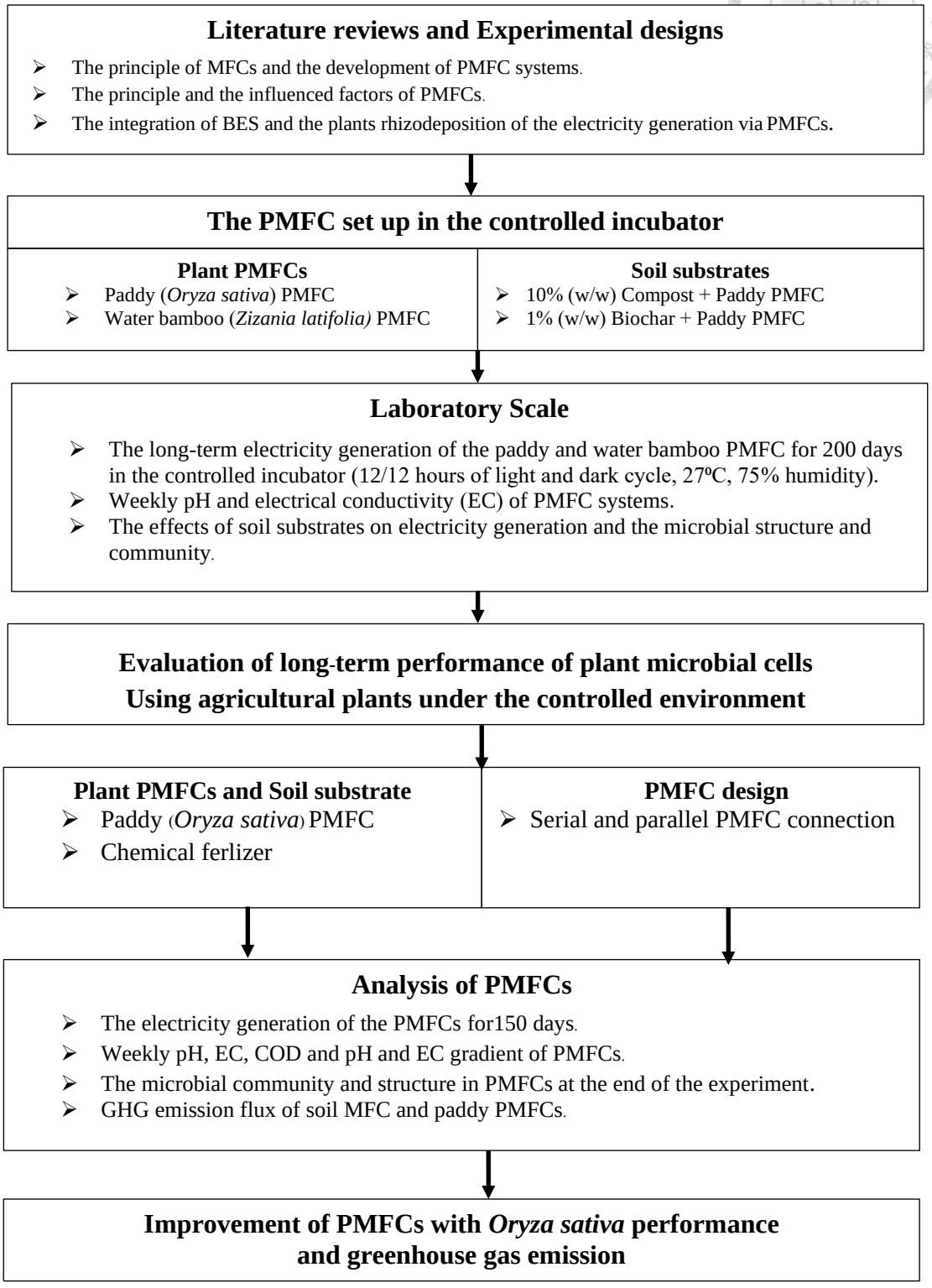

Figure 1-1 Research framework

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Chapter II Literature Review

2.1 Microbial fuel cell (MFC)

Microbial fuel cell (MFC) is an emerging biotechnology which microorganisms convert chemical energy to electrical energy. In 1910, Potter inculcated the idea of the potential of microbes in the electricity generation (Potter, 1911). Initially, it did not attract much attention. Afterwards, the advent of this technology became more interest by the research communities since it converted the waste into the energy with no environmental footprint (Cohen,1931; Davis, 1962). However, the experiments that were conducted needed the use of chemical mediators, or electron shuttles, which could carry electrons from inside the cell to exogenous electrodes. The breakthrough in MFCs occurred in 1999 when it was recognized that mediators do not need to be externally added (Kim et al. 1999).

Traditionally, two-chamber MFC was developed which consisted of anode and cathode, separated by a proton exchange membrane (PEM, Figure 2-1). Microorganisms grow and oxidize organic compounds, then produce electrons and protons through extracellular electron transfer (EET) at the anode chamber. The anode and cathode are connected by the wire containing an external resistance. Meanwhile, electrons would be transported to the cathode and protons transferred through the PEM in a cathode chamber. In principle, the membrane is permeable to protons that are produced at the anode and the proton migrate to the cathode where they can combine with electrons conducted via the wire and oxygen to finally form water. The current produced by an MFC is typically calculated in the laboratory by monitoring the voltage drop across the resistor using either a voltmeter (intermittent sampling) or a multimeter or potentiostat hooked up to a computer for essentially continuous data acquisition (Logan, 2006).

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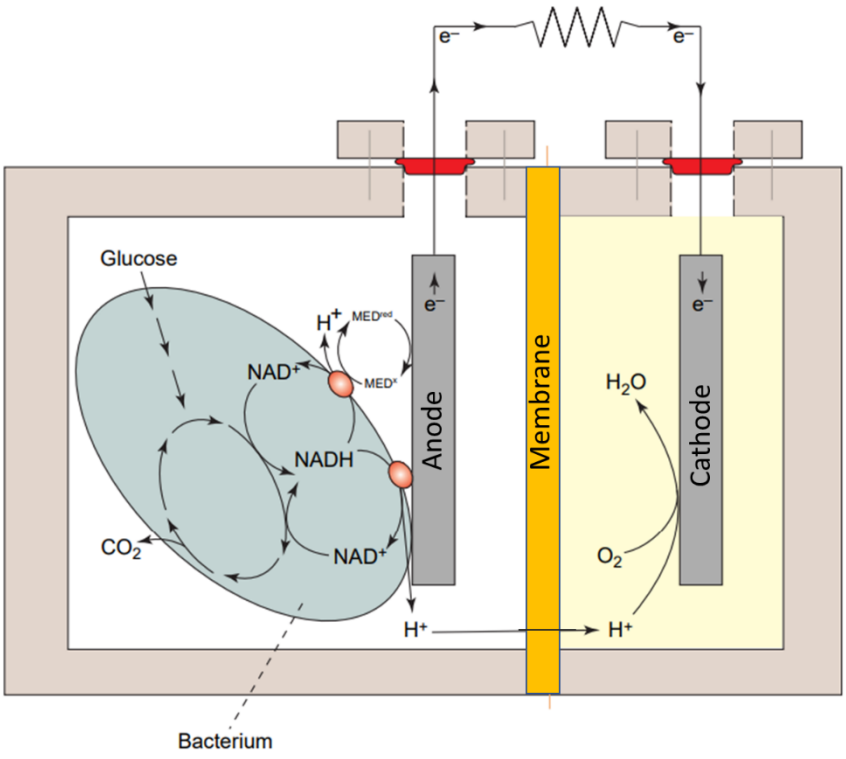

Figure 2-1 Schematic of MFC system (modified from Rabaey and Verstraete, 2005)

2.1.1 Role of microorganisms in MFCs

Generally, several microorganisms transfer the electrons which derived from the metabolism of organic matters to the anode. The marine sediment, soil, wastewater, freshwater sediment and even activated sludge are all rich sources for these microorganisms (Niessen et. al., 2004). A number of researches conversed the screening and identification of microbes and the construction of a chromosome library for microorganisms that are able to generate electricity from oxidizing organic matters (Logan et. al., 2006). As stated above, microorganisms transfer electrons to the electrode compartment over an electron transport reaction that either consists of a series of components in the bacterial extracellular matrix or together with electron shuttles dissolved in the bulk solution. *Geobacter* is known as one

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genus of metal-reducing microorganisms, which yield energy in the form of ATP during the reduction of metal oxides under anaerobic conditions in soils and sediments. The anodic reaction in mediator-less MFC constructed with a metal reducing bacteria belonging primarily to the genera of *Shewanella*, *Rhodoferax,* and *Geobacter* are alike since the anodic electrode as a final electron acceptor is similar to the solid mineral oxides.

2.1.2 Electron transfer mechanisms in the biofilm

In MFCs, some microorganisms which are electrochemically active bacteria has the ability to accept electrons from an outside source or provide electrons to an external material such as an electrode. These microorganisms are acknowledged as the electrogenic microorganisms. Not all microorganisms are electrogenic bacteria, but non-electrogenic microorganisms can still be part of a synergistic electrogenic biofilm as they accomplish other jobs such as providing some organic nutrients to the electrogenic microorganisms. Microbial cells are usually non-conductive since their cell membranes typically contain non-conductive materials such as polysaccharides, lipids and peptidoglycans. Electron transfer between microorganisms and electrodes depends on two points which are direct electron transfer (DET) and mediated electron transfer (MET). It should be noted that some electrogenic microorganisms, such as some microorganisms in the biofilm consortia or activated sludge, have yet to be characterized although such uncharacterized mixed-culture biofilms have been used widely. DET needs direct physical contact between the microbial cell membrane or a membrane organelle and the anode electrode surface, without the requirement for any diffusional redox species.

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2.2 Plant microbial fuel cell (PMFC)

Plant microbial fuel cell (PMFC) is a kind of MFC derived technology in which plant roots excrete rhizodeposits to directly fuel electrochemically active bacteria (EAB) at the anode to generate bioelectricity (Powell et al., 2014). Plants convert the solar energy into the chemical energy, and so that the organic compounds can be synthesized. The excessive organic compounds are entered into the rhizosphere, which can be utilized by microbes as substates (Strik et al., 2008; Deng et al., 2012; Nitisoravut et al., 2017). PMFC has been developed on the basis of rhizodeposition which is the flux of substrates and organic matters at the root-soil interface (Strik et al., 2008). Reed mannagrass (*Glyceria maxima*) PMFC was firstly explored to utilize root exudate via the EABs at the anode, and it also yielded a maximum power output of 67 m/Wm^2 (Strik et al., 2008; 2011). Undoubtedly, PMFC is able to add plants to MFC can increase biomass and the power outputs without harvesting the plants (Helder et al., 2010; Wetser et al., 2015; Moqsud et al., 2015). PMFC can produce sustainable power 18 times higher than the conventional sediment microbial fuel cell (SMFC). The increase in power generation is due to the flux in organic matter availability at the anode for microbial oxidation (De Schamphelaire et at., 2010; Cabezas et al., 2015; Ramadan et al., 2017).

As shown in Figure 2-2, PMFC works on the basis of plant roots excrete of organic compounds e.g., secretion, lysates during photosynthesis process. Subsequently, the electrochemically active bacteria (EAB) oxidzes the root exudates and produce the electrical energy in PMFC systems (Gregory, 2008). PMFCs can also be used with or without a membrane. Notably, membranes were applied to PMFC for separating the anode and cathode chambers in the early development as shown in Figure 2-2. (Strik et al., 2008; Helder et al.,

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2012; Sudirjo et al., 2019). However, membranes are less preferred in a field application because of its cost (Wetser, 2016). As an alternative, membranes can be replaced with a non-conductive spacer or not used at all by placing the anode and the cathode at a proper distance (Kaku et al., 2008; Kouzuma et al., 2013; Ueoka et al., 2016; Sudirjo et al., 2019). The distinction between MFC and PMFC is that the latter uses plants as a source of additional substrates for bacterial methabolism. (Strik et al., 2008). Therefore, the key in PMFC system is a plant-microbe harmony at the rhizosphere, driven by rhizodeposition coupled with efficient engineering. The performance of PMFC is influenced by the biological component, design configurations and the environmental condition during the operation. Since the first reed mannagrass PMFC, not only the intensive research has been conducted to obtain the high electricity levels, but also a various of the PMFC application has been made a great progress. Up to date, PMFCs have been extended to constructed wetland, to generate bioelectricity from paddy fields and agricultural land, wetlands, green roofs and pollution treatment (Yadav et. al, 2012; Xu et. al., 2016; Takanezawa et al., 2010; Sudirjo et al., 2018; Helder et al., 2013). Furthermore, PMFC can be used as a biosensor for determining of water content levels in soil (Tapia et al., 2017). PMFCs are capable of generating sustainable green energy with living plants as the only source of organic matters, making PMFCs more attractive as potential sources of cheap, clean and renewable bioenergy (Moqsud et al., 2015; Deng et al., 2012; Nitisoravut et al., 2017).

doi:10.6342/NTU202203744

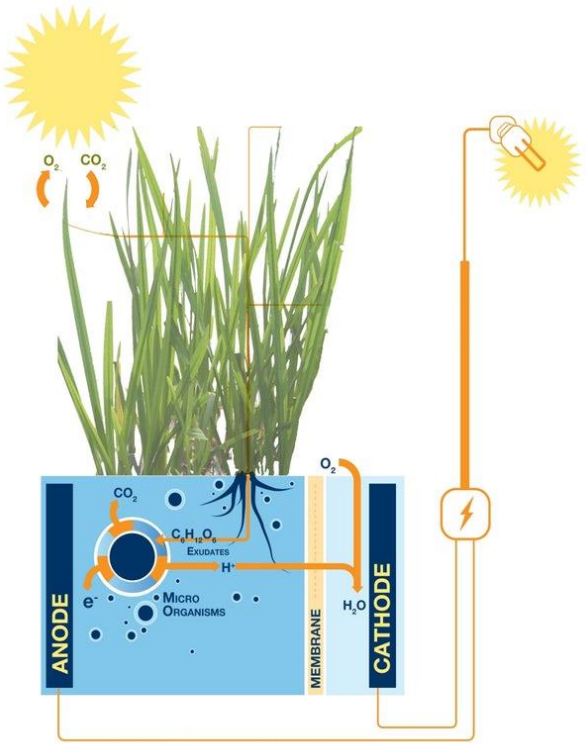

Figure 2-2. Schematic diagram of plant microbial fuel cell (Strik, et. al., 2008)

2.3 Analysis of PMFC performance

Microbial activity around the root of the plants is more than 10 times higher than in the rest of the soil (Kuzyakov, 2010). At the rhizoshere, organic matters are released from the root excretion (either actively or passively) and directly oxidized by microoganisms (Darrah et al., 1991; Toal et al., 2000). However, the oxidation at the anode of PMFC may occure from different electron donors or sources due to the plant rhizodeposits. In addition, other soil redox processes also occur, like sulphide anaerobically oxidized to elemental sulphur (S^0) and SO_4^{2-} by phototrophic sulphur bacteria (*Chlorobium* spp.) (Muyzer et al., 2008). Due to complex processes available from the interaction of plants and microoganisms. It is difficult to determine the potential of the systems. In this research, we use the most common approach to estimate the theoretical cell potential is using thermodynamics of the

doi:10.6342/NTU202203744

anode (e.g acetate) and the cathode (e.g oxygen) reaction (Hamelers, et al., 2010). In this approach, the anode and the cathode potential are calculated based on Gibbs free energy for a specific condition as extensively described by Logan et al (2006). The anodic oxidation reaction for acetate and the cathodic reduction reaction from oxygen to water are given as an example in Table 2-1.

Table 2-1: Standard and actual potential of acetate oxidation and oxygen reduction

Reactions	**E^0** (V vs Ag/AgCl)	**E** (V vs Ag/AgCl)
Acetate oxidation $C_2H_3O_2^- + 4\ H_2O \rightarrow 2\ HCO_3^- + 9\ H^+ + 8\ e^-$	-0.018	-0.494
Oxygen to water $O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$	1.024	0.600

Where as E^0 is standard potential under standard condition and the E is the actual potential (Acetate concentration 0.05M, pH = 7, pO_2 = 0.2 bar, T = 298K). In the PMFC, the electricity production depends on the diffierence of anodic and cathodic potentials like shown in Eq. (2-1) (Rozendal et al., 2008).

$$E_{cell} = E_{cat} - E_{an} \qquad (2\text{-}1)$$

In which E_{cell} = cell voltage, E_{cat} = cathodic potential and E_{an} = anodic potential.

The total amount of electricity that can be obtained from the PMFC system is determined by two factors: voltage and current. The product of the voltage and current leads to the power output of the system, according to Eq. (2-2).

$$P = E_{cell} \times I \qquad (2\text{-}2)$$

In which: P = power (W), E_{cell} = cell voltage (V), I = current (A).

doi:10.6342/NTU202203744

In reality, internal resistance causes the cell potential of PMFC to be far below its theoretical value. These losses are caused by several factors leading to increased internal resistance in the system and lead to a lower voltage than a theoretical one.

Polarization has provided the standard method of presenting MFC and PMFC performance as is the case with chemical fuel cells (Zhao et. al., 2009). A polarization curve is a powerful experiment to analyze and characterize the quality of fuel cells in terms of power generation and could provide a lot of information. Polarization curves can be obtained by varying external resistances using a resistance box or using programmed liner sweep voltammetry. Maximum power density is obtained by constructing polarization curve, and internal resistance is calculated by a slope of I-V curve.

2.4 Factors affecting electricity generation in PMFCs

PMFCs have distinctive characteristics different from MFCs such as living plants, rhizosphere, substrates, microbes, and electron transfer mechanisms. Although intensive research on PMFCs has been conducted in the last decade, PMFCs are still infants. A deep understanding of those factors influence PMFC performance would be benefit for development of design configuration and scale up the in situ PMFC application.

2.4.1 The role of microorganisms

In the PMFCs, microorganisms play a vital role at the root zone of the plants. Microbes at the rhizosphere oxidize root exudates as a substrate and then release the electron to anode via either direct electron transfer or mediated electron transfer. (Logan and Regan, 2006). The rhizosphere supplies microorganisms by providing the rhizodeposits and microbes simplify the nutrient forms for better uptake by the plants (Moulin et al., 2001).

doi:10.6342/NTU202203744

Understanding the microbial community could assist the better performance of PMFC including the competition among the electron donors in the system. (Timmers et al., 2012). In MFCs, a wide range of microbial communities such as α-, β-, γ- or δ-Proteobacteria, Firmicutes and many unknown classes are likely identified for electricity generation (Logan and Regan, 2006). However, the interaction between bacteria and plants in PMFCs are still unclear. A widely diverse species was observed in the both PMFCs and MFCs. This means that the electricity generation might be affected by the variety of bacteria species (Logan and Regan, 2006; Kan et al., 2010). Nevertheless, the microbial community depends on the phylogeny and the species of the plants (Berg and Smalla, 2009). The power generation of PMFC technology depend upon various types of plants-associated microbial community which is shaped by substrates, plant species and rhizodeposit, pH, humidity, temperature, electron donors and accepters, and electrodes.

According to the different microbial community in rhizosphere between plant species related supporting matrix and operation conditions, PMFC system can be different in each specific configuration constructed bioelectrochemical systems (Nitisoravut and Remi, 2017; Cabezas da Rosa, 2010; Rusyn, 2021). In PMFC, three main groups of microbial population in rhizosphere can be determined by their functions and localizations such as anode, cathode the root surface and the electrodes (Rusyn, 2021). Most of the PMFC studies have confirmed the role of *Geobacter* at the anode. Some species such as *Geobactor sulfurreducens* are electrochemically active species which are presenting and active on the electrodes (Bond and Lovely, 2003). Timmer et al (2012) reported that *G. sulfurreducens*, *G. metallireducens*, *G. grbiciae* and *G. hydrogenophilus* were more often found on the anode made of graphite granules rather than on the root of *G. maxima*. In the rhizosphere of paddy

doi:10.6342/NTU202203744

PMFC with potting soil, the dominant bacterial groups related to *Desulfobulbus* spp. and *Geobacter* spp. were isolated on the anode compartments and these were in charged of electricity generation (De Schamphelaire et al., 2010). Futhermore, the anode microbiome of PMFCs have demonstrated high biodiversity and the abandance of different species other than *Geobacteriaceae*. Nitrogen fixing bacteria of *Rhizobiales*, *Beijerinckiaceae*, and *Natronocella acetinitrilica*, *Myxococcus*, *Deferrisoma* and archaea also were found on the anode of paddy PMFC (Kaku et al., 2008). The cathodic microorganisms are named biocathode which is diverse the same as anode microorganisms (He and Angenent, 2006). The cathodic microoganisms consist of the aerobic microoganisms and that use oxygen as the oxidant and the anaerobic microorganisms that use compound such as nitrate, sulphate, iron, manganese, fumurate and carbon dioxide as an electron acceptor (Chen et al., 2008). A 16S rRNA sequencing analysis of microbial PMFC with *O. sativa* revealed that *Gammaproteobacteria* were dominated on the cathode while *Deltaproteobacteria* were enriched on the anode (Ahn et al., 2014). *Rhodobacter gluconicum* was detected on the cathode of paddy PMFC (Kaku et. al., 2008). Among the diversity of microorganisms on the cathode uncultured microbes were found likely to *Gammaproteobacteria* such as *Thiotrichales*, *Chromatiales*, *Legionellales*, *Methylococcales* and *Acidithiobacillales* including many sulfur bacteria. Apparently, sulfur and iron bacteria were found on the cathode as electron acceptors in the PMFC systems. However, the archaea was undetected in the cathode compartment by PCR, suggesting that the archaea played a minor role in the cathodic reaction (Ahn et al., 2014). Nevertheless, the composition of cathodic bacteria in PMFC systems is due to the influence of plant species, substrates, and operating conditions, and light. In PMFC, there is a high diversity of bacteria as a specific ecosystem (Rusyn,

doi:10.6342/NTU202203744

2021). Most of them are cellulolytic and fermentative bacteria that provided the breakdown of complex organic compounds of root excretion and plant residues for EAB (Wang et al., 2017; Ueoka et al., 2016; Timmer et al., 2012). *Clostridium cellulolyticum* and *G. sulfurrducens* were found in the medium with carboxymethyl cellulose and cellulose as electron donors, while a single culture of these bacteria did not found the organic compounds. (Ren et al., 2007; Timmer et al., 2012). Cellulolytic and fermentative bacteria are mediators of electron transfer from plants to EAB and serve as electron donors for EAB that directly transport electrons to the anode. (Ueoka et al., 2016). Thus, these groups of bacteria play valuable roles in the conversion of complex organic compounds to simpler compounds and feed EAB in PMFC systems.

2.4.2 Rhizodeposition

The roots excretion of organic compounds consists of sugar, organic acids, carbohydrates, enzymes and dead-cell materials which provide for EAB. The amount of root exudates, root morphology, phosynthesis and plant-microbe relationship the performance of PMFC is associated with the power output of PMFCs (Bacilio-Jiménez et al., 2003; Takanezawa et al., 2010; Kaku et al., 2008). Therefore, performance of PMFCs can be improved with better optimized rhizodeposition and the suitable of plants. Exploiting the maximum rhizodeposition for electricity production is obligatory for sustainable and extended operation (Strik et al., 2008). Rhizodeposition accounts for approximately 20-40% of the plant's photosynthetic productivity, and these compounds can be oxidized by a diverse of microorganisms (Lynch, 1990). PMFC research elucidated the hydrolysis of root exudates in current generation and claimed that current was limited by oxygen loss in the anodic region. Besides, plants with high root biomass were suggested for PMFCs. (Timmers et al.,

doi:10.6342/NTU202203744

2012). Mechanisms of rhizodeposition have been explored over many decades by researchers working in plant sciences. PMFC researchers need to apply those already understood mechanisms for long term and maximum bioenergy harvest from the system.

In principle, when plants get older, the rhizodeposition decreases. Hence, it can be hypothesized that power output of PMFC declines near the end of the life cycle. The highest currents were recorded at the seedling and tillering stages in the paddy PMFC was operated for 98 days through five different stages; seedling, tillering, midseason aeration, filling and ripening (Deng et al., 2016). A possible explanation could be high microbial activities and more exudates at early stages (Bacilio-Jiménez et al., 2003) or higher photosynthetic compounds utilized by the plants for fruit formation, rendering less to the root at latter stages (Moqsud et al., 2015). Therefore, plants can generate more power at a vegetative stage than a reproductive stage. However, a decrease of power output in the marshy grass PMFC was attributed to the vitality of plants rather than the effect of growth stages (Strik et al., 2008). Apart from root exudates, the power output of PMFCs are increased with an organic amendment. When compared to the control, the addition of compost to the rhizosphere of a paddy plant PMFC resulted in a significant improvement in power density. Besides, organic waste such as wastewater sludge from various food industries that contained high organic content (Bermek et al., 2014; Guo et al., 2013; Zhang et al., 2013) and kitchen were utilized in MFCs and PMFCs (Wang et al., 2012; Zhang et al., 2013). Furthermore, biomass or by-products during harvesting such as straw were used as substrates for the operation (Zeng et al., 2010; Hassan et al., 2014). Therefore, additional substrates in PMFCs might enhance the bioenergy output.

doi:10.6342/NTU202203744

2.4.3 Selection of plants

Mostly, aquatic plants, wetland or waterlogged plants, marshy plants or even salt-tolerant plants have been utilized in PMFC systems. *Glyceria maxima* was provided for the first time for the bioelectricity generation (Strik et al., 2008). Later, wetland species, *Arundinella anomola*, along with marshy species *Spartina anglica* and *Arundo donax* were selected to compare their performance of PMFCs (Helder et al., 2010). The maximum power reported for *Spartina*. *anglica* PMFC was 222 mW/m^2, twice that of the result obtained using the same plant earlier (Helder et al., 2010). This may be due to the difference in electrode materials. Thus, the same plant can perform differently under varied operating conditions. Many other grass species were used in PMFCs because of their adaption to this system, high biomass production, and salinity tolerance (Timmer et al., 2010) e.g., *Pennisetum setaceum*, *Cyprus involucratus*, *Lolium perennee*, *Echinorriea crassipes*, *Acorus calamus*, *Ipomoea aquatica*, *Typha latifolia* and *Canna indica* (Chiranjeevi et al., 2012; Nattawut, 2014; Mohan et al., 2011; Yan et al., 2015; Liu et al., 2013; Oon et al., 2015; Habibul et al., 2016; Lu et al., 2015). Furthermore, paddy plant is applied to PMFC on the basis of its anaerobic conditions developed in the rhizosphere that favor the oxidation reaction of exudates by microbes. The initial outdoor experiments on paddy PMFC confguration resulted in less power density (6 mW/m^2) since the cathode was submerged and covered with soil because of active agitation of the flooded field (Kaku et al., 2008).

As the selection and the utilization of plants depend on the area of the operation, the local species are preferred to avoid invasive species (Remi et al., 2018; Xu et al., 2015). The plant species that secrete large amounts of rhizodeposits are preferentially selected. Nonetheless, paddy is the most preferred plant on the basis of easy access, adequate

doi:10.6342/NTU202203744

hardiness and flexibilities to grow under different conditions within various ecological zones. Addiontionally, plant species with C4 photosynthetic pathways are suitable provided in PMFC because they have high rates of solar energy conversion. C4 plants (e.g., monocots/grass plants) exhibit high photosynthetic efficiency, which leads to an enriched rhizodeposition to serve as substrates for microbial oxidation (Remi et al., 2018; Deng et al., 2012). The criteria for the selection of plants include growth rate, microbiome at the rhizosphere, amount of root system, tolerance and bioaccumulation abilities, local availability, adaptability and rhizodeposition (Nitisoravut and Remi, 2017). Other attributes of plants should also be considered e.g., the sensitiveness of aquatic plants to conductivity and the negative effect of conductivity on plant growth (Eynard et al., 2005).

2.4.4 Soil and supporting matrix

Soil is a common source for inculum utilized in biological systems. According to the presence of rich and diverse natural microbes, it uses as the substrates in PMFC systems (Wolińska et al., 2014). Soil applied to PMFC operation includes natural soil, agricultural and forest soil, paddy field soil, flooded soil, red soil, peat soil, marshy soil, compost soil mix, sediment, garden and potting soil (Nitisoravut and Remi, 2017; Jiang et al., 2010). The soil-root consortium is a vicinity supporting microbes and maintains the relationship between microbes and plants (Gobat et al., 2004). Without understanding the role of soil, an efficient PMFC would hardly be achieved. Soil inoculum bacterium generated higher voltage but lower columbic efficiency than pure culture of *Geobacter sulfurreducens* as well as the presence of the non electrogen bacteria in the soil (Jiang et al., 2010). Due to the species richness and lower C/N ratio, MFC using agricultural soil as an inoculum was shown to be 17 times greater than MFC using forest soil in a previous study. (Lui et al., 2005; Roesch et

doi:10.6342/NTU202203744

al., 2007). A diverse microbial community was observed, e.g., Deltaproteobacteria, *Geobacter*, in the best performing reactors, while low-power MFC anode communities were dominated by *Clostridia*. Therefore, soil physicochemical and biological properties affect the power performance in PMFCs. The increase of power was related to lower C/N ratios in the anode of system (Feng et al., 2010). The lower C/N ratios of agricultural soil might be another reason for system performance. Soil structure, soil texture, nitrogen availability and soil pH are driven shape of the bacterial community (Wakelin et al., 2008; Lauber et al., 2009; Sessitsch et al., 200). Moreover, the organic decomposition and inorganic matter presented in the soil can affect the redox potential (Patrick Jr, 1981). Soil not only can yield electrons via chemical decomposition, such as sulphur species, humic acid, and iron (II) but also continuously undergo redox reactions (De Schamphelaire, Rabaey, et al., 2008; Meek and Chesworth, 2008). These findings concluded that the nature of soil, with its microbial world, plays a major role for electricity generation in PMFCs. Therefore, to develop the PMFC performance, the clearly understanding of soil roles is required for bioelectricity production.

According to improve the power production of PMFCs, the rate of photosysthesis, amount of rhizodeposition and energy recovery should be more studied (Strik et al., 2011). Photosynthetic reaction influences PMFC power output because it affects the performance of microbes and plants. Besides, the use of soil or the supporting matrix such as vermiculate, sediment, sludge or others growth media have been provided for the better performance of PMFCs. The maximum power of *S. anglica* reached 100 W/m^2 when Hoagland medium was added to anolyte and potassium ferricyanide chemical at the cathode (Timmer et al., 2010). The additional of rice amendment as compost in paddy PMFC could be obtained the maximum power of 23 mW/m^2 (Moqsud et al., 2015). Similarly, constructed

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wetland coupled with microbial fuel cell (CW-MFC) with *I. aquatica* was inoculated with anaerobic sludge produced a power density of 12.42 mW/m^2 which higher than that unplanted CW-MFC (Liu et al., 2013). Furthermore, ryegrass PMFC with graphite granule anode showed the current density at the range of 30-70 mA m^{-2} and could be removed 99% of Cr(VI) in soil (Habibul et al., 2016). Therefore, the enrichment of the supporting matrix plays an important role in the enhancement of electricity generation in PMFCs.

2.4.5 Light

The photosynthesis reaction which converted process solar radiation into carbohydrates and, finally, it is a series of the transformation of biomass (Alocilja, 2000). Similarly, the PMFC arrests the root exudate from photosynthesis and converts it to bioelectricity via the microbial metabolisms (Strik et al., 2008; De Schamphelaire et al., 2008; Kaku et al., 2008). The effect of the light cycle to a power generation have been studied in MFC since it is directly involved the activity of the microbes (Wu et al., 2013; Juang et al., 2012). The optimal light intensity is also related to the microbial community in the systems. (Xiao and He, 2014). Besides, the previous PMFC studied reported the effect of light on the power production. For example, the shading of the plants can decrease the electricity output in PMFC due to inhibition of the photosynthesis accompanied with a decline in rhizodeposition (Kaku et al., 2008). Differences in time for achievement in the maximum power were accounted for the physiology of the plant, such as synthesis of the organic compounds, transportation of compounds to the root, release of the exudates and oxidize of the exudates by bacteria and release of the electrons. Futhermore, the addition of the acetate as a substrate increased the electricity output in the dark elucidating the role of light in triggering root exudates. *O. sativa* and *E. glabrescens* exhibited different times in

doi:10.6342/NTU202203744

achieving the maximum power in light phase e.g., 3–4 h and 6–8 h, respectively (Bombelli et al., 2013).

2.5 Design and configuration of PMFCs

The various PMFC configurations have been developed based on the utilization of plants in MFCs and SMFCs. Although there are different system designs and configurations, the basic principle of PMFC operation is the same (Strik et al., 2011). The practical PMFC technology depends on simple design with cost-effective configurations and operation. The distinctiveness of PMFC is the use of non-catalyzed electrode and avoiding an expensive proton exchange membrane. Some of important factors that need to be considered such as the anode placement, the distance between anode and cathode and size and the dimension of electrodes (Shaikh et al., 2020). Up scaling of PMFC technology has to consider various of factors including substrate inculums, cell resistances and types of ion exchange membranes. Various designs have been ulilized for PMFCs that classified into single (sediment PMFC) and double chambered PMFCs (Pandit et al., 2012; 2014).

2.5.1 Sediment type PMFCs

The sediment PMFC includes a single chamber with both electrodes where the cathode is on the soil surface. It has been known that it is cost-efficient and it can be operated without an ion exchange membrane. However, the low power production is the major limitation. The influenced factors such as the electrode sizes, position, the depth of anodes and the distance between the electrodes need to be considered (Nattawut, 2014; Takanezawa et al., 2010; Oon et al., 2015). In the paddy PMFC, the anode was normally placed approximately 2–5 cm under the soil and the cathode was located on the interface of soil and water (Kaku et al., 2008; Moqsud et al., 2015; Takanezawa et al., 2010). On the other

doi:10.6342/NTU202203744

hand, the soil cathode of paddy PMFC was affected the oxygen released by the plant roots due to the small distance between both electrodes (Chen et al., 2012). According to the study of factors influenced PMFC performance, it has been reported that a 5 cm dipped anode produced the power output almost 3 times than that of 2 cm depth anode (Takanezawa et al., 2010). Similarly, the soil MFC showed a better performance when the anode was placed at the depth of 5 cm in soil (Deng et al., 2016). It could be concluded that the suitable anodic zone is an essential for providing the anoxic conditions and utilization of the substrates by microbes in PMFCs (Takanezawa et al., 2010). Similarly, varying the distances of anode from the root zone can alter the power output (Chiranjeevi et al., 2012). The effect of the electrode sizes on the performance of the paddy PMFC was recently noticed in the study. It has been suggested that the anode is the limiting factor until the microbial community has acclimatized, while in the long run, decrease in the cathode performance limited the efficency of the system (Ueoka et al., 2016).

2.5.2 Double chambered PMFCs

The two types of configurations, tubular and flat plate, have been applied in the double chamber system (Helder et al., 2012; Timmers et al., 2013). Tubular PMFC is the most commonly used PMFC for bioelectricity generation. The design setup consists of a tubular anode with a membrane attached at the tubular end and the cathode is placed underneath the anode (Timmers et al., 2013). The different materials were employed for the construction of tubular PMFC. The glass chamber was provided in the previous PMFC. The anode compartment was comprised of a glass tube wrapped with aluminium foil filled with graphite granules, while the cathode was made of a glass beaker containing graphite, separated by the cation exchange membrane (Strik et al., 2008). Polyvinyl chloride is one of

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the most frequently used in tubular PMFC. Similarly, tubular PMFCs were setup with *S. anglica* and *P. australis*, a vertical PVC tube placed in a wetland with the bottom side covered with a PVC cap attached to the membrane. Besides, electrodes were made of graphite felts linked to the golden wires (Wetser et al., 2015). Plexiglass is also provided in tubular PMFC with graphite felt as the electrode materials (Helder et al., 2010). Although this design is popular, the drawback is that the power production is largely deterred due to the distance travelled by protons from anode to cathode (Shaikh et al., 2020).

The flat plate PMFC is the design that a cathode and an anode that are adjacent to one another and separated by a membrane (Helder et al., 2012). This design was also performed in paddy field with a 5 cm depth anode under the soil whereas the cathode was on the top of the soil (Kaku et al., 2008). The two flat plate PMFCs were operated for a long period (370 and 703 days) by varying the anode materials (Helder et al., 2013). The flat plate PMFC resulted in lower internal resistance and it was claimed to have better performances than the tubular PMFCs. The previous study of paddy PMFC also revealed that the size of the electrodes affects the power output of PMFC (Kaku et al., 2008). Studies also suggested that the anode was the limiting factor until the microbial community had adapted, which during the long-term operation decreased the cathode performance and limited the efficiencies of the PMFC systems (Mohan et al., 2011). Therefore, understanding the designs and engineering methods are necessary to improve the efficiency of the system including the integration of technogy for better power output (Xu et al., 2015). Such a hybrid mode of the application would be equally applicable to a PMFC system based upon the purpose of study.

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2.5.3 PMFC based flow-through systems

Flow-through systems (FTSs) relate ecologically engineered systems (EESs) and assorted constructed wetland systems. This is designed to personate the natural cleasing function of wetlands with simultaneous electricity generation. An EES consists of the rectangular tank fabricated with sheets of polymethyl methacrylate that are connected in succession with various functions for wastewater treatment. The bottom of the tank was filled with lake sediment as the biocatalyst for organic metabolization systems and delivered electrons and protons along with metabolic intermediates. The electrodes were assembled with the ecologically engineered systems (EESs) by placing an anode at the bottom of the tank. Thus, this created the potential gradient between the electrodes. Inlets and outlets have been provided for the wastewater flow. Diverse aquatic and wetland plants, microbial communities such as bacteria, protozoa, algae, and planktons, as well as fish, were used to treat wastewater. Wastewater treatment is done with a series of tanks with a controlled environment and a huge diversity of organisms with bioelectrochemical mediation (Mohan et al., 2011).

A floating macrophyte-based system was designed with a polymethyl methacrylate (Perspex) tank by integrating a fuel cell with non-catalyzed electrodes and was applied to treat domestic sewage for 210 days (Mohan et al., 2011). Submerged and emergent macrophytes such as *Hydrilla verticillate*, *Myriophyllum*, *Bophyllumry pinnatum*, *Lycopodium mesculentum, Coriandrum sativum*, *Capsicum annuum* and *O. sativa* were utilized for the treatment of distillery wastewater. The results showed an adequate power output for a longer period (Chiranjeevi et al., 2013). Similarly, constructed wetland PMFCs have also been used in EES with the acrylic column and the PVC container for simultaneous

doi:10.6342/NTU202203744

wastewater treatment and electricity generation (Schröder et al., 2007). The designed EES investigated mechanical simplicity, low energy consumption, and cost-effectiveness. Moreover, it also provided esthetic values from natural cleansing technology.

2.6 Application of PMFCs

PMFCs are the alternative sources to generate sustainable and renewable energy. To date, several studies have combined the biosystem principle of PMFC with the feasible application (Deng et al., 2016). PMFCs are cost-effective and can be used to generate electricity in developing and remote areas, such as wetlands and agricultural land. PMFCs can also be integrated with wastewater treatment, soil pollutant remediation greenhouse mitigation, biosensing, and urban greening.

2.6.1 Wastewater treatment

PMFC systems have been employed in the bioelectricity generation from various wastewater and they have been proven to be eco-friendly with high organics removal and stable power generation capabilities than other treatment systems (Habibul et al., 2016). Flow-through systems (FTSs) as ecologically engineered systems (EESs) with PMFCs were provided to wastewater treatment and recycling in wetland (Mohan et al., 2011; Chiranjeevi et al., 2013; Ren et al., 2015; Habibul et al., 2016). Floating macrophyte, *Eichornia*, was applied in EESs to treat distillery wastewater and evaluate the electricity generation. The results revealed the effective removal rates of COD (86.67%) and VFA (72.32%) and the electricity generation of 224.93 mA/m^2 (Mohan et al., 2011). Furthermore, PMFCs also has been integrated to constructed wetland based on the benefits of MFC which named the constructed wetland-MFC (CW-MFC) (Doherty et al., 2015). CW-MFC treats wastewater in

doi:10.6342/NTU202203744

the presence of plants which act as aeration (Xu et al., 2016; Fernández et al., 2014). The compatibility of this hybrid system is that two biological systems are used to degrade organic matters. However, the difference is that the EABs in PMFCs are fed by rhizodeposits with the aim of producing green electricity (Strik et al., 2008), while the EABs in CWs are fed with both rhizodeposits and wastewater for the purpose of wastewater treatment (Doherty et al., 2015). The benefit of CW-MFC system is that it takes the advantages of MFC and CW to achieve wastewater treatment and bioenergy generation concurrently (Feng et al., 2013).

Plants in the CW-MFC undergo photosynthesis to produce rhizodeposits and exudates at the roots which are used as sources of organic matters at the anode for microbial oxidation to produce electrical power (Timmers et al., 2012). The insertion of plant roots at the cathode excretes O_2 via the rhizosphere to improve the performance of the CW-MFC (Deng et al., 2011). Plant biomass will also absorb dissolved nutrients and heavy metals to improve removal efficiency during wastewater treatment (Ju et al., 2014; Song et al., 2011). The vertical flow subsurface system was the first design of CW-MFC (Yadav et al., 2012; Fang et al., 2013). Later, the horizontal subsurface flow system (Villasenor et al., 2013) and surface flow system with floating macrophytes (Mohan et al., 2011; Chiranjeevi et al., 2013) have been developed. CW-MFC with common reed (*P. australis*) was used to treat swine wastewater using aeration at the catode to enhance the performance (Zhao et al., 2013). The maximum power density and COD removal were 9.4 mW/m^2 and 76.5%, respectively. In an upflow CW-MFC with *Typha latifolia* revealed the maximum power density of 6.12 mW/m^2, 100% removal of COD, 40% of NO_3^- and 91% of NH_4^+ (Oon et al., 2015). Another CW-MFC reported 3.4% boron removal and produced 78 mW/m^2 of the maximum power (Türker et al., 2017). CW-MFC showed the range of 53.1 to 75.4% of nitrogen removal from

doi:10.6342/NTU202203744

synthesis wastewater and generated 0.5 to 2 mW/m^3 of the power density (Xu et al., 2017). Although the bioelectricity generation in PMFCs during wastewater treatment is comparatively low, PMFCs still represent the promising ability to continuously supply stable power for low energy devices (Bajracharya et al., 2016).

2.6.2 Remediation of pollutants

Contamination of water and soil with organic or inorganic pollutants demonstrates severe environmental impacts that cause many health threats for humans and all life forms. Plants play a significant role in removing contaminants with their attendant microorganisms via the phytoremediation process (Ashraf et al., 2019; Rezania et al., 2016). During this process, the plant accumulates organic and inorganic pollutants, metabolizes the organic wastes, and stimulates the microbial decay of organic wastes in the rhizosphere zone (Yadav et al., 2018; Vymazal et al., 2016). PMFCs have been attracted more attention for sediments and surface water remediation through the degradation of organic matters and rhizodeposits in sediments by EABs (Strik et al., 2008). In an experiment of wetland systems, sediment PMFC with *Typha domingensis* degraded the organic matter and achieved a maximum power density of 6.12 ± 2.53 mW/m^2 without the addition of other carbon sources (Cervantes-Alcalá et al., 2012). Submerged aquatic plants coupled with SMFC revealed that nitrogen could be removed by 25.3%, while a power density of 4.42 and 3.16 mW/m^2 was generated in the planted and closed-SMFCs, respectively (Xu et al., 2017). Furthermore, PMFCs with different plant species including *Oryza sativa*, *Acorus calamus*, *Spathiphyllum petite* and *Chamaedorea elegans* were built and assessed for sulfide removal from the sediment and surface water (Liu et al., 2019). This suggests that PMFC systems can be fully incorporated into aquatic ecosystems to contribute to ecological restoration.

doi:10.6342/NTU202203744

Phytoremediation is an autonomous method for pollutant removal that is environmentally friendly, cost-effective, relevant for surface and groundwater, sediments, soil, and sludge (Kumar et al., 2018). Phyto-power system is a hybrid system of PMFC and phytoremediation for simultaneous bioenergy generation and contaminants removal (organic and/or inorganic) from the soil or water (Saba et al., 2019). This system can sustainably harvest energy from anaerobic respiration of microorganisms around the plant root in a clean, low-cost, and effective way (Pamintuan et al., 2019; Türker et al., 2017). To date, PMFCs have been utilized to treat various pollutants with different configurations, e.g., heavy metal removal and organic pollutants.

2.6.3 Greenhouse gas mitigation

The soils in the rice paddy fields and wetlands are rich in organic substrates, which support the growth of hydrophytes and microbes for bioenergy producing (Kaku et al., 2008). PMFCs have been successfully installed in wetlands and paddy fields to reduce methane gas emission and produce bioelectricity (Wetser et al., 2015; Arends et al., 2014). The anoxic-oxic interface created at the soil/sediment–water interface and rhizodeposits provides the suitable conditions necessary for the installation and operation of PMFCs in the ecosystem (Salomons et al., 1987; Du Laing et al., 2009). The reduction of methane gas has been reported to occur spontaneously by inserting the anode of a PMFC at the rhizosphere in a paddy field or wetland while the cathode is placed in surface water (Kaku et al., 2008; Arends et al., 2012). In pot cultured paddy PMFCs have been observed about 50% methane reduction (Arends et al., 2012; Cabezas da Rosa et al., 2010). Moreover, methane gas mitigation and electricity harvesting of the wetland have removed 71–82% of methane and obtained a maximum current density of 187 mAm^{-2} from the CW-MFC system (Lui et al.,

doi:10.6342/NTU202203744

2017). In the study of rice plant PMFC with a mixture of vermiculite and graphite granules supporting matrix, it showed the reduction of methane gas with simultaneous bioelectricity generation (Cabezas da Rosa et al., 2010). *Geobacter psychrophilus* and related species were discovered to be capable of growing on anodes and producing a maximum power density of 19 3.2 mWm^{-2} (Kouzuma et al., 2013). Also, paddy PMFC with a graphite felt anode inserted into the rhizosphere and the cathode placed in the flooded water yielded a maximum power density of 6 mW/m^2 (Kaku et al., 2008). An assessment of the electricity generation in an earthen membrane double chamber PMFC under greenhouse conditions recorded a maximum power output of 60 mW/m^2 in the double chamber PMFC, which was 55% greater than that achieved in the single chamber PMFC with methane gas emitted from the microcosm. Thus, the installation of PMFCs in wetlands and rice paddy fields is shown to be a coping strategy in greenhouse gas mitigation and bioelectricity generation.

2.6.4 Biosensing

MFC-based technology has been developed for a wide range of applications, including biosensors (Das, 2018). Phytosynthetic organisms are used in biosensors for the detection of environmental pollutants, photovoltatic devices for power generation, photosensors and phototransistors as fuel cells for biosensing (Sanders et al., 2001; Cho et al., 2008). The PMFC was built with the intention of biosensing plant health and generating power in order to supply a wireless electronic system that correlated the quality of environmental factors (Tapia et al., 2017). PMFCs based on drought-tolerant *Sedum* species can be used as soil moisture sensors on green roofs in arid and semiarid environment. *Sedum* PMFCs showed a maximum power density of 114.6 μmW^{-2} and a positive relationship between electricity generation and water content. This represented the first effort to use

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PMFC systems as low-cost water content biosensors for green roofs (Tapia et al., 2017; 2018). Consequently, this technology enables monitoring irrigation in semiarid areas and could assist in efficient water management.

According to their high tolerance to low moisture content and ability to survive in a wide range of temperatures, bryophyte MFCs based on the moss species *Dicranum montanum* were also used as biosensors (Bombelli et al., 2016; Hubenova et al., 2011). *Physcomitrella patens* PMFCs developed on the basis of a mixture of six moss species have been capable of powering devices such as LCD and meteorological stations. Furthermore, ten series connected moss PMFCs generated an average of 6.7 mW/m^2 and 53 mA and were capable of charging batteries with a nominal voltage of 3.6 V. After 10 h of charging, the battery could power the radio for approximately 80 sec (Bombelli et al., 2016). Although a PMFC generated sub-millwatt power during the operation, it was enough to self-sustain a wireless sensor node to monitor both the surrounding environment and the plant's health (Brunelli et al., 2016). As a result, the bioelectricity generated by the PMFC systems was adequate to power biosensors and distant devices that required constant and low-power sources.

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Chapter III Materials and methods

3.1 Soil preparation

In this research, the soil was collected from a natural paddy field in Taoyuan City (24° 53′ 21″ N, 121° 17′ 20″ E) at the topsoil (0-15 cm depth). The rocks and the plant debris were manually removed via screening. The screened soil was air-dried to remove the moisture content and sieved through 2 mm sieved-mesh. Before setup of soil-MFCs and PMFCs, the soil was incubated by adding tap water until the saturated condition and mixed with NH_4NO_3 (120 mg N/kg soil) and K_2HPO_4 (30 mg P/kg soil and 75 mg K/kg soil), which function as the essential nutrients for crop farming (Khan et al., 2013).

3.2 Paddy and water bamboo cultivation

The two species of waterlogged agricultural plants, including paddy (*Oryza sativa*) and water bamboo (*Zizania latifolia*) were used to evaluate the electrical energy generation in PMFCs. Rice seeds were obtained from the National Taiwan University farm. All rice seeds were cultivated at 27 ºC in the incubator without light. After the root shooting, the paddy was transplanted into the soil until 3 weeks old and then transplanted in PMFCs. Meanwhile, the water bamboo plants were obtained at the ages of 2 weeks from Honglin Garden Company, Changhua, Taiwan and continuously cultivated from their stem until they had 5 cm length, and they were set up in PMFCs afterward.

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3.2 PMFC configuration and operation

3.2.1 PMFC setup in the controlled incubator

In this study, round polyvinyl chloride (PVC) buckets (24 cm height and 17 cm diameter) were designed in all cases and set up in the light incubator (LG-600RH, LIAN SHEN ENTREPRISE CO., LTD., Taiwan). Each bucket contained 3 kg of soils and 1.5 L of water. The experimental setup included soil-MFCs without plants and different PMFCs, including two species of plants (paddy and water bamboo) and two soil conditioners (biochar and compost) for PMFCs. The soil-MFC was set up as a control for comparison. P-PMFC and water bamboo-PMFC (W-PMFC) were set up to evaluate the impact of different waterlogged agricultural plants on electricity production in PMFCs. Two additional sets of P-PMFC which were mixed with 10% (w/w) of compost (Moqsud et al., 2015) and 1% (w/w) of biochar (Khan et al., 2013), respectively, to test the effect of soil conditioners. The compost, which was made from food waste, was obtained from Musta Refuse Incineration Plant, Department of Environmental Protection, Taipei City government. The commercial biochar made from waste wood biomass was obtained from the GreenPros CO., LTD, Taiwan. The basic properties of soils, compost and biochar are reported in Table 3-1 In this study, all experimental setups are triplicated.

Table 3-1 The basic properties of soils, compost and biochar

Parameters	Paddy soil	Compost	Biochar
pH	5.53	5.05	5.85
EC (μS/cm)	65.7	295	294
Moisture content (%)	60	44	37
Organic matter (%)	4.65	8.15	-
Total Organic carbon (%)	2.7	4.73	-

doi:10.6342/NTU202203744

The electrode material for both anode and cathode were made from carbon felt in a round shape (Gansu Haoshi Carbon Fiber Company, China) with 13 cm of diameter and 3 mm of thickness. The cathode was placed on the top of the soils but in the waterlogged condition, while the anode was buried in the soils. The distance between cathode and anode was about 5 cm. Both cathode and anode were connected via titanium wire, and the circuit was connected with 1 kΩ of an external resistor. The experimental configurations are showed in Figure 3-1. All experiments were conducted in the controlled environment in the lighting incubator with 27 °C and 75% of humidity, and carried out for a period of 200 days (December 2018–June 2019). An artificial light which includes fluorescent and LEDs lighting was controlled at 12/12 h of the light and dark cycle. The average light density monitored via a light sensor (UA-002-64, HOBO, USA) was 2095.4 Lux within the 63 × 65 × 50 cm space of the incubator. To keep all experiments in the waterlogged condition, all cases were irrigated with tap water every day.

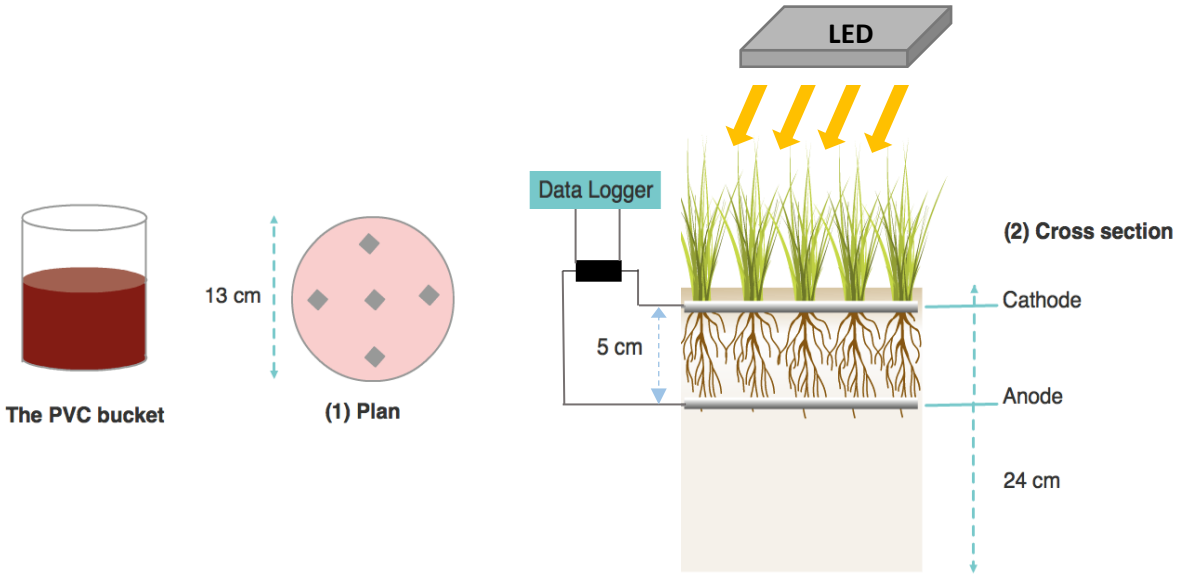

Figure 3-1 Schematic diagrams of the PMFCs configuration in the controlled incubator

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To enhance the electricity production of *Oryza sativa* PMFCs and understand more the bioelectrochemical systems, the round acrylic reactors with 14 cm of diameter and 35 cm of height were provided in this study, including the ports (Figure 3-2). Homogenized soil (3 kg) and deionized water (1.5 L) were added to each reactor. The electrodes were made of carbon felts in a round shape with 130 mm in diameter and 3 mm in thickness (Gansu Haoshi Carbon Fiber Company, China). The distance between the cathode and anode was about 5 cm, and both electrodes were connected via titanium wire. The circuit was connected with 1 kΩ of an external resistor and the voltage output was investigated through the data logger and computer. After transplanting the paddy into the reactors, all experiments were conducted in the controlled incubators under the same conditions and carried out for a period of 150 days. The experimental setup consisted of soil MFCs, paddy PMFCs (P-PMFC) and paddy PMFC adding chemical fertilization (F-PMFC). Chemical fetilizers (NH_4NO_3 and K_2HPO_4) were applied to F-PMFC during the vetgetative stages (day 60, 70, 90, and 110, Khan et al., 2013) for comparision the electricity enhancement among the treatments. Weekly pore water was collected and analyzed after applied the chemical fertilizers during the rice productive stage via a pore water sampler (Rhizon type, Eijkelkamp, Netherland).

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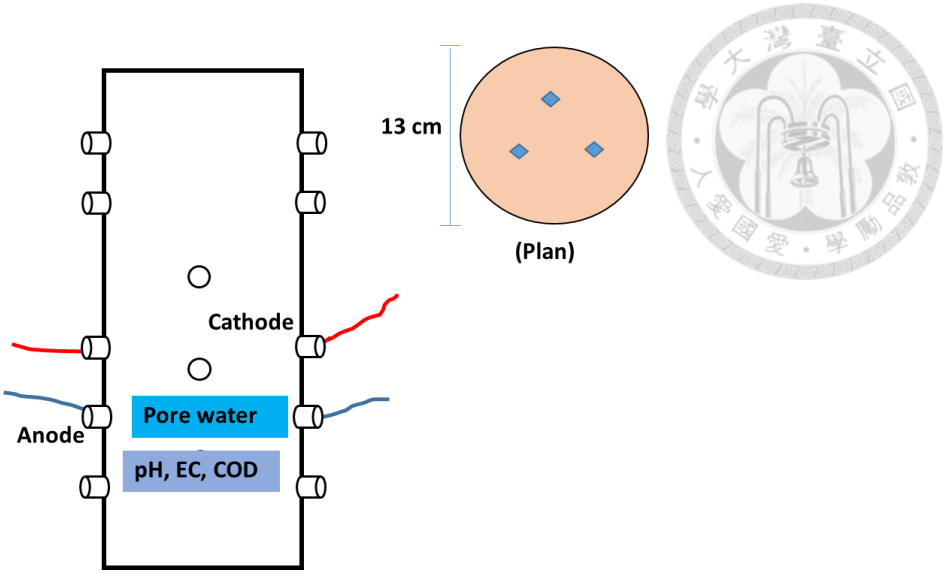

Figure 3-2 The design of acrylic reactors in the controlled incubator

3.2.2 PMFCs setup in the greenhouse

Cylinder PVC buckets with high-density polyethylene plastic bags placed inside were also used in the paddy (*Oryza sativa*) PMFC systems. Homogenized soil (3 kg) and deionized water (1.5 L) were added to each bucket. Round-shaped carbon felts (Lansu Haoshi Carbon Fiber Company, China) with 130 mm in diameter and 3 mm in thickness interwoven with copper wire were provided for the electrodes. The anode was buried in the soil approximately 10 cm from the cathode, with the cathode installed at the topsoil-water interface. To investigate the enhancement of bioelectricity generation, three paddy PMFCs were connected in series and parallel as shown in Figure 3-3. All experiment setup in this study were triplicated and soil MFCs were used as a controlled treatment for comparison. The external circuit was completed with 1 kΩ external resistors under the closed-circuit condition and connected with the data logger and computer. All experiments were conducted in the greenhouse of NTU farm, National Taiwan University. Inside the greenhouse, the wind

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and the rain sheltering were provided and the light, temperature, humidity and sunlight duration varied along with weather changes. During the experiments, the plant growth was regularly monitored by measuring the height of the plant leaves of each plant using measuring tape. To keep all experiments in the waterlogged condition, all cases were irrigated with tap water every day.

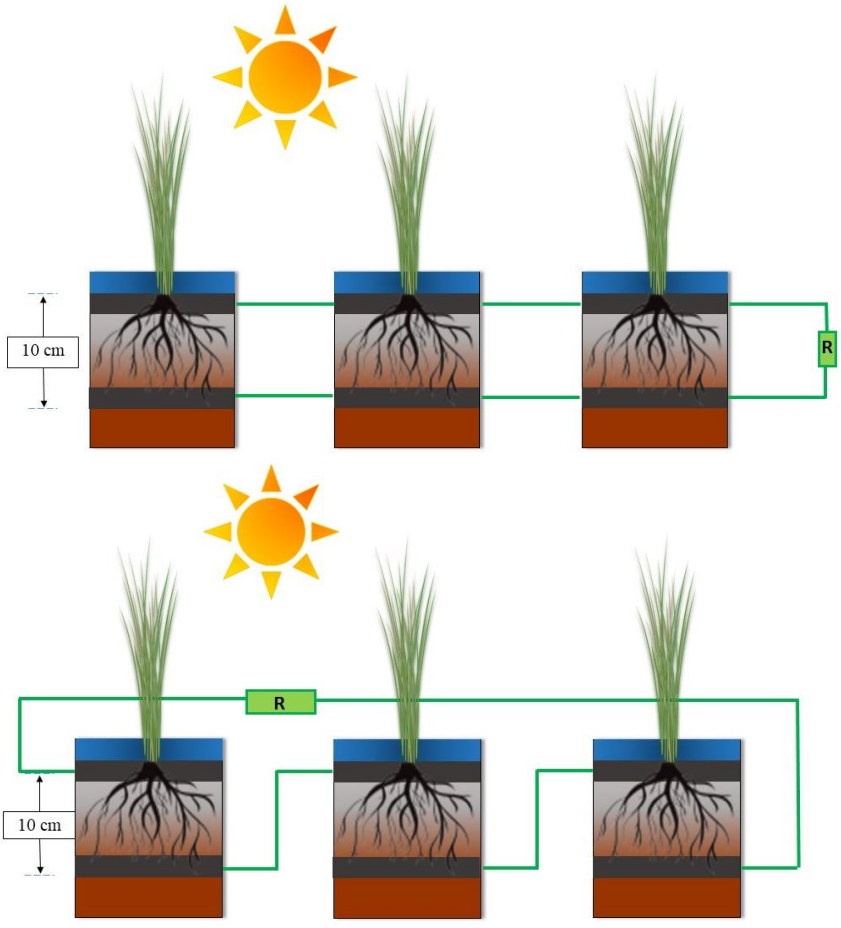

Figure 3-3 The diagram of paddy PMFCs with series and parallel connection

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3.3 Analytical method

To monitor power performance, the voltage across the resistor of all PMFCs was monitored every 5 min via connection to the data acquisition system (2700, Keithley, the USA) controlled with KE 302 Kick start data logger software, and the data were saved to a computer. After 60 days, the polarization tests were made by using different resisters. Internal resistances and power density were calculated as described in the previous literature (Logan et al., 2006). The electricity output was measured in voltage (V) against time, and the current was calculated by using Ohm's law. The current density was calculated based on the anode surface area according to Eq. (3-1) (Moqsud et al., 2015).

$$I = V/\alpha R \qquad (3\text{-}1)$$

where V is the measured voltage in volts (V), R represented the value of the external load resistor in Ohms, and α is the electrode surface area. The power output (P) was calculated following Eq. (3-2)

$$P = V \times I \qquad (3\text{-}2)$$

The internal resistance (R_{int}, Ω) was calculated by the peak power density method with the aid of polarization and power density curves. When the maximum power density (P_{max}, mW/m^2) is acquired, the internal resistance is equal to the external resistance (R_{ext}, Ω) following Eq. (3-3) (Logan et al., 2006).

$$R_{int} = R_{ext} = P_{max}/i^2 \qquad (3\text{-}3)$$

where i represented the current corresponding to the maximum power density.

The organic matter content of the soils and compost was determined by “loss on ignition” method (LOI). The dry weight of the sample was weighted before and after combustion at 600 °C for 2 h. The LOI was calculated according to Eq. (3-4)

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$$LOI\ (\%) = ((DW105-DW600)/DW105) \times 100 \quad (3\text{-}4)$$

where DW105 is the dry weight of sample at 105 °C to constant weight, and DW600 is the weight of dry sample after combustion in the furnace at 600 °C for 2 h (Zhao et al., 2016).

The soil sampling and the analysis of soil followed the methods of the Environmental Analysis Laboratory, Environmental Protection Administration, R.O.C. (Taiwan). The electrode methods were used to analyze the pH and EC values (NIEA S410.62C). The procedures also included mixing dried soil samples with deionized water, waiting for the precipitation, filtering, and measuring the pH values using a pH meter (HQ40D, HACH, USA). For EC of soil measurement, the EC meter (HQ40D, HACH, USA) was used after mixing the soil and deionized water (1:5), shaking for 1 h at 140 rpm, and filtering using Whatman 5 filter (NIEA W203.51B). Meanwhile, pore water from the PMFC systems with different depths of soil was collected by a pore water sampler (Rhizon type, Eijkelkamp, Netherland).

3.4 Measurement of greenhouse gas emission

At the productive stage of paddy, methane and nitrous oxide of paddy PMFCs and soil-MFCs in the greenhouse were measured in weekly for one month. The static chamber method was provided to quanlify the gas emission from soil-MFCs and PMFCs. Each PMFC was closed with the headspace chamber (30 x 30 x 80 cm) for 60 mins at 9.00 a.m. in each sampling day in order to enclose paddy plants. Thirty minutes prior to gas sampling, the internal blower was switched on to mix the air in the headspsce chamber. Gas samples were taken through 1 mL plastic syringes (all done in triplicate) for 30 mins interval of each sample

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into the glass vial tubes. All gas samples including the ambient air were brought to the laboratory for analysis.

The concentration of methane and nitrous oxide were measured by injecting 0.25 mL of gas samples into gas chromatography equipped with ECD and FID (Agilent 7890 GC, Agilent Technologies, USA). The gas emission flux (*F*) was expressed in ppm h^{-1} and ppb h^{-1} as the following equations (3-5) and (3-6) (Minamikawa et al., 2015):

$$Flux_{CH4} = (\triangle C/\triangle t) * (V/A) \times (p) * (273/273+T) \quad (3\text{-}5)$$

$$Flux_{N2O} = (\triangle C/\triangle t) * (V/A) \times (p) * (273/273+T) * (28/44) \quad (3\text{-}6)$$

where $\triangle C/\triangle t$ is the concentration change over the time (ppm CH_4 or ppb N_2O h^{-1}); V is a chamber voume (m^3); A is a chamber area (m^2); *p* is gas density (0.717 kg m^{-3} for CH_4 and 1.977 kg m^{-3} for N_2O at 0 ºC; T represents the air temperature inside the chamber (ºC).

3.5 Analysis of the electrodes

At the end of the experiment, anodes were analyzed to detect the electrode biofilm after pretreatment by using scanning electron microscopy (SEM). The anode pretreatment processes included cleansing, fixation, cooling, and dehydration steps. First, the anode was cleaned with phosphate buffer and soaked into a different serial concentration of alcohol for the dehydration process. Subsequently, the samples were coated with copper seat and gold plate by a sputter coater (E Ion sputter, Hitachi, Japan). The biofilm on the electrodes was observed using the scanning electron microscope (TM-3000, Hitachi, Japan).

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3.6 Microbial community analysis

At the end of the experiment, the anodes were carefully removed and the soil samples of all treatments were sampled to analyze the microbial community for analyzing microbial community structures using 16S rRNA gene amplicon high-throughput sequencing. The DNA was extracted from the anodes of soil-MFCs and PMFCs by using the commercial DNA extraction kit (DNeasy PowerSoil Kit, QIAGEN, Germany). The fragments of 16S rRNA genes were amplified using the universal primers: 16S V3–V4; 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′GACTACHVGGGTATCTAATCC-3′). The DNA was amplified using the thermal cycling with the initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, elongation at 72 °C for 30 s, and the final extension at 72 °C for 5 min. All 16S rRNA gene amplicons were sent to sequencing (Genomics company, Taiwan). The sequencing libraries were generated using Truseq nano DNA Library Prep Kit (Illumina, the USA) following the manufacturer's recommendations, and index codes were added (Caporaso et al., 2010; Schloss et al., 2009). The mothur (Caporaso et al., 2010) and QIIME (Schloss et al., 2009) softwares were used to analyze raw sequencing data as mentioned previously. Every representative sequence was described as an operational taxonomic unit (OTU) using the RDP classifer with the SILVA database version 132 (Quast et al., 2013).

3.7 Statistical analysis

The descriptive statistics and analysis of variance (ANOVA, IBM SPSS Statistics version 22.0) with Tukey post hoc test were used to compare the electrical voltage, pH, and EC values of P-PMFC, W-PMFC, soil-MFC, PMFCs with conditioners. Using α = 0.05,

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statistical significance (*P* value) was provided to compare the diferent treatments of soil-MFCs and PMFCs. UniFrac principle coordinate analysis (PCoA) which is a distance metric using phylogenetic information was also used to compare microbial communities among the soil-MFC and PMFCs (Lozupone et al., 2011). Meanwhile, the unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering was used to classify the average linkage of the microbial community in soil-MFCs and PMFCs based on their pairwise similarities (Saitou and Nei, 1987). The method can be more effective in revealing ecological patterns than taxon-based methods (e.g., use of lists of species, genera, and OTUs) used in the previous study (De Schamphelaire et al., 2010). In this study, UniFrac coupled with PCoA and UPGMA hierarchical cluster was carried out by PALSTAT software package version 3.21.

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Chapter IV Results and discussions

4.1 Evaluation of long-term performance of plant microbial fuel cells using agricultural plants under the control environment

4.1.1 Electricity generation in PMFCs

The output voltages of the multiple PMFCs tested in this study are shown in Figure 4-1. After operating soil-MFC and PMFCs for 10 days, the closed-circuit voltage of all treatments increased gradually and showed varied voltage outputs, which were similar to the previous study (Timmers et al., 2010). For the soil-MFC, the highest output voltage was 468.57 ± 34.64 mV, while the P-PMFC and W-PMFC had the highest output voltage of 668.28 ± 32.53 and 618.11 ± 45.38 mV, respectively, as shown in Figure 4-1(a). Comparing soil-MFC with PMFCs, PMFCs with paddy and water bamboo had significantly higher voltage values ($P < 0.05$) than soil-MFC (Table A1). The significantly higher output voltage in PMFCs should be related to the available carbon sources from rhizodeposition, which is the root excretion of organic compounds to the soils including sugars, organic acids, polymeric carbohydrates, enzymes, and dead-cell material (Timmers et al., 2010). As also shown in the previous study, it revealed that PMFC with paddy was still able to generate electricity even using inert vermiculite as the growing medium, where the plants were the only sources of organic compounds (De Schamphelaire et al., 2010). These results suggest that living plants like rice paddy and water bamboo used in this study are important to enhance the bioelectricity generation, since electroactive bacteria (EAB) could access more carbon sources from the rhizodeposition in PMFCs. In addition, the declining trend of output voltage in soil-MFC after 20 days in Figure 4-1(a) implied the depletion of readily biodegradable organic matter in soil. However, after 60 days, there was a gradual increase in

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output voltage in soil-MFC again, which might suggest after a period of acclimation, microorganisms were likely able to use the complex organic matter in soil as the new substrate for electricity generation. Overall, plants are critical to supply the organic compounds through rhizodeposits in PMFCs, especially in the long-term operation. From the result in Figure 4-1(a), the P-PMFC showed significantly higher output voltage than W-PMFC ($P < 0.05$), suggesting that different plants could cause the different performances of electricity production in PMFCs. However, plant roots in PMFCs could fuel the EAB at the anode by providing rhizodeposits (Md Khudzari et al., 2018), previous studies mentioned that it also related to root architecture, quality and the quantity of plant rhizodeposition would vary over the growth stages and plant species (Aulakh et al., 2001). The variation in amount and speciation of rhizodeposits from different plants probably resulted in different performances of electricity production in P-PMFC and W-PMFC. Furthermore, the varied plant rhizodeposition over the growth stages likely caused changes in the flux of organic compounds and therefore caused fluctuation in anode potential as a consequence cell potential of PMFCs (Timmers et al., 2010), since all environmental conditions were maintained under the constant conditions throughout the operation.

As shown in Figure 4-1(b), it is found that P-PMFC with compost (PC-PMFC) reached the highest voltage at 894.39 ± 53.44 mV (34.78 mW/m^2), which was the highest voltage values among all treatments. The previous study of outdoor paddy PMFCs observed the increase in voltage generation with the addition of compost made from kitchen and yard waste (Moqsud et al., 2015). However, the compost experiments in the previous study were conducted based on one single PMFC without replicate, and the observation was not yet statistically confirmed. Our PMFCs were triplicated and clearly demonstrated that PC-PMFC

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with the addition of compost made from food waste could produce significantly higher voltage than those without compost (P< 0.05) (Table A1). In addition, compared with other treatments with largely varied electricity production, P-PMFC showed more consistent output voltage with the addition of compost. A stable output voltage suggested that the organic substrates in compost could provide sufficient foods for EAB to minimize the impact from varied plant rhizodeposition and resulted in additional capacity to enhance the bioelectricity in P-PMFC. Food waste management has been a critical environmental issue in different countries (Gustavsson et al., 2011). Therefore, our results demonstrate that compost converted from food waste can be considered as an efficient organic fertilizer to apply in paddy PMFCs to enhance electricity production.

On the other hands, the P-PMFC with biochar (PB-PMFC) had quite low electricity generation in the first and second month of the experimental duration. Even though it largely increased after 120 days, the output voltage still showed high fluctuation and significantly lower than PCPMFC (P<0.05). This result implied that adding the biochar in soils might cause some adverse effects to PMFCs, e.g., the higher EC after adding the biochar in the soils could impact some microorganisms which have a low tolerance to higher EC (Tremouli et al., 2010). In addition, although the previous study reported the addition of biochar produced by sewage sludge significantly stimulated rice growth (Khan et al., 2013), our study observed the inhibited rice growth after the addition of biochar produced from waste wood biomass (Figure A1). One study has shown that free radicals can be detected in biochar produced from biomass charring, and these free radicals were persistent and could inhibit the germination and growth of rice seedlings (Liao et al., 2014). The authors also found that lignin in the biomass played an important role in the free radicals generation during biochar production.

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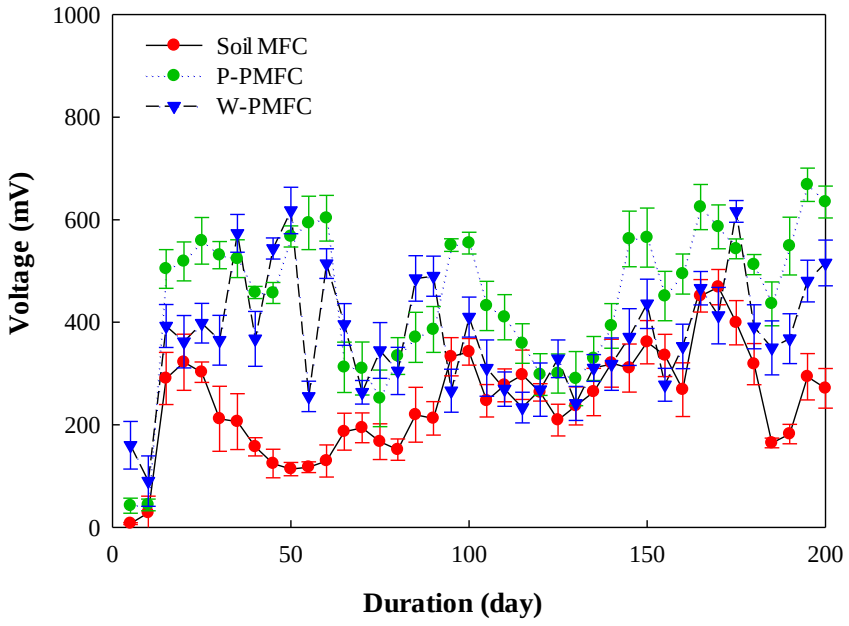
As mentioned earlier, the biochar used in our experiments was made from waste wood biomass, which may contain sufficient amount of lignin to produce free radicals and inhibit rice growth, and further influence the performance of electricity production in PMFCs. Furthermore, the low electricity generation also might be caused by the electrical resistivity of biochar granules and the oxygen intrusion occurred through the porous of the biochar near the cathode (Md Khundzari et al., 2019). According to biochar made from numerous and abundantly feedstock, e.g., forest, agricultural residues or even wastewater sludge (Huggins et al., 2014), the chemical and physical properties of biochar could have different effects on the crops and soil microorganisms when added into the soil. Therefore, our results warrant the need for future studies to identify biochar quality requirements for PMFC application

(a)



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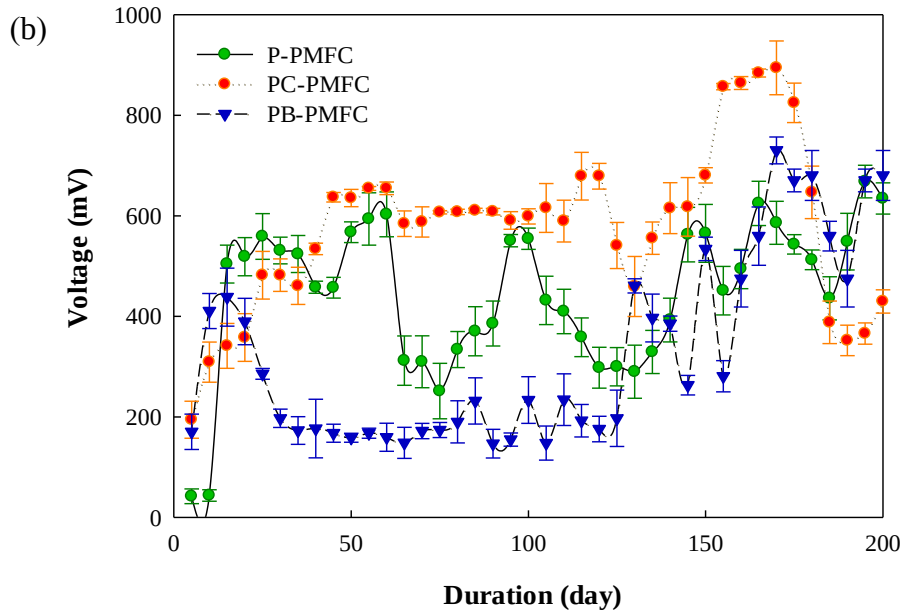

Figure 4-1 Variation of voltage generation versus time. (a) Soil-MFC and PMFCs with different plants; (b) P-PMFCs with or without soil conditioners

4.1.2 The changes of pH and EC in PMFCs

The pH and EC values of the surface soils close to the cathode of soil-MFC and PMFCs varied with time as shown in Fig. 3. After operating for 15 weeks, pH values of soil MFC showed the range of 7.23 ± 0.65 to 8.57 ± 0.57, while pH of the P-PMFC and W-PMFC varied from 6.00 ± 0.69 to 7.89 ± 0.44 and 6.95 ± 0.41 to 8.30 ± 0.12 as shown in Figure 4-2(a), respectively. Comparing pH values of P-PMFC, PC-PMFC and PB-PMFC, it is found that adding compost significantly increased pH values ($P< 0.05$) (Table A1). The pH values of PC-PMFC and PB-PMFC as demonstrated in Figure 4-2(b) were at the range from 7.5 ± 0.11 to 8.3 ± 0.29 and 6.9 ± 0.15 to 7.8 ± 0.34, respectively. Generally, pH values showed increasing trend in the top soil close to the cathode, although a variation of pH was also

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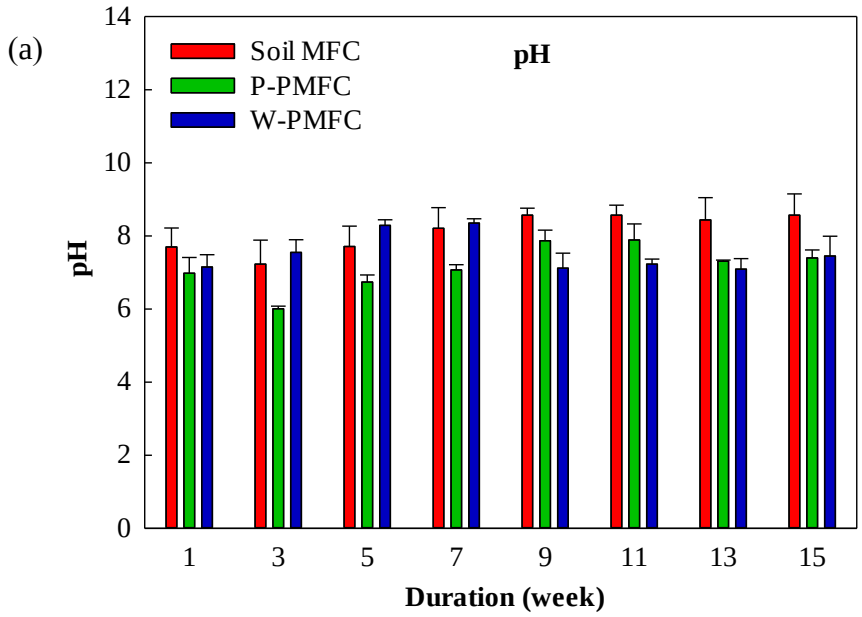
observed during operation, likely due to the heterogeneity of soil samples. Similar findings were also reported in the PMFCs study of remediation of metal contaminated soils, which showed significantly higher pH values of soils close to the cathode than those close to the anode after long-term operation (Guan et al., 2019b). As shown in the previous study, rapid consumption of H^+ in the oxygen reduction reaction in the cathode could cause the increase in pH values in the cathode chamber of MFCs (Wu et al., 2019), and therefore, increasing pH values in the top soil close to the cathode could be due to the redox reaction effects driven by the electrochemical reactions through the relationship among microorganisms, plants, soils, substrates, and electrode systems in the soil MFC and PMFCs.



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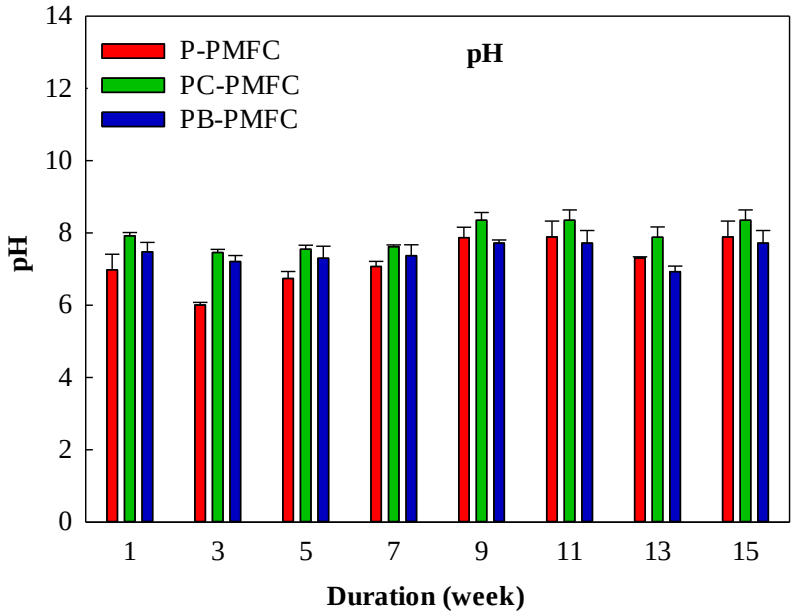
(b)



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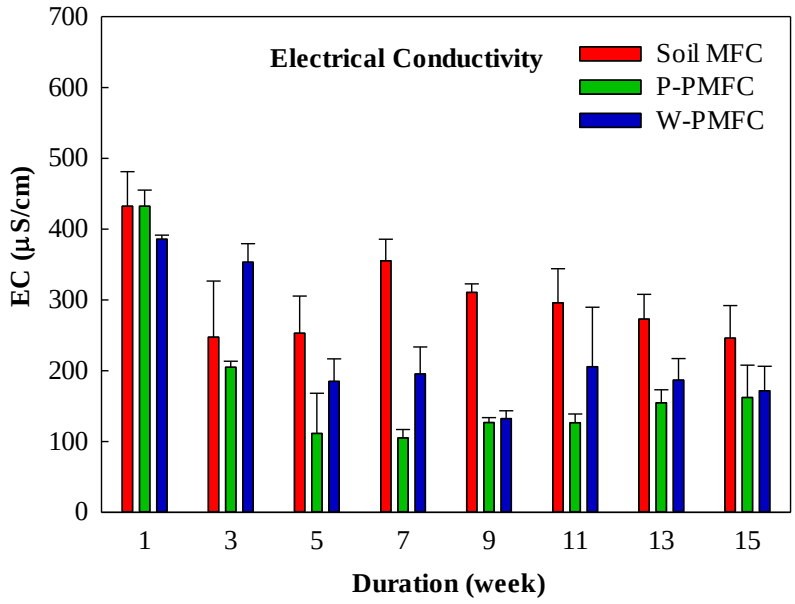

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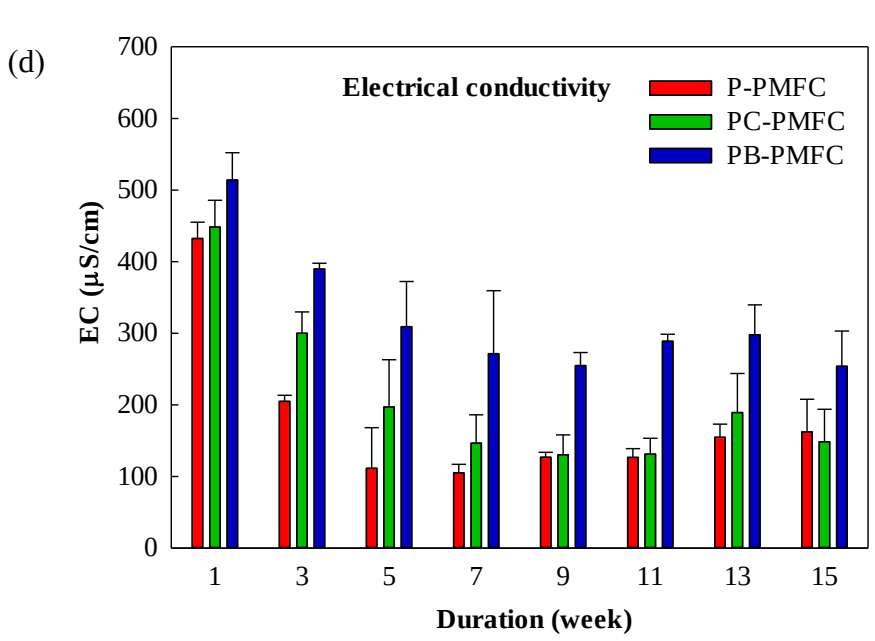

Figure 4-2 Variation of pH and EC of the soil-MFC and PMFCs versus time. (a) pH of soil-MFC and PMFCs with different plants; (b) pH of P-PMFCs with or without soil conditioners; (c) EC of soil-MFC and PMFCs with different plants; (d) EC of P-PMFCs with or without soil conditioners

The weekly EC was monitored at the soil surface near the cathode in the experiment. Generally, EC values of all treatments showed a decreasing trend since the 1st week of operation, which was similar to the previous study (Guan et al., 2019a), as shown in Figure 4-2(c), d. After 15 weeks of operation, the EC of soil-MFC, P-PMFC, and W-PMFC decreased to 246 ± 46.11, 162.30 ± 45.57, and 171.80 ± 34.55 µS/ cm, respectively. The EC of PC-PMFC and PB-PMFC were 115.75 ± 40.77 and 254.01 ± 49.11 µS/cm, respectively. From the results, the EC of soil-MFC was significantly higher than that of P-PMFC and W-PMFC ($P < 0.05$) (Table A1), indicating that the electrokinetics mechanism of PMFCs driven by the bioelectrochemical process (Guan et al., 2019b) and the ion absorption by plant root

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systems could cause the decrease of EC in soils. In addition, compared with W-PMFC, P-PMFC had significantly lower EC (P<0.05). As mentioned earlier, P-PMFC had better performance in electricity production than W-PMFC, and therefore, the stronger electrokinetic effects in P-PMFC might cause the more significant migration of soluble ions. Compared with other treatments, PB-PMFC had the highest EC values (254.01 ± 49.11 to 514.00 ± 38.18 μS/cm) from the beginning till the end of the operation as shown in Figure 4-2(d), although the weekly EC of PB-PMFC decreased during operation. The high EC values should be caused by biochar, whose properties have changed the soil physiochemical properties such as pH, EC, cation exchange capacity, salinity, and redox potential in the PMFC systems (Palansooriya et al., 2019). Even though sometimes the high soil EC could imply more nutrients in the soils, the high EC or saline soils could likely affect the plant growth (Corwin and Lesch, 2005) and consequently affect the bioelectrochemical performance of the anodophilic bacteria with a lower tolerance and cause lower coulombic efficiency and electricity generation of PMFCs (Tremouli et al., 2010; Lefebvre et al., 2011).

4.1.3 The polarization curve of PMFCs

The polarization curve, which presented the voltage as a function of current, was provided as the characteristics of our P-PMFC to compare with the previous study. However, as shown in Figure 4-3, the output voltage of P-PMFC varied during operation and could achieve around 600 mV on the 60th day, which was roughly the highest values achieved by P-PMFC in our study. As shown in Figure 4-3, the polarization curve of P-PMFC started with initial open-circuit voltage (OCV), and then, the voltage was evaluated across several different resistors (10, 39, 68, 100, 320, 510, 820, 912, 1 k, 1.5 k, and 22 kΩ). Starting with

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the OCV of 608.8 mV, this point means no current in the system, and during the polarization, the cell potential decreased stepwise. After measuring with different resistors, the power density reached the peak value with decreasing external resistance. The maximum power density of P-PMFC was 8.66 mW/m^2. At the maximum power density point of polarization, the internal resistance was estimated to be 328.85 Ω according to Eq. (3-3), which indicates the internal resistance will be equal to the external resistance at themaximum power density. Afterward, the power density began to drop with an increasing current density which indicated a typical fuel cell behavior. The polarization trend of P-PMFC showed similarity to the polarization curves reported in previous MFC studies and PMFC studies (Logan et al., 2006; Moqsud et al., 2013, 2015). The previous reed mannagrass (*Glyceria maxima*) PMFCs study reported the internal resistance in the range of 450–600 Ω, and their maximum voltage was at 253 mV (Strik et al., 2008). It indicated that the high internal resistance could affect the electron transfer in the bioelectrochemical systems and could influence the power density of PMFCs. Kaku et al. (2008) estimated that the maximum power density, internal resistance, and OCV via polarization curve of the rice paddy PMFCs were 5.75 mW/m^2, 156 Ω, and 701 mV, respectively. Watanabe et al. (2017) reported paddy PMFC using carbon graphite felt as electrodes achieved power density around 12 mW/m^2. In this study, the polarization curve was generally similar to most of the previous PMFCs studies, with the compatible maximum power density and internal resistance values. However, this study used the simple setup of PMFCs, and the tubular PMFCs with biocathodes have been reported to improve the power generation to 82 mW/m^2 (Wetser et al., 2017). Therefore, the better design of PMFCs can be considered to further increase the electrical performance.

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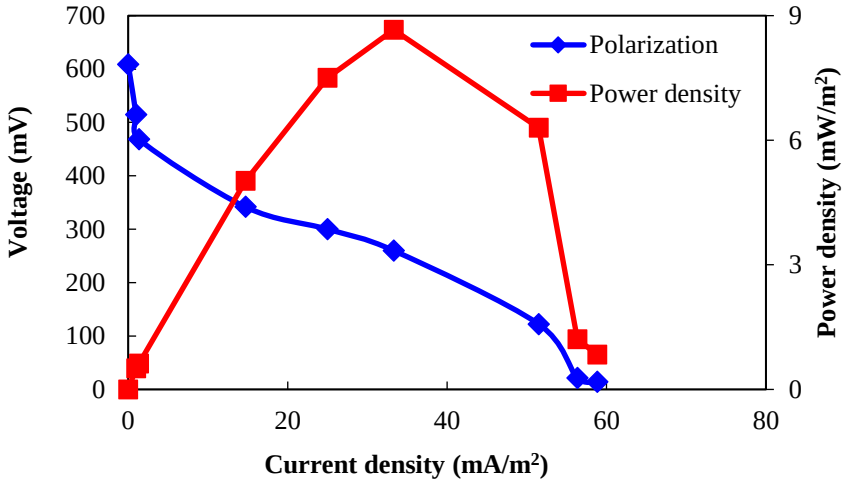

Figure 4-3 Polarization curve of the P-PMFC at the 60th day of the experiment

4.1.4 Microbial community structure

After 200 days of long-term incubation, anode samples of all experimental setups were analyzed for their constituents of the microbial community. High-throughput sequencing of 16S rRNA genes amplified using 16S V3–V4:341F- 805R primers was adopted for the microbial community analysis. Overall, total 406,044 high-quality 16S rRNA gene sequences were obtained and classified into OTUs with 97% of similarity. Species richness and evenness of community distribution indicated by Shannon index and Chao1 are showed in Table 4-1. The results of taxonomic classification demonstrated that Proteobacteria was the most predominant phylum with relative abundance ranging from 20.25 to 34.10% followed by Patescibacteria, Bacteroidetes, Chlorfexi, Verrucomicrobia, *and* Planctomycetes, accounting for 16.39–21.02%, 9.56–15.17%, 5.02–13.24%, 4.40–16.09%, and 3.54–8.13%, respectively (Figure 4-4(a)). The most abundant classes were *Gammaproteobacteria* and *Deltaproteobacteria* which comprised 5.44–14.62% and 5.48–11.54% of the microbial communities as showed in Figure 4-4(b). A previous study showed

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that Proteobacteria (31.7–38.7%), *Chlorfexi* (8.1–8.9%) and Bacteriodetes phyla (1.7–6.9%) were enriched at the anode of *Canna indica* PMFCs, and the results also showed the dominance of *Gammaproteobacteria* at the class level (Lu and Xing, 2015). Furthermore, the other study found that Proteobacteria was the most abundant phylum of the anode rhizosphere bacterial community in *Glyceria maxima* PMFCs (Timmers et al., 2012). In addition, the phyla of Bacteriodetes and Chlorfexi, which were considered as the rhizosphere bacterial groups, were also found enriched on the anodes of the previous study of paddy PMFCs (De Schamphelaire et al., 2010). Generally, our results of microbial communities in the anodes of PMFCs were in agreement with the previous PMFC studies at the phylum and class levels.

Table 4-1 OTUs and species richness and diversity estimated by 97% of similarity.

Sample	Read	OTUs	Chao 1	Shannon
Soil-MFCs	86576	44815	1668	9.27
Paddy-PMFCs	77707	43000	1794	9.14
Paddy-PMFCs with compost	76189	43775	1838	9.17
Paddy-PMFCs with biochar	91281	52607	1694	9.11
Water bamboo-PMFCs	74291	52607	2100	9.52

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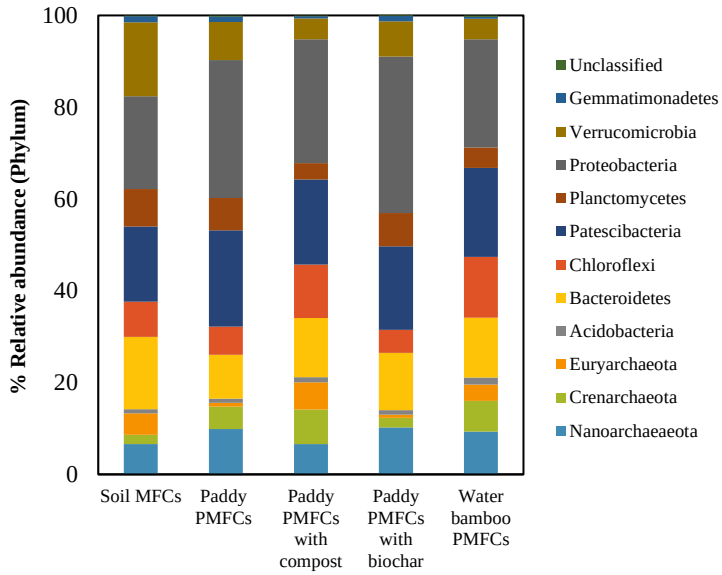
(a)



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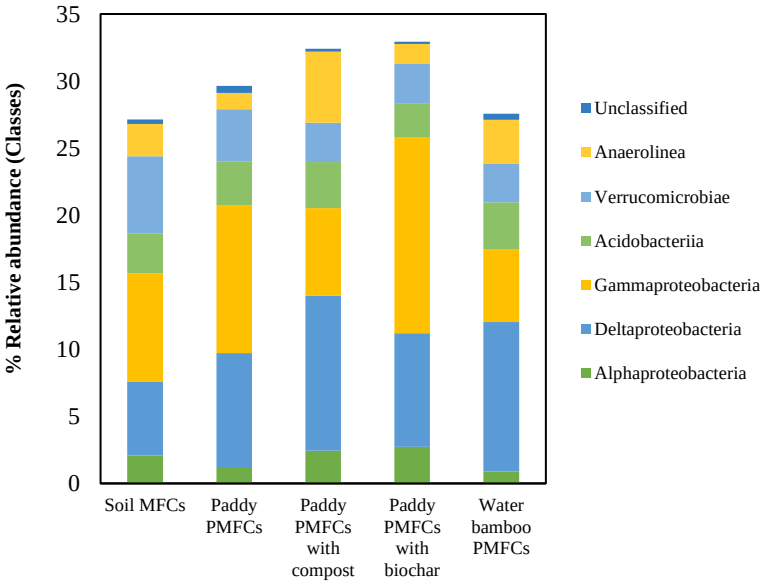

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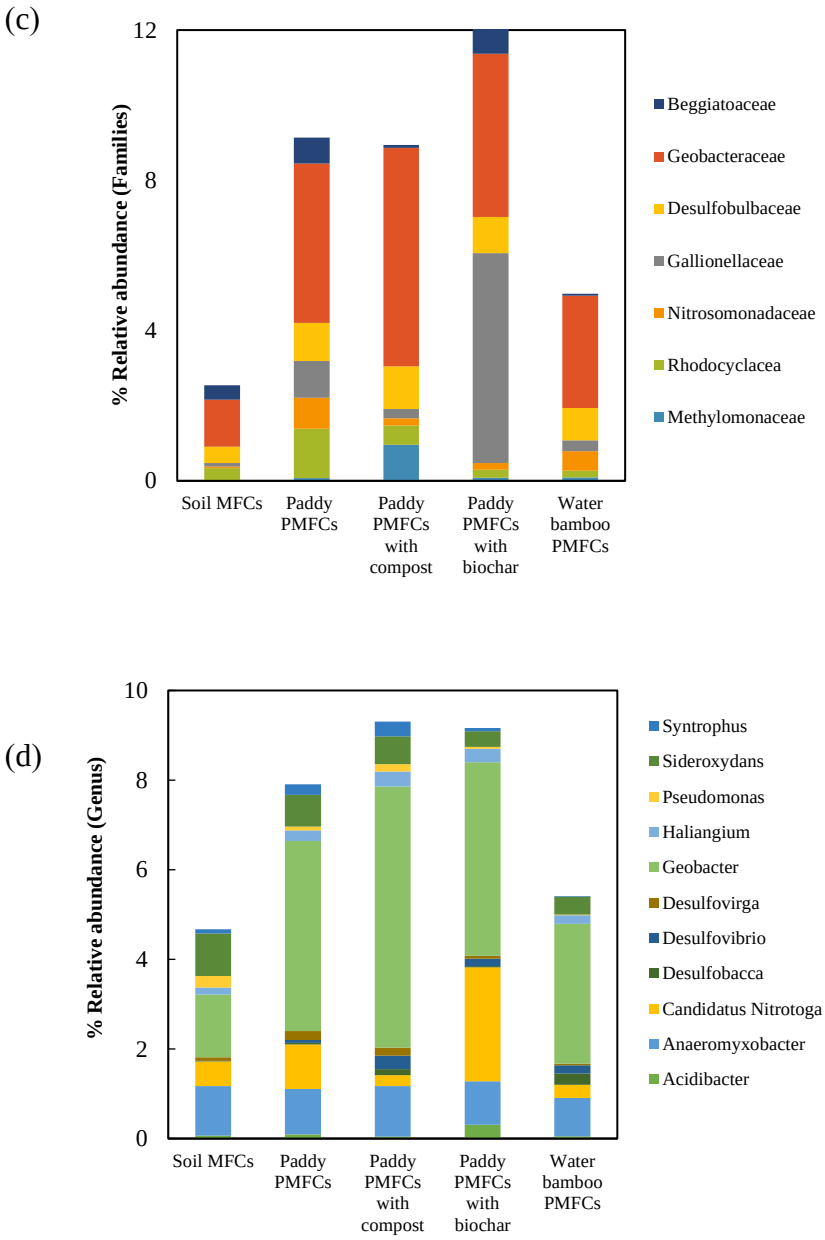

Figure 4-4 Microbial communities at the anode after 120 days of operation. (a) Relative abundance of phyla of the soil-MFC and multiple PMFCs; (b) relative abundance of classes of the soil-MFC and multiple PMFCs; (c) relative abundance of families of the soil-MFC and multiple PMFCs; (d) relative abundance of genera of the soil-MFC and multiple PMFCs

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Geobacteraceae, which consisted of the commonly known EAB genus *Geobacter*, was the most abundant family of the whole microbial communities and was found more abundant in PMFCs (2.99–5.82% of the total microbial community) than the soil-MFC (1.3%) as showed in Figure 4-4(c). In addition, the family *Desulfobulbaceae*, containing the sulfate-reducing bacteria, was also enriched at the anode. *Desulfobulbaceae* is known to contain filamentous bacteria, cable bacteria, and mesophilic sulfate-reducing bacteria, which can use sulfate, thiosulfate and sulfate and nitrate as electron acceptors (Kuzyakov et al., 2005). *Desulfobulbaceae* was also found more abundant in PMFCs (0.85–1.13%) than soil-MFC (0.43%). *Geobacter* was the most dominant genus which accounted for 6% of the whole microbial communities followed by *Anaeromyoxobacter*, *Candidatus* Nitroga, and *Sideroxydans* as shown in Figure 4-4(d). Since *Geobacter* is the well-characterized EAB with high electrical production capacity (Bond and Lovely, 2003), the result indicated that *Geobacter* should be involved in electricity generation in PMFC systems in this study. *Anaeromyoxobacter* has been reported to use acetate, lactate, and pyruvate as the electron donor (Hwang et al., 2015). Since the root exudation or rhizodeposition of PMFCs could provide acetate or other organic compounds as electron donors for microorganisms, whether the functions of microbial population found in this study were related to current generation or related to the carbon, nitrogen, sulfur, and iron cycling in paddy soil (Lu et al., 2006) needs further validation. Competition for electron donors among the different microorganisms could result in the decrease in electron donors available for EAB and thus lower current generation (Timmers et al., 2012).

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The microbial community structure was analyzed by using UniFrac analysis coupled with PCoA and UPGMA hierarchical clustering to compare the linkages of microbial community among different samples of soil-MFC and multiple PMFCs based on beta diversity and their phylogenetic assignments as shown in Figure 4-5. Figure 4-5(a) showed the comparison of microbial communities among five samples by PCoA, and the dendrogram cluster analysis of the microbial communities (at the family level) of the anode is showed in Figure 4-5(b). From the results, PCoA and cluster analysis of microbial community structure roughly showed three different groups, i.e., (1) soil-MFC and W-PMFC, (2) P-PMFC and PC-PMFC, and (3) PB-PMFC (Figure 4-5). Thus, the results suggest that microbial communities could be influenced by the soil fertilizers and conditioners, the plant root systems, and root exudates. However, the PB-PMFC had the most distinct anode microbial community from other samples (Figure 4-5). From the microbial community analysis in PB-PMFC, we found *Gallionellaceae* as the predominant family, which is considered to be involved in the iron cycling (Hallbeck and Pedersen, 2014), but *Geobacteraceae* was the most dominant family in other PMFCs (Figure 4-4(c)). As mentioned above, the electricity generation of PB-PMFC.

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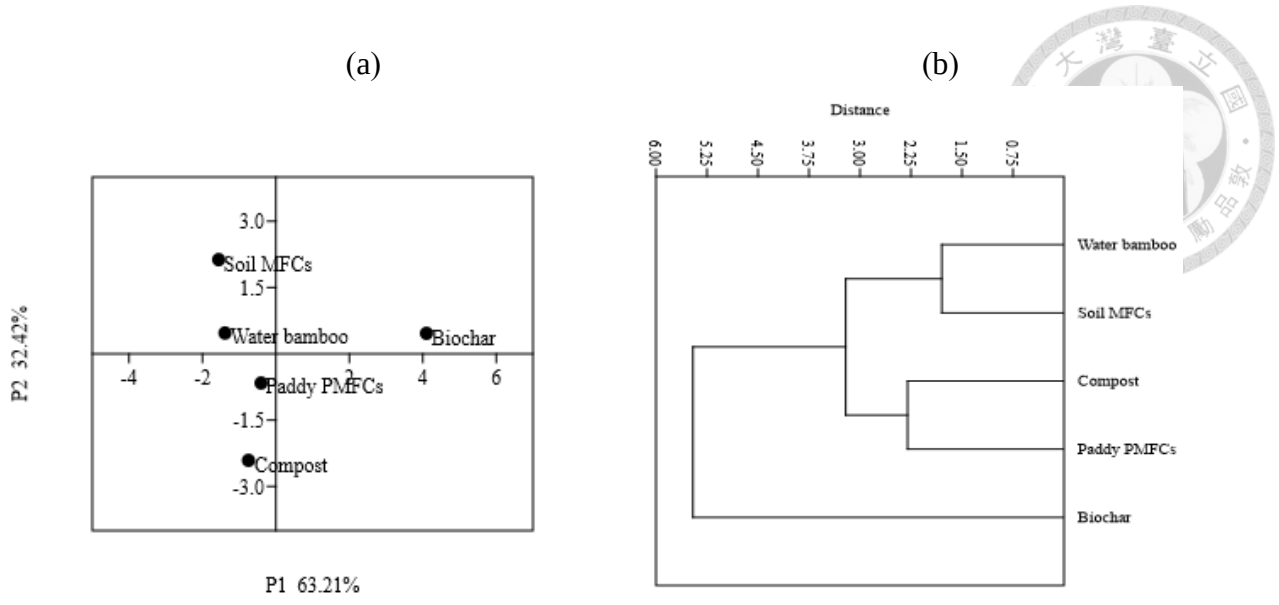

Figure 4-5 Analysis of microbial community structures of the soil-MFC and multiple PMFCs. (a) PCoA of microbial community structures in the soil-MFC and different PMFCs; (b) Cluster analysis of the microbial community structures in the soil-MFC and different PMFCs.

4.1.5 Assessment of plant species health

Paddy (*Oryza sativa*) and water bamboo (*Zizania latifolia*) performed well and produced rice seeds during the long-term in the incubator. The plant height of the experiment was shown in Figure 4-6. In this study, we measured the height of all PMFCs after cultivation and set them up in the light incubator for a week. The highest height was the water bamboo PMFC (89.3 ± 28 cm) followed by PC-PMFC (89.1 ± 23.7 cm), P-PMFC (81.9 ± 21.2 cm) and PC-PMFC (73.9 ± 17.2 cm), respectively (Table 4-2). All plants were healthy at the vegetative and reproductive stages. The paddy turned yellow after the ripening stage (week of 11-14). However, they still survived and generated electricity. This indicated

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that the microorganisms around the rhizosphere might oxidize the decay plants and supply carbon sources and nutrients for EAB until the end of the experiment. The roots of the paddy grew well and penetrated the anode. On the other hand, the water bamboo roots did not observe that passed through the carbon fiber. At the end of the experiment, the water bamboo's health was not good and they consumed lots of water, which caused the sharp fluctuation and negative voltage from the dried cathode during the experiment. Moreover, we observed that PC-PMFC grew sharply in the vegetative stage. The results suggested the LEDs light drives the photosynthesis of plants. Paddy can survive in the growth chamber longer than water bamboo. Furthermore, it can be upscaled and integrated into other applications such as soil remediation, biosensors, or even in-situ agricultural land.

Table 4-2 *Oryza sativa* (Paddy) and *Zizania latifolia* (Water bamboo) height.

Sample	Initial height (cm)	Final height (cm)	Average height (cm)
Paddy-PMFCs	42.7 ± 1.5	105.0 ± 9.5	81.9 ± 21.2
Paddy-PMFCs with compost	42.3 ± 2.1	120.0 ± 2.3	89.1 ± 23.7
Paddy-PMFCs with biochar	40.0 ± 6.1	86.0 ± 2.6	73.9 ± 17.2
Water bamboo-PMFCs	5.0 ± 1.0	124.0 ±10.3	89.3 ± 2.8

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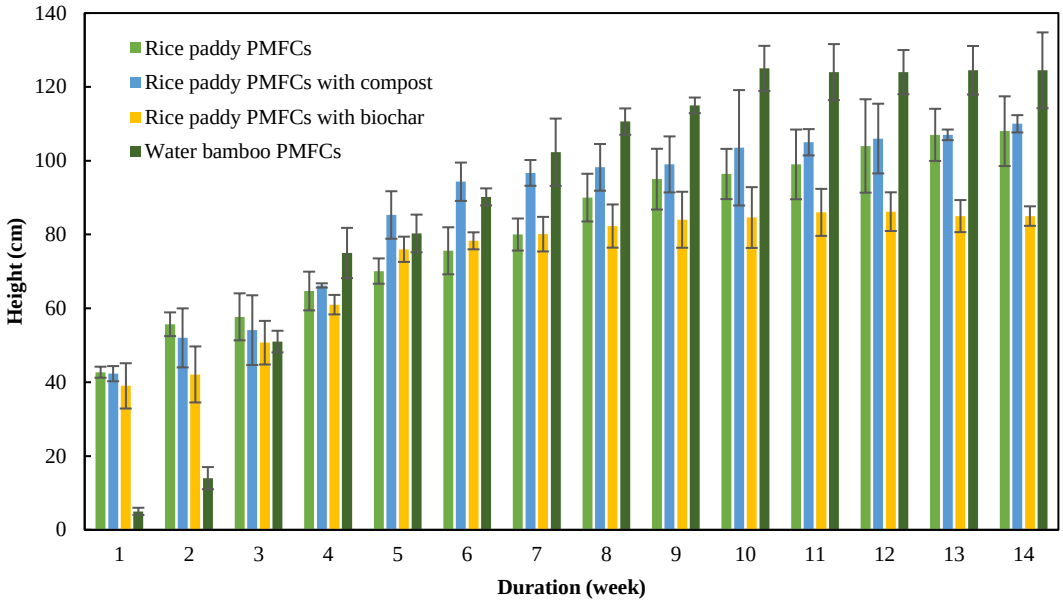

Figure 4-6 The height of paddy and water bamboo PMFCs

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4.2 Improvement of PMFCs with *Oryza sativa* performance and greenhouse gas emission

4.2.1 Influence of conventionally fertilizer on electricity generation of *Oryza sativa* PMFCs

The voltage generation during 150 days of the experiment is shown in Figure 4-7. All experiments were conducted in the growth chamber with 12/12 h of the LED light and dark cycle, 27ºC and 75% of the humidity. At the beginning of the experiment, all soil MFC and PMFCs were operated in an open-circuit for 2 weeks. Later, the closed circuit of all soil MFC and PMFCs was monitored throughout the experiment. The average votage output of soil MFC, P-PMFC and F-PMFC was 252.44 ± 55.20, 320.93 ± 58.74 and 429.84 ± 141.60 mV, respectively. The maximum voltage output of the soil MFC, PMFC, and F-PMFC was 360.06 ± 50.10 mV, 499.26 ± 60.27 mV, and 775.96 ± 230.21 mV, respectively. However, the zero-voltage output were observed during the experiment. It probably caused by the computer connecting the data logger shut down during the operation. The stable output voltages were attributed the acclimation of EAB to carbon sources or orgarnic substrates in the soil. Next, the output voltages gradually increased in PMFCs. It was clear that PMFCs had significant higher power output (P<0.05). This is because PMFCs can access to more carbon sources from the rhizodeposition, and it is probably related to additional carbon sources from root exudates serving anode bacteria (Guan et al., 2019a). On the 60th, 70th, 90th, and 110th days of the paddy plant's productive stage, chemical fertilizers were applied to paddy PMFC (F-PMFC). The voltage output of F-PMFC sharply increased and reached the maximum voltage and then the voltage output gradually decreased (Figure 4-7). Similarly, the previous study reported the voltage output of paddy PMFC adding fertilizer increased

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fast and reached to 0.5 V. Afterwards, the voltage output dropped quickly (Omine et al., 2018). This could be influenced by chemical fertilizer which work quickly but not have a long-lasting effect. The COD concentration in pore water was shown in Table 4-3. Overall, the COD in pore liquid was high during the first of the measurement and gradually decreased to the end. The high COD concentration would be influenced by the chemical fertilizer that added to the rhizodeposition. Subsequently, it was immediately oxidized by EAB as can be noticed by the almost instantanouse increase the current as reported in previous study (Arends et al., 2014). Futhermore, the previous study has reported that the methane emission was significantly reduced when the electricity production was increased in rice PMFC adding chemical fertilizer (Jung and Regan, 2011). It was noteworthy that the increase of EAB at the anode of bioelectrochemical systems reduced methane emission in PF-MFC application (Da Rosa et al, 2011; Kamaraj et al., 2020).

Table 4-3 The concentration of COD (mg/L) in pore water of the day 63th, 73th and 93th

Day	Soi MFC COD (mg/L)	P-PMFC COD (mg/L)	F-PMFC COD (mg/L)
63th	53.0 ± 12.6	107.5 ± 15.1	94.7 ± 9.6
73th	68.0 ± 7.1	80.8 ± 3.7	123.2 ± 11.3
93th	21.3 ± 6.1	16.0 ± 2.6	26.9 ± 4.2

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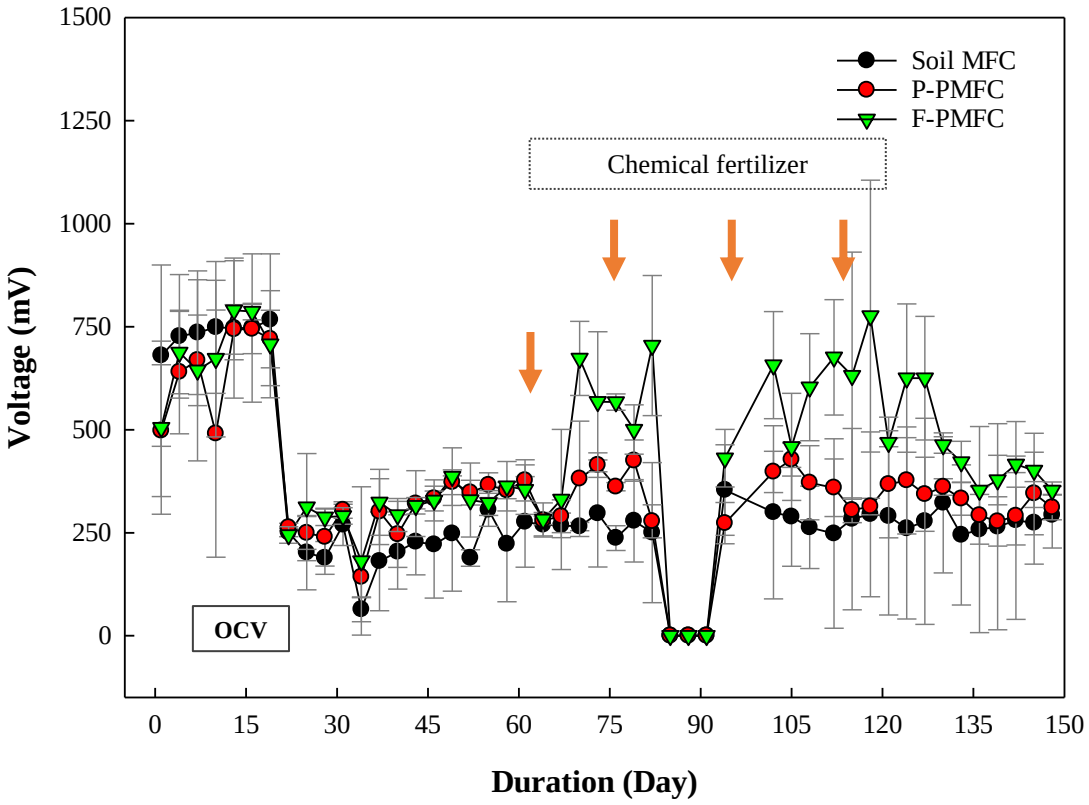

Figure 4-7 The voltage generation of paddy PMFCs and soil MFC against the time

(1) pH and EC changes

The variation of the homogenized soil pH and stratified soil pH from the top to the bottom of PMFCs were measured and are presented in Figure 4-8 (a) and (b). The range of homogenized soil pH of all treatments were 6.86 ± 0.25 to 7.65 ± 0.17 (soil MFC), 6.56 ± 0.50 to 7.48 ± 0.06 (P-PMFC) and 6.25 ± 0.10 to 7.12 ± 0.21, respectively. The initial pH of all treatments was slightly acidic and increased to neutral at the end of the experiment. It indicates that the bioelectrochemical process leads to increase pH values. The soil pH values of P-PMFC and F-PMFC were likely to be lower than soil MFC. This result probably was due to more H^+ released from the anodic bacteria, the rhizodeposition, and less alkaline

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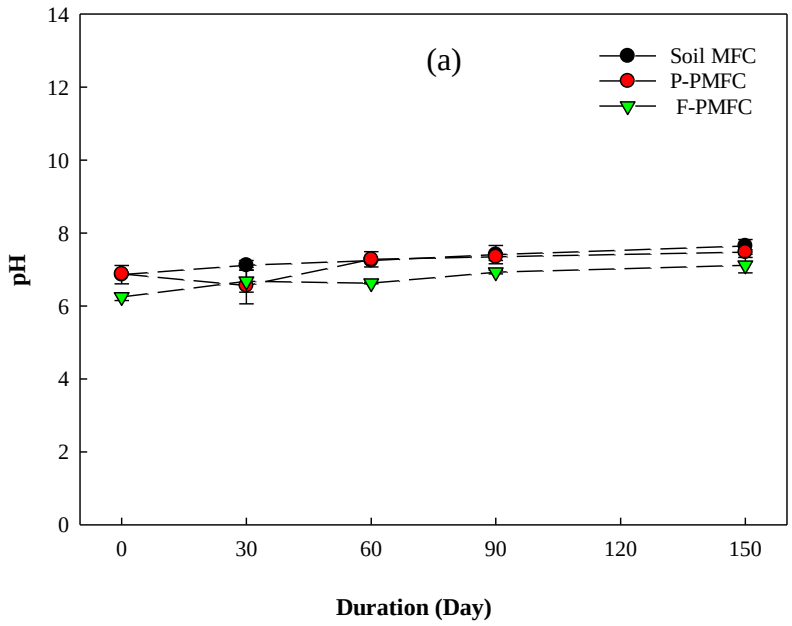
substances being exuded from the plant microbial fuel cell (Han et al., 2021). Besides, the stratified soil pH was monitored at the end of the experiment. The soil pH was at the range of 6.90 ± 0.06 to 7.65 ± 0.17 ((soil MFC), 6.18 ± 0.02 to 7.48 ± 0.06 (P-PMFC) and 5.70 ± 0.20 to 7.12 ± 0.21 (F-PMFC), respectively. The result revealed that pH values at the bottom was the most acidic followed by those the anode and cathode, respectively. It could be associated with the bioelectrochemical systems driving the electrokinetic mechanisms, which would enhance the stratified soil pH values. Additionly, the rapid consumption of H^+ of the redox reaction in the cathode may cuase the increasing of pH values at the cathode (We et al., 2019; Mena et al., 2012). Nonetheless, these pH values were all near neutral which is the most optimal pH condition for the microorganisms (He et al., 2008).



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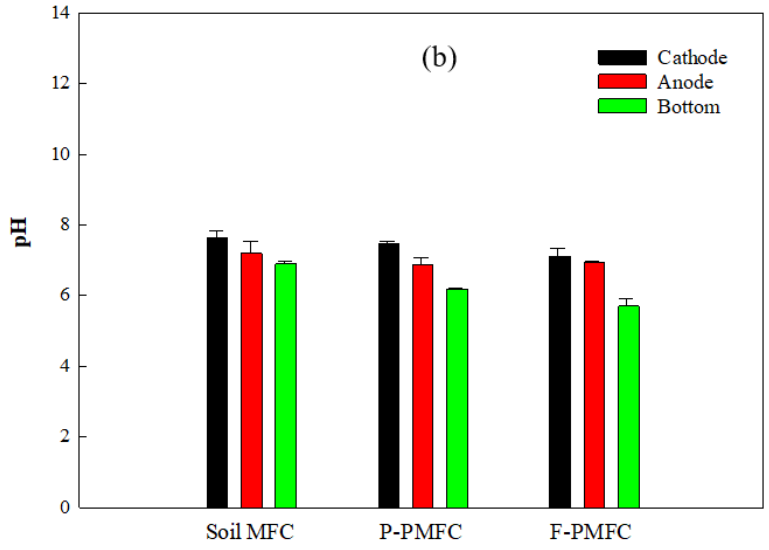

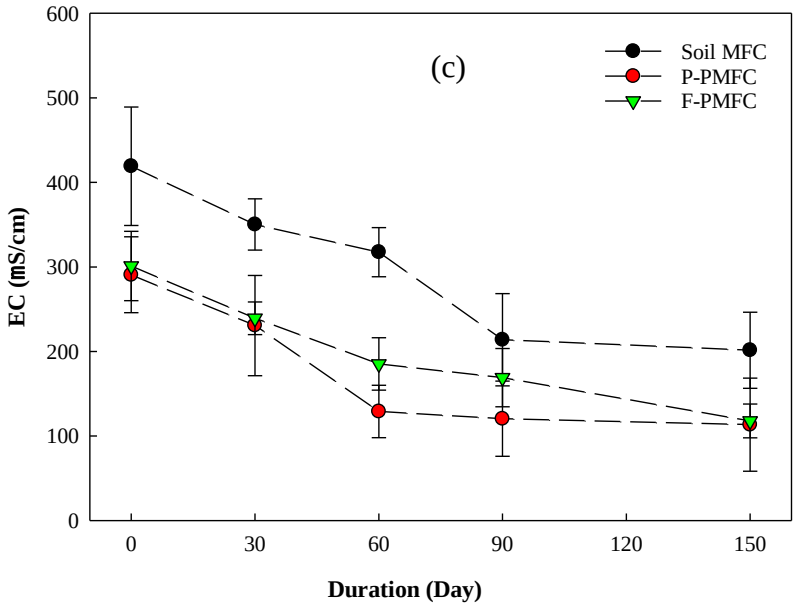

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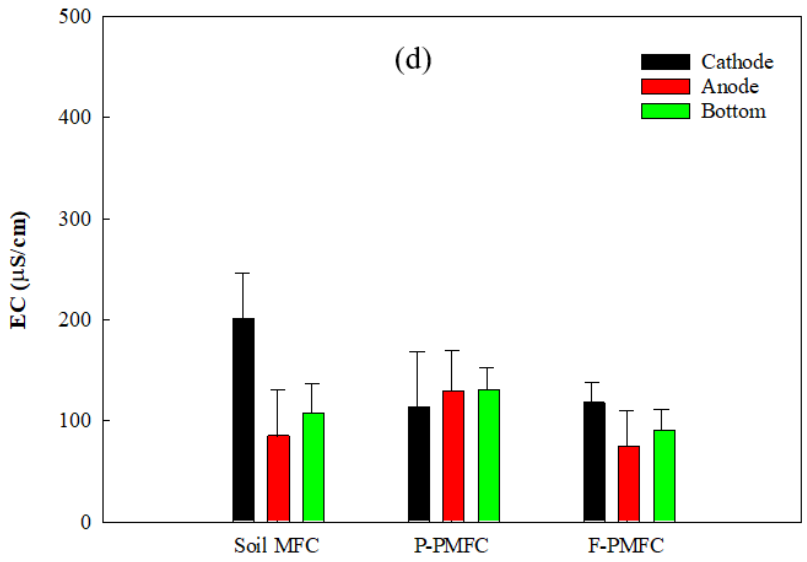

Figure 4-8 pH and EC of PMFCs (a) pH variation; (b) The stratified soil pH of all treatment; (c) EC variation; (d) The stratified soil EC of all treatment

Figure 4-8 (c) and (d) showed the homogenized soil EC and stratified soil EC from the top (cathode region), middle (anode region), The EC of all treatments dramatically decreased from the initial EC of 419.17 ± 70.0 to 201.40 ± 45.0 (Soil MFC), 290.77 ± 45.0 to 113.40 ± 55.0 (P-PMFC) and 301.11 ± 41.0 to 117.80 ± 20 (F-PMFC), respectively. By comparison, the EC of soil MFC was significantly higher (P<0.05) than all PMFCs. It is assumed that plant roots couple with bioelectrochemical process of PMFCs can promote the absorption the ions in the soil, similar to the previous studies (Guan et al., 2019a). The stratified soil EC of all treatments was measured by collecting the top, the middle and the bottom soil at the end of the experiment. The result showed the variation of EC values within the depth of soil. From the result, only clearly stratified EC soil was investigated in F-PMFC which showed it gradually dropped from the anode to the cathode. It might be assumed that the redox reaction occurring in PMFC systems seems to be influenced by EC in the soil.

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However, the ion migration in the soil is influenced by a variety of factors, including the soil properties, soil pH, soil addition, etc. (Li et al., 2018).

(2) The polarization tests

Polarization analyses were conducted to compatatively the PMFCs with a chemical fertilizer addition and their control. The polarization test performed on the soil MFC and PMFCs during the vegetative stage of the plants (day 70th) as shown in Figure 4-9. Starting with the initial OCV of each treatment and then the volatage was measured across several various of resistors (20, 51, 120, 390, 510, 680, 820, 1k, 1.2k, 1.8k and 5.6kΩ). The highest maximum power density (P_{max}) of polarization test was F-PMFC (7.90 mW/m^2) followed by P-PMFC (4.68 mW/m^2) and soil MFC (4.41mW/m^2), respectively. The result showed the range of 50-510 Ω and their maximum voltage was at 72.40 mV (soil MFC), 111.00 mV (P-PMFC) and 163.7 mV (F-PMFC), respectively. The polarization curves of this study were generally similar to the previous PMFC studies that reported the maximum power density at 5.75 mW/m^2 and the internal resistance was 156 Ω via polarization curve of the rice paddy PMFCs (Kaku et al., 2008). It indicated that the bioelectrochemical process of EAB at the anode region probably influenced the power production of soil MFCs and PMFCs. Additionally, the relationship between the low internal resistances in the PMFC and the high-power output was likely affected by the number of EAB communities within the anode chamber (Ueno and Kitajima, 2012). However, a comprehensive analysis of EAB in PMFC systems including electrodes and substrate serving as an electrochemically active bacteria's energy source would be required for improved power generation.

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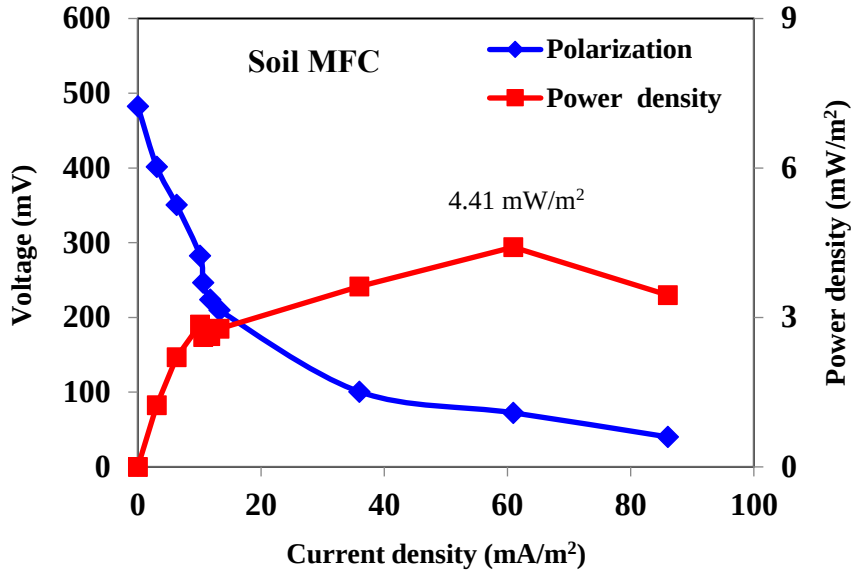
(a)



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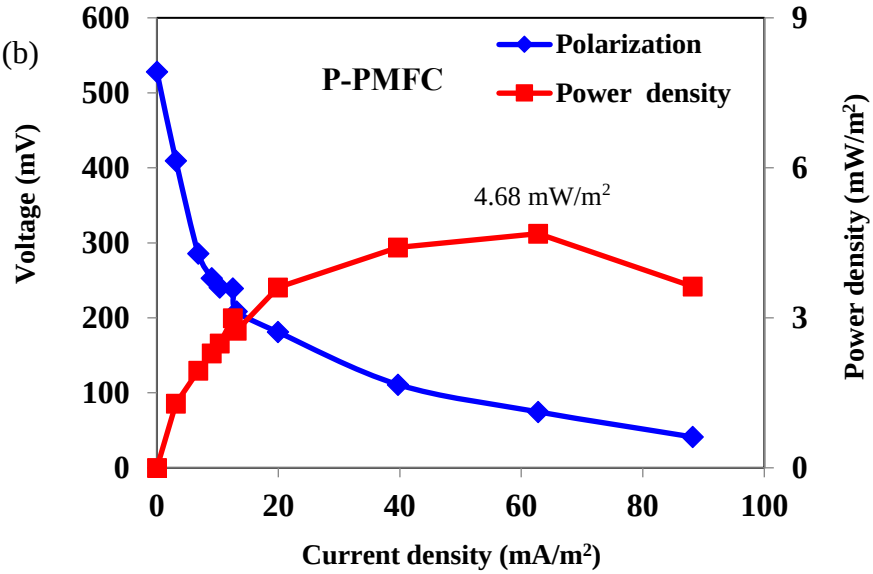

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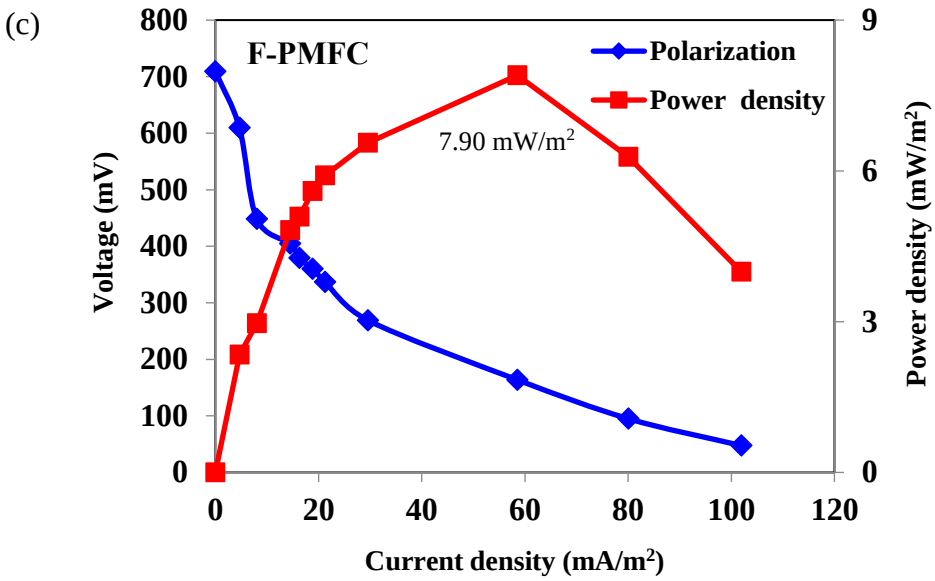

Figure 4-9 Polarization curves at the 70th day of the experiment

(3) Microbial community in PMFCs

At the end of the experiment, the anode samples of all samples were collected and analyzed for the microbial community. Three bacterial 16S rRNA gene sequencing libraries were constructed with around 234,373 high-quality reads. Each community obtained 33,866–35,298 operational taxonomic units (OTUs) with 97% similarity. The richness and diversity of the microbial community are shown in Table 4-4. The Shannon index, which represented the diversity of the microbial community including the number and everness of species, showed 6.72 (soil MFC), 7.29 (F-PMFC) and 7.45 (P-PMFC), respectively. The Chao1 and Shannon indexes indicated that PMFCs had higher species diversity than soil PMFCs. Similarity, the previous studies reported that the diversity of the anodic species of plant microbial desalination cell (PMDC) was higher than soil microbial desalination cell (SMDC) (Han et al., 2021). The high Shannon indexes of this

doi:10.6342/NTU202203744

study were distinguished as that of hydrocarbon contaminated soil (6.99) as reported previously. (Lu et al., 2014). This indicated that the species richness and diversity were attributed to the bioelectrochemical process of anodic-electrogenic bacteria in PMFC systems. The nutrients, the substrate addition, and the complex rhizodeposits would influence the biodiversity in the anode region as the use of activated sludge as fuel for anodic bacteria in the previous study (Lu et al., 2012).

Table 4-4 Species richness and diversity with 97% of similarity of soil MFC, paddy PMFC and paddy PMFC with fertilizer

Sample	OTUs	Chao 1	Shannon
Soil-MFC	33,866	421	6.7228
P-PMFC	34,171	486	7.4542
F-PMFC	35,298	470	7.2909

The taxonomic classification (OTUs) showed that Proteobacteria was the dominant phylum with a percent relative abundance of 27.94 to 36.20%, followed by Acidobacteria (14.20 to 15.42%), Bacterodites (9.37 to 16.92%), Chloroflexi (7.38 to 9.62%), Firmicutes (3.41 to 8.12%), Planctomycetes (3.91 to 6.33%), Nitrospirae (0.93 to 6.05%), Verrucomicrobia (2.52 to 3.11%), and Actinobacteria (0.98 to 2.08%), respectively (Figure 4-10). *Deltaproteobacteria*, *Gammaproteobacteria*, *Alphaproteobacteria*, *Acidobacteria*, and *Bacteroidia* were discovered to be the dominant classes of paddy PMFCs, which is similar to the previous study that evaluated the anodic bacteria of MFC systems (De Schamphelaire et al., 2010). However, the microbial community structure varied between the power production of MFCs. The previous study reported that the high-performance paddy MFC was dominated by *Deltaproteobacteria* at the anode while *Betaproteobacteria* was

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established as a dominant group in the low electricity production of paddy MFC (Wang et al., 2015). *Geobacter*, highly electrogenic bacteria, dominated in both P-PMFC (5.38%) and F-PMFC (6.60%) at the genus level, respectively, (Figure 4-10). As shown in Figure 4-10(c), the percentage relative abundances of the top 8 species at the family level were processed and showed the difference in abundance of the families. The microorganism genus was distributed widely among soil MFC, P-PMFC, and F-PMFC. *Geobacteraceae* were scattered and higher in F-PMFC and P-PMFC than soil MFC, as well as *Desulfovibrionaceae* and *Chitinophagaceae*. On the other hand, *Methylomonaceae* were observed to be the most abundant family in soil MFC. It indicated that the soil properties involving carbon sources, including organic matter, soil TC, and DOC, might influence the electrogenic bacteria and microbial structure, as previous studies have reported that *Geobacter* was related to the high DOC concentration in paddy MFC (Wang et al., 2015). On the other hand, the *Methylobactor* genus, the methanotrophs, which are the methane-oxidizing bacteria, was enriched at the anode of soil MFC. It might indicate that the bioelectrochemical process not only influenced the dynamics of the microbial community but it also related to methane emission of the systems. However, methane-oxidizing bacteria in microbial fuel cell systems need more in-depth study.

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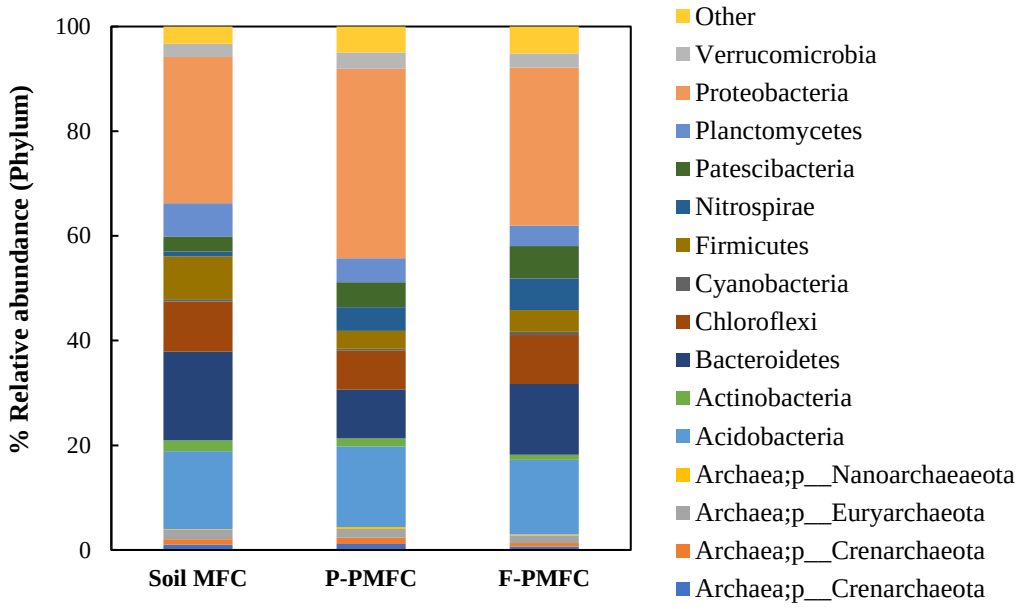
(a)



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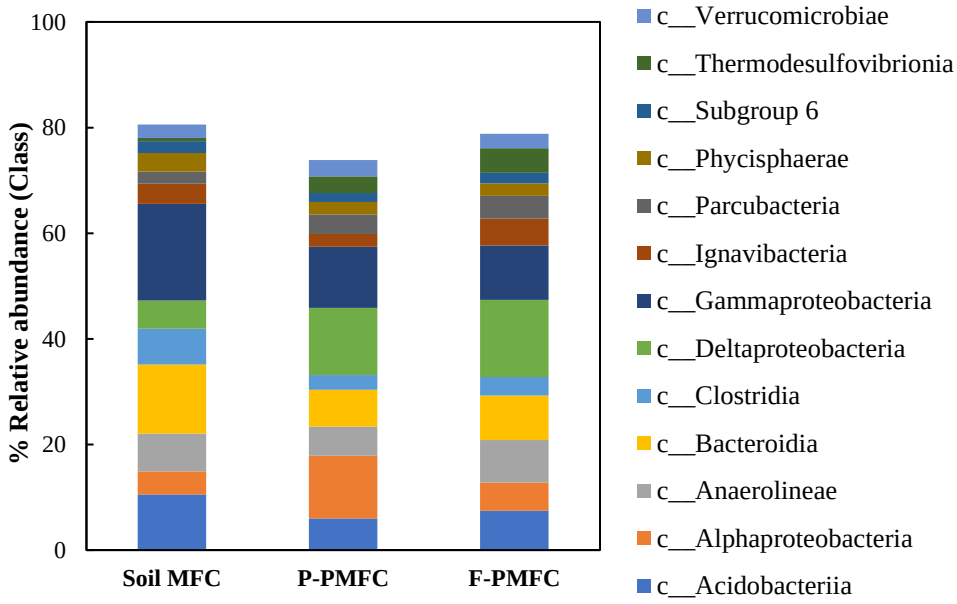

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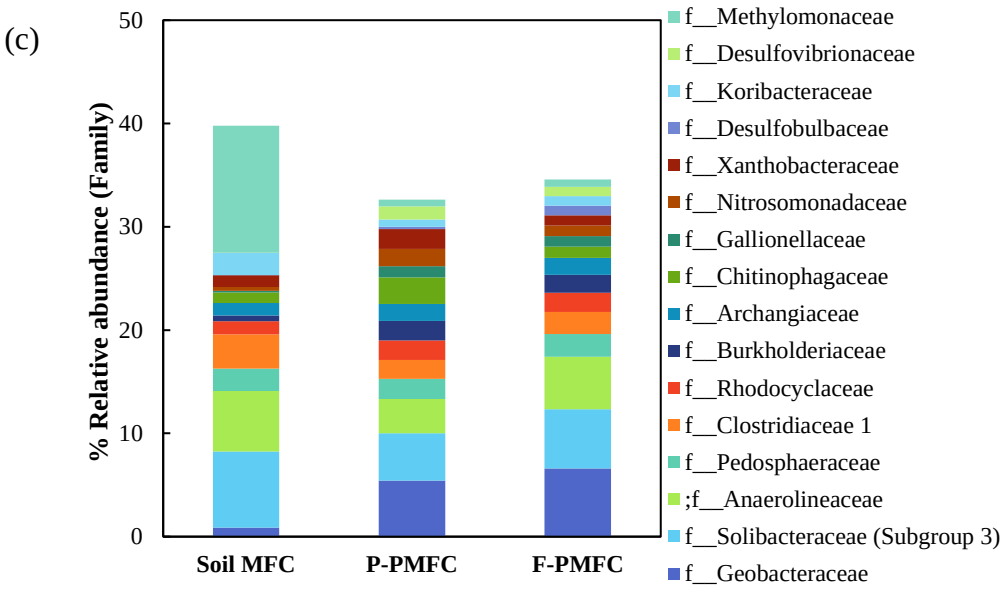

Figure 4-10 Microbial communities at the anode after 150 days of the operation. (a) Relative abundance of phyla of multiple treatments; (b) Relative abundance of classes of multiple treatments; (c) Relative abundance of family of multiple treatments

4.2.2 Performance of soil MFCs and *Oryza sativa* PMFCs with different connections

Three buckets of paddy (*Oryza sativa*) PMFCs were connected in parallel and series with 1 kΩ resistor. By comparison, three soil MFCs were also set up as the control of the experiment (n = 3). The voltage outputs through 130 days of operation in the greenhouse is shown in Figure 4-11. After two weeks, the closed circuit of all treatments was observed to generate electricity. Serial soil MFCs, serial paddy PMFCs, parallel soil MFCs, and parallel paddy PMFCs showed a variety of voltage output. Paddy PMFC connected in series (PMFC-series) showed the maximum voltage at 1,131.06 ± 34.27 mV followed by soil MFC-series (598.20 ± 93.34 mV), PMFC-parallel (834.12 ± 26.60 mV) and soil MFC-parallel

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(494.34 ± 83.64 mV), respectively. At the beginning of the experiment, the voltage outputs for all these MFCs gradually increased and subsequently decreased at the end of the experiment. It is most likely related to EAB oxidation to organic or carbon sources in the soil. As shown in Figure 4-11, the substantial voltage fluctuation might be related to the proper growth of biofilm on the electrodes and the acclimation of the exoelectrogenic bacteria. On the other hand, phytoplankton was found in both soil MFC, most likely as a result of voltage output fluctuation caused by oxygen depletion in water at the cathode region. Another reason relative the low outputs during the ripening stage were considered to be due to sudden of cold weather and the mouse in the greenhouse. In the end, PMFCs of both connections showed slightly steady voltage outputs. This is associated with the rhizodeposits from plant roots serving the EAB in paddy PMFCs.

P-PMFC connected in series (PMFC-series) showed the highest average voltage of 474.97 ± 164.84 mV, followed by P-PMFC-parallel (396.89 ± 149.91 mV), soil MFC-series (283.03 ± 128.79 mV) and soil MFC-parallel (235.50 ± 102.69 mV), respectively. By comparison, the voltage output of PMFCs is not only significantly higher than soil MFCs, but the series connection also has a higher power output than the parallel connection ($P<0.05$). The previous rice PMFC study also reported the average voltages of PMFCs in series (1.19 V) higher than that parallel connection (0.33 V) (Omine et al., 2018). Several studies have focused on the number of electrodes and the different configurations to improve the power production of microbial bioelectricity in MFC by connecting them into stacks but that is not well studied in PMFC (Ren et al., 2012; Jung and Pandit, 2019). Serial and parallel connection of multi-electrodes *Caltha palustris* with PMFCs generated 10.1 times more electricity than mono-electrode plant microbial fuel cells, and serial connection

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with three multi-electrodes increased voltage generation by 2.9 times (Rusyn et al., 2020). Similarly, PMFCs in series connection with *A. africanus* demonstrated a 1.9-fold increase in electric potential (Gómora-Hernández et al., 2020). The proper quality of serial and parallel connected plant microbial fuel cells can be generated by the high current. Nevertheless, the factors influencing the electricity generation of PMFCs include plant species, electrode materials, soil substances, and different configuration needs for in-depth research for the future up scale or in situ application.

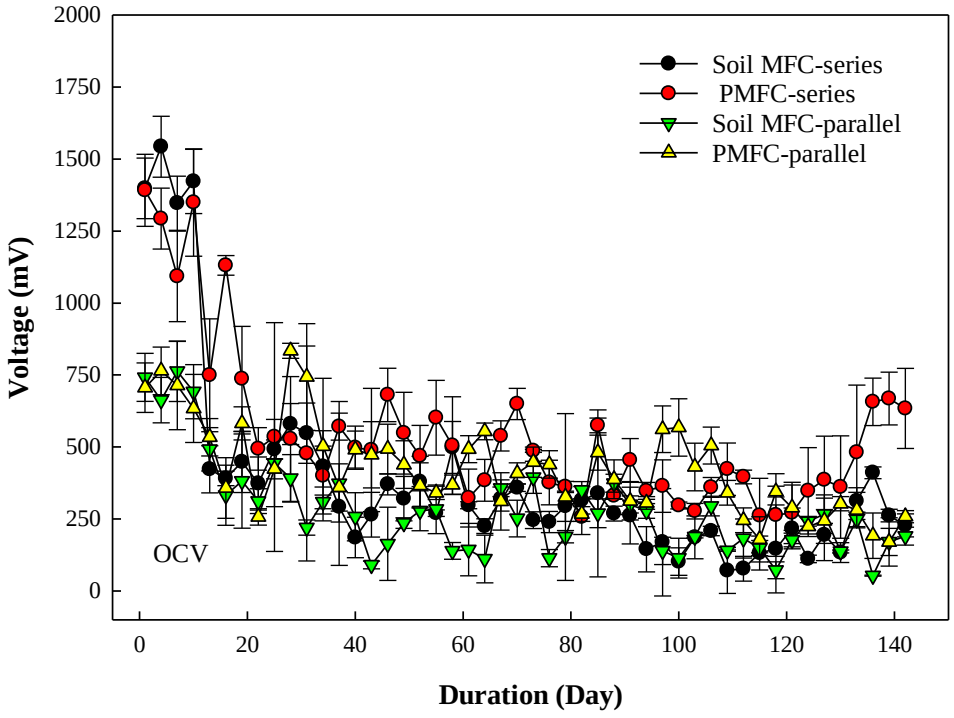

Figure 4-11 Voltage generation of PMFCs against time

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4.2.3 GHG flux in bioelectrochemical systems with different connections

For gas emission, methane and nitrous oxide production of soil MFCs and PMFCs were collected from 9.00 to 10.00 a.m. every Wednesday for one month during the rice vegetative stage (day 60-90). Each bucket had a removable head space chamber equipped with an electric fan for mixing the gases through the sampling. As shown in Figure 4-12 (a), the average CH_4 emission flux of PMFC-series was 3.82 ± 0.92 mg m^{-2} h^{-1} followed by soil MFC-parallel (3.25 ± 1.39 mg m^{-2} h^{-1}), PMFC-parallel (3.20 ± 1.03 mg m^{-2} h^{-1}) and soil MFC-series (3.04 ± 1.41 mg m^{-2} h^{-1}), respectively. Overall, the trend of CH_4 emission flux gradually decreased towards the end of the fifth week. From the study, there had not clearly different in methane emission between soil MFCs and paddy PMFCs as well as between serial and parallel connection. However, for the entire measurement, the methane flux from paddy PMFC was slightly higher than that of soil MFCs. This is associated with the physiological changes in rice plants (Lancashire et al., 1991). Plant aerenchyma, the vassel tubes within the porous tissue of rice stems, plays a major role in the methane emission in rice fields over the entire vegetation period (Butterbach-Bahl et al., 1997). Previous rice PMFC studies have reported that methane flux generally peaks towards the end of the vegetative stage and then decreases at the end of the harvesting stage (Deng et al., 2016). Futhermore, it was also investigated that the decrease of methane flux might relate to the increase of methanotrophic bacteria in soil by adding biochar to the rice fileds (Feng et al., 2012).

Figure 4-12 (b) shows the emission of nitrous oxide (N_2O) from all treatments. Soil MFC-parallel showed the highest average N_2O emission flux at 106.48 ± 80.58 μg m^{-2} h^{-1} followed by soil MFC-series, paddy PMFC-series and paddy PMFC-parallel at 89.41 ±

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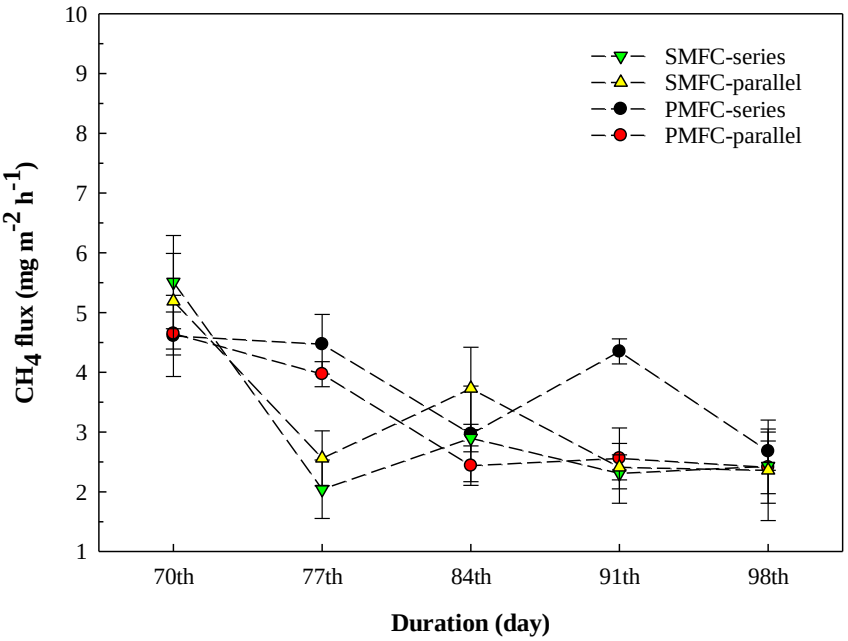
36.11 μg m^{-2} h^{-1}, 63.87 ± 23.38 μg m^{-2} h^{-1} and 43.25 ± 20.29 μg m^{-2} h^{-1}, respectively. All the trends of N_2O emission flux sharply decreased in the second week and then gradually decreased towards the end. However, there was no significant difference between soil MFCs and paddy PMFCs, the same as the different connections. Changes in N_2O emission flux was likely related to redox potential, as previously study reported that a decrease in redox potential at the anode resulted in an increase in both CH_4 and N_2O of rice PMFCs (Arends et al., 2013). The flooding and drying cycle of rice fields would also be expected to increase N_2O more than continuously flooded ones (Bronson et al.,1997). Moreover, nitrogen ferlizer application probably influenced N_2O accumulation in rice paddy fields. Therefore, the minimization of greenhouse gas emission (GHG) by bioelectrochemical system can be envisioned and cooperated with substrates addition and the agricultural practice such as water management (Xu et al., 2007; Arends et al., 2013).

(a)



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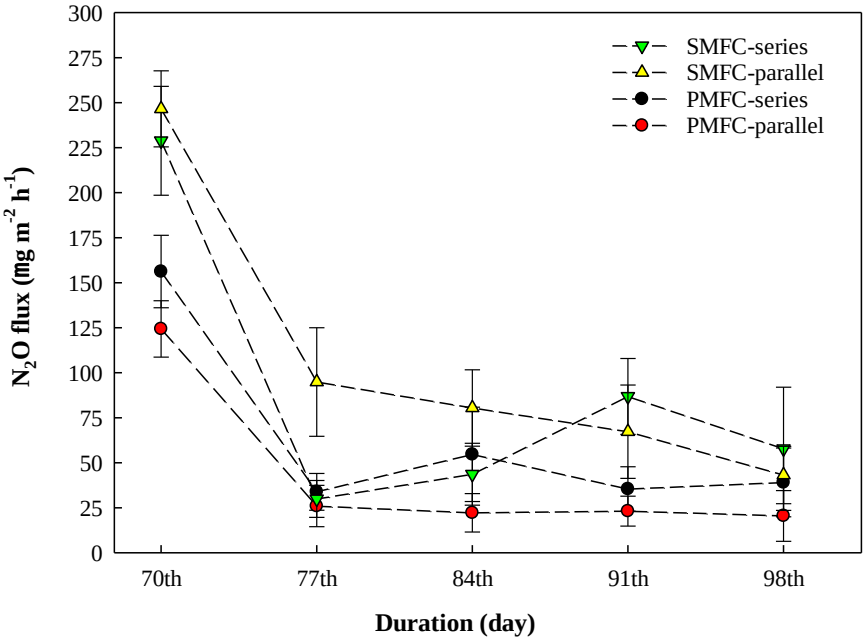

Figure 4-12 Greenhouse gas emission from the multiple soil MFC and PMFCs;

(a) Methane emission flux; (b) Nitrous oxide emission flux

4.2.4 Microbial and archaeal community and structure

After the long-term experiment, the bulk soil at the anode of all treatments was collected and analyzed for the bacterial community by using 16S rRNA gene sequencing with high-quality reads. All experiments were constructed with 318,906 high through-quality reads and a range of 32,052 to 41,782 operational taxonomic units (OTUs) with 97% similarity. Soil MFC-series showed the highest Shonnon index at 7.30, followed by soil MFC-parallel, paddy PMFC-series, and paddy PMFC-parallel at 7.07, 6.99, and 6.92, respectively. The microbial community structures of all treatments consisted of 21 major phyla of microbial and archea communities as shown in Figure 4-13(a). Proteobacteria with relative abundance percentages range from 21.29-32.08% was the most predominant phylum

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followed Acidobacteria (17.49-26.03%), Bacteroidetes (9.12-16.25%), Chloroflexi (4.71-7.92%) and Verrucomicrobia (4.69-5.59%), respectively. It indicated that Proteobacteria phyla is not only consisten with EAB but also have the root-associated microbial community in the rhizosphere. Therefore, the bioelectricity obtained from PMFCs were likely higher than SMFC (Wang et al., 2007).

On the level of genus, the microbial communities mainly consisted of 10 genera as shown in Figure 4-13(b). *Geobacter* was the predominant genus with the relative abundance range of 0.33-8.54%, followed by *AKIW659* (2.03-8.31%), *Candidatus* Solibacter (4.45-6.06%), *Anaeromyxobacter* (1.85-2.55%) and *Anaerolinea* (0.60-2.74%), respectively. Among them, *Geobacter* is a known EAB in the anode microbial communities and widely distributed in freshwater and groundwater sediments with organic compounds (Bond et al., 2002). The relative abundance values of EAB *Geobacter* in PMFC-parallel and series connection (8.54 and 5.58% of all OTUs) were significantly higher (P<0.05) than soil MFC in both parallel and series connection (0.70 and 0.33% of all OTUs). The hierarchical clustering at the genus level based on their phylogenetic assignments as shown in Figure 4-13 (c) was analyzed to compare the linkage of the microbial community among the different soil MFCs and paddy PMFCs with serial and parallel connections. The diagram of cluster analysis showed the clearly different groups of soil MFCs and paddy PMFCs. Therefore, it demonstrated that EAB not only influenced the electricity production of plant microbial fuel cells but also the specific microbiome composition in the plant rhizosphere, which drives the electricity generation through the various pathways (Logan and Regan, 2006; Nevin et al., 2010).

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The archaeal microbiome at the anode bulk soil was discovered to be dominated by the phyla Crenarchaeota, Euryarchaeota, and Nanoarchaeota, with the percentage relative abundance of all OTUs ranging from 0.23-1.68%, 0.22-1.16%, and 0.15-0.71%, respectively. Figure 4-13 (d) presents the different archaeal communities of all treatments at the order level. The results showed that the *Bathyarchaeia* class was observed to have the highest abundance in paddy PMFC with parallel connection (1.59%) followed by soil MFC in parallel connection (0.92%), while *Methanomicrobiales* was found in PMFC in parallel connection at 0.51% of all OTUs. *Bathyarchaeia* wildely distributed in estuarine or marine sediment with a unique metabolic function in carbon metabolism (Wang et al., 2020). Previous study also reported that *Bathyarchaeia* is able grow with lignin as the energy source and the bicarbonate and the carbon source (Yu et al., 2018). Besides that, *Methanomicrobiales* which is H_2- based methanogensis was reported that it associated to a high COD concentration in pore water and resulting high methane emission (Arends et al., 2013).

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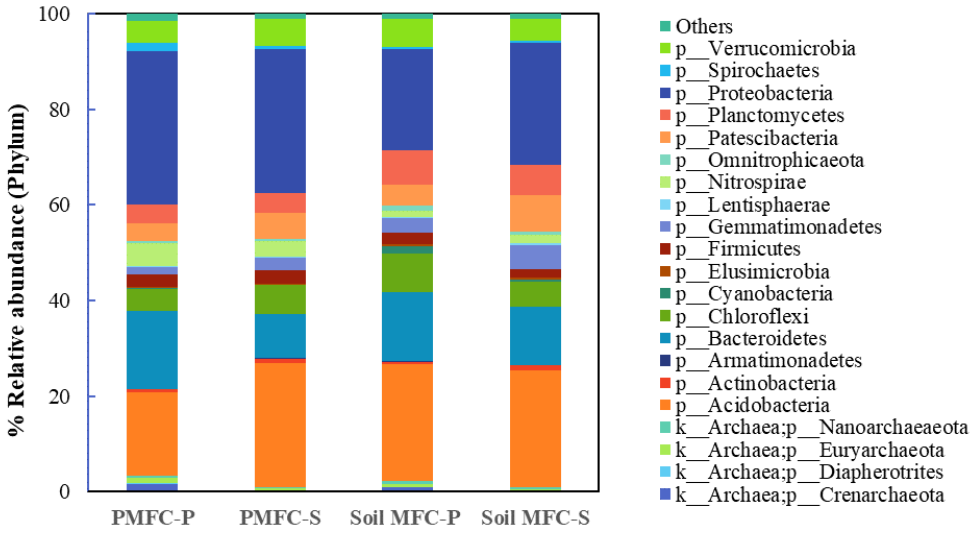

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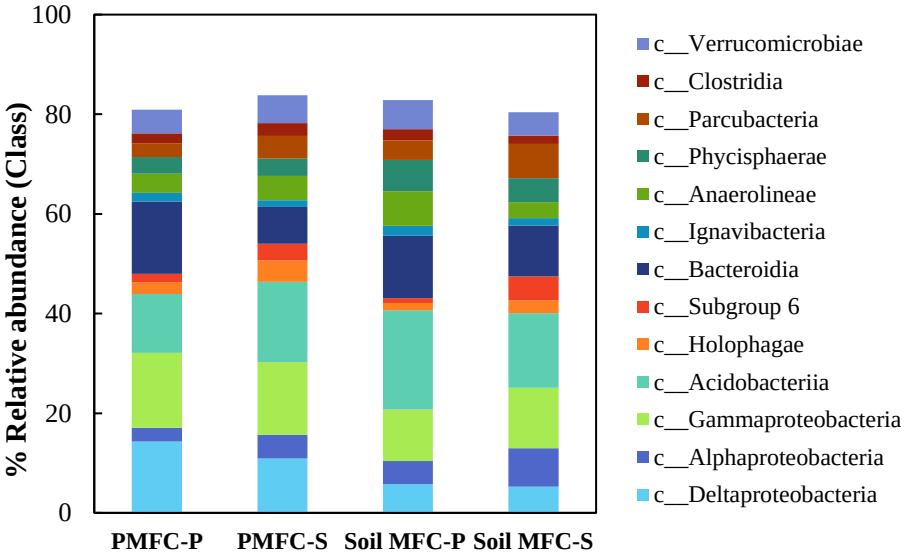

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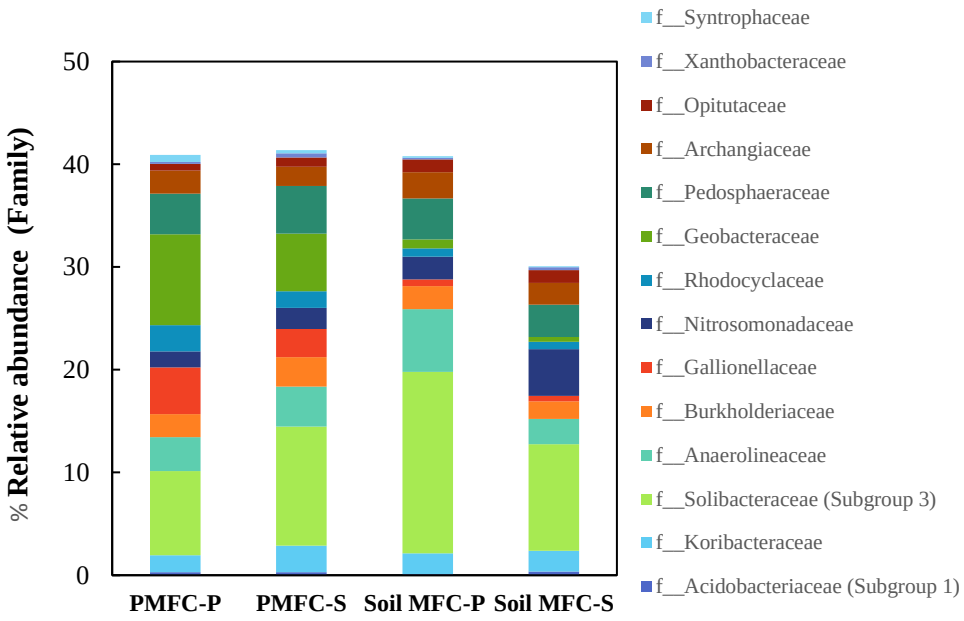

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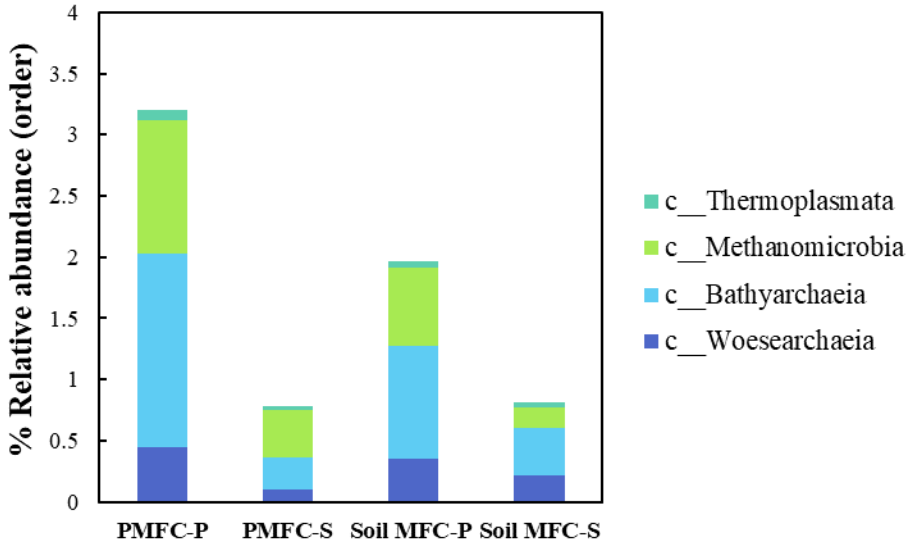

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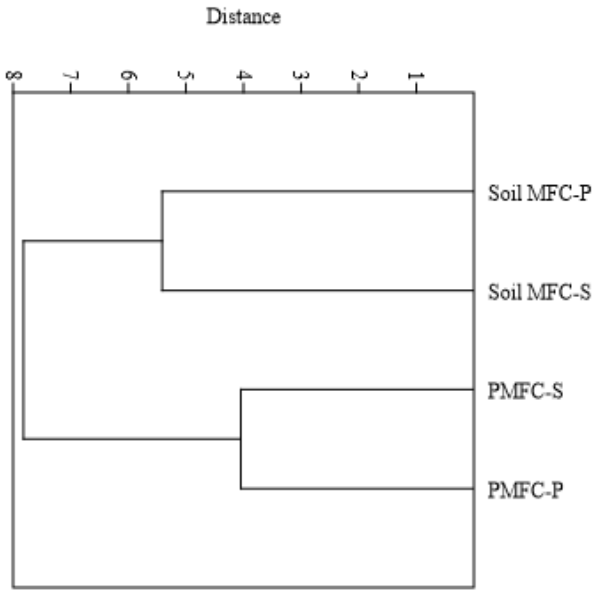

Figure 4-13 Microbial communities at the anode after 130 days of operation. (a) Relative abundance of phyla; (b) Relative abundance of class;(c) Relative abundance of family; (d) Relative abundance of archaea at the order level; (e) cluster analysis at the genus level

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Chapter V Conclusion

5.1 Evaluation of long-term performance of plant microbial fuel cells using agricultural plants under the control environment

Multiple PMFCs were operated under the controlled environments, and the voltage output of PC-PMFC, which was added with compost made from food waste, reached the highest value of 894.39 ± 53.44 mV (34.78 mW/m^2). PB-PMFC demonstrated significantly lower voltage production than those without biochar, likely due to the inhibitory action of biochar made from waste wood biomass. All PMFCs had significantly higher voltage outputs than soil MFC. The significantly higher output voltage of P-PMFC than W-PMFC indicated that plant species would affect electricity generation of PMFCs. The 16S rRNA gene high throughput sequencing revealed *Proteobacteria*, *Bacteroidetes* and *Chlorofexi* were the most abundant phyla of the anode microbial community. The exoelectrogen *Geobacter* was the most dominant genus of anode microbial communities and showed the highest abundance in PC-PMFC. This study has demonstrated that the power output of PMFC systems can be influenced by different agricultural plants and soil conditioners, and soil conditioners made by suitable waste biomass could be applied in PMFCs to enhance the performance of electricity production.

5.2 Improvement of PMFCs with *Oryza sativa* performance and greenhouse gas emission

The enhancement of bioelectricity production in PMFCs with *Oryza sativa* was continuously explored. To improve power production, chemical or conventional fertilizer was added to the paddy PMFC (F-PMFC). Paddy PMFC adding chemical fertilizer reached

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the maximum voltage at 775.96 ± 230.21 mV, followed by P-PMFC and soil MFC, respectively. The high COD concentration of pore water in F-PMFC (123.2 ± 11.3 mg/L) likely influenced the EAB at the anode and the electricity increased in F-PMFC. The bioelectrochemical systems increased the pH values and decreased the EC of soil. Moreover, the stratified soil pH of all samples gradually increased from the anode to the cathode region at the topsoil, while the stratified soil EC did not show clearly the EC gradient in all samples. Besides, paddy PMFCs with different connections were conducted in the greenhouse. PMFC-series showed the highest average voltage of 474.97 ± 164.84 mV, followed by paddy PMFC-parallel at 396.89 ± 149.91 mV. By comparison, the voltage output of PMFCs was not only significantly higher than soil MFCs, but the series connection also has a higher power output than the parallel connection (P<0.05). The GHG flux measurement from soil MFCs and PMFCs revealed that the highest average CH_4 emission was PMFC-series (3.82 ± 0.92 mg m^{-2} h^{-1}) and soil MFC-parallel showed the highest average N_2O emission flux of 106.48 ± 80.58 μg m^{-2} h^{-1}. The 16S rRNA gene high throughput sequencing showed that Proteobacteria were the most abundant phyla of the anodic microbiome. *Geobacter* is also the most abundant group that is associated with the bioelectricity generation in PMFCs. An Archaea community analysis observed Crenarchaeota, Euryarchaeota, and Nanoarchaeota as predominant phyla in the bulk soil of the anode. Overall, this study demonstrated the enhancement of the bioelectricity production from the paddy PMFCs with various soil additions, and electricity production and GHG emission from the serial and parallel connections of PMFCs, which will be beneficial for the future scale-up of PMFCs.

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5.3 Recommendation

5.3.1 The different electrode materials and the design configurations could be more focused on the scale-up of electricity generation from paddy PMFCs.

5.3.2 The stack connections or designs of paddy PMFCs could be continuously researched, such as the multi-electrodes connected with current collecting devices of paddy PMFCs.

5.3.3 The relationship between the bacterial dynamics in bioelectrochemical systems and GHG emissions of paddy PMFCs should be more in-depth investigated for future study.

5.3.4 Paddy PMFCs could be further developed for biosensor applications e.g., water management in paddy fields.

doi:10.6342/NTU202203744

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VII Appendix 1 Published journals

Part of the results has been published in two journals as shown below:

1. Tongphanpharn, N., Guan, C. Y., Chen, W. S., Chang, C. C., & Yu, C. P. (2021). Evaluation of long-term performance of plant microbial fuel cells using agricultural plants under the controlled environment. *Clean Technologies and Environmental Policy*. https://doi.org/10.1007/s10098-021-02222-9

2. Tongphanpharn, N., Chou, C. H., Guan, C. Y, & Yu, C. P. (2021). Plant microbial fuel cells with Oryza rufipogon and Typha orientalis for remediation of cadmium contaminated soil. *Environmental Technology & Innovation*, 24, 102030. https://doi.org/10.1016/j.eti.2021.102030

doi:10.6342/NTU202203744

VII Appendix 2 Supplementary Information

Table A1 *P* values of One-way ANOVA with Tukey post hoc test in various treatments for voltage generation, pH and EC

Sample	Parameters		
	Voltage Generation	pH	EC
Soil-MFCs vs Paddy-PMFCs	< 0.001	< 0.001	< 0.001
Soil-MFCs vs Water bamboo-PMFCs	< 0.001	0.0358	0.5212
Paddy-PMFCs vs Water bamboo-PMFCs	< 0.001	0.2946	< 0.001
Paddy-PMFCs vs Paddy-PMFCs with compost	< 0.001	< 0.001	0.3059
Paddy-PMFCs vs Paddy-PMFCs with biochar	0.0044	0.3148	< 0.001
Paddy-PMFCs with compost vs Paddy-PMFCs with biochar	< 0.001	0.03243	0.0145
P-PMFC vs Soil MFC	< 0.001		
F-PMFC vs Soil MFC	< 0.001		
F-PMFC vs P-PMFC	< 0.001		
Soil MFC-S vs Soil MFC-P	0.038		
PMFC-S vs PMFC-P	< 0.001		

Table A2 Relative abundance of phyla (%) of paddy PMFC, paddy PMFC with compost, paddy PMFC with biochar and water bamboo PMFC

OTUs	**P-PMFC (%)**	**PC-PMFC (%)**	**PB-PMFC (%)**	**W-PMFC (%)**
k__Archaea;k__Archaea_unclassified	0.02093	0.006885	0.013306	0.04226
k__Archaea;p__Crenarchaeota	3.362791	5.767068	1.564431	4.977344
k__Archaea;p__Diapherotrites	1.472093	0.75043	0.610185	1.274857
k__Archaea;p__Euryarchaeota	0.581395	4.514056	0.547456	2.603714
k__Archaea;p__Nanoarchaeaeota	6.834884	5.005164	7.784135	6.916629
k__Archaea;p__Thaumarchaeota	0.132558	0.052783	0.093143	1.072946
k__Bacteria;k__Bacteria_unclassified	0.137209	0.153758	0.121657	0.199563
k__Bacteria;p__Acidobacteria	10.96744	9.611015	9.084342	9.452257
k__Bacteria;p__Actinobacteria	0.602326	0.833046	0.735644	1.145728
k__Bacteria;p__Armatimonadetes	1	0.587493	0.958047	0.917991
k__Bacteria;p__BRC1	0.013953	0.039013	0.026612	0.030521
k__Bacteria;p__Bacteroidetes	6.602326	9.794607	9.527249	9.675299
k__Bacteria;p__CK-2C2-2	0	0	0.007604	0
k__Bacteria;p__Caldiserica	0.009302	0.016064	0.011405	0.04226
k__Bacteria;p__Calditrichaeota	0.013953	0	0	0.007043

doi:10.6342/NTU202203744

Table A2 (cont.)

OTUs	P-PMFC (%)	PC-PMFC (%)	PB-PMFC (%)	W-PMFC (%)
k__Bacteria;p__Chlamydiae	0.327907	0.100975	0.526546	0.068086
k__Bacteria;p__Chloroflexi	4.248837	8.906483	3.828388	9.818515
k__Bacteria;p__Cloacimonetes	0	0	0	0.007043
k__Bacteria;p__Cyanobacteria	0	0.252438	0.887715	0.13852
k__Bacteria;p__Dadabacteria	0	0	0	0.007043
k__Bacteria;p__Deinococcus-Thermus	0.427907	0	0.089342	0
k__Bacteria;p__Dependentiae	0.053488	0.00918	0	0
k__Bacteria;p__Elusimicrobia	0.013953	0.31899	0.269926	0.394431
k__Bacteria;p__Epsilonbacteraeota	0.048837	0.071142	0.121657	0.136173
k__Bacteria;p__FBP	0.195349	0	0.026612	0
k__Bacteria;p__FCPU426	0	0.110155	0.074135	0.063391
k__Bacteria;p__Fibrobacteres	0.586047	0.289157	0.268025	0.26765
k__Bacteria;p__Firestonebacteria	0.84186	0	0.007604	0
k__Bacteria;p__Firmicutes	0.27907	2.177854	0.479024	1.288944
k__Bacteria;p__Gemmatimonadetes	0	0.355709	0.925732	0.359214
k__Bacteria;p__Hydrogenedentes	0.037209	0.167527	0.682419	0.143216
k__Bacteria;p__Kiritimatiellaeota	0.525581	0.045898	0.066531	0.039913
k__Bacteria;p__LCP-89	0.013953	0.059667	0.011405	0.068086
k__Bacteria;p__Latescibacteria	0	0.335055	0.391583	0.319301
k__Bacteria;p__Lentisphaerae	0	0.006885	0.032315	0.025826
k__Bacteria;p__Margulisbacteria	0.006977	0	0.007604	0.007043
k__Bacteria;p__Modulibacteria	0.011628	0.091796	0	0
k__Bacteria;p__Nitrospinae	0	0.027539	0.081738	0.007043
k__Bacteria;p__Nitrospirae	11.97442	7.052209	5.447944	8.304181
k__Bacteria;p__Omnitrophicaeota	0.523256	0.234079	0.505636	0.692602
k__Bacteria;p__Patescibacteria	14.51395	14.08147	13.90689	14.36386
k__Bacteria;p__Planctomycetes	4.830233	2.6965	5.510673	3.282229
k__Bacteria;p__Poribacteria	0	0.006885	0	0
k__Bacteria;p__Proteobacteria	0.006977	20.5852	26.00985	17.49348
k__Bacteria;p__Rokubacteria	0.011628	0.006885	0.062729	0.147912
k__Bacteria;p__Schekmanbacteria	20.79302	5.767068	0	0.037565
k__Bacteria;p__Spirochaetes	0.209302	0.068847	0.747049	0.370953
k__Bacteria;p__Verrucomicrobia	0	0	5.82432	3.261099
k__Bacteria;p__WOR-1	0.618605	0.920252	0.007604	0.007043
k__Bacteria;p__WPS-2	5.697674	3.430866	0.039919	0.072782
k__Bacteria;p__WS2	0.011628	0	0.055126	0.105651
k__Bacteria;p__WS4	0.018605	0.016064	0.133062	0.018782
k__Bacteria;p__Zixibacteria	0.044186	0	1.866672	0.176085
unknown;unknown_unclassified	0.053488	0.027539	0.019009	0.147912

doi:10.6342/NTU202203744

Table A3 Relative abundance of phyla (%) of soil-MFC, paddy PMFC and paddy PMFC with fertilizer

OTUs	Soil-MFC (%)	P-PMFC (%)	F-PMFC (%)
k__Archaea;p__Crenarchaeota	1.0163164	1.1700984	1.0163164
k__Archaea;p__Diapherotrites	0.0138274	0	0.0138274
k__Archaea;p__Euryarchaeota	1.8113938	1.65798396	1.8113938
k__Archaea;p__Nanoarchaeaeota	0.1279038	0.35557761	0.1279038
k__Archaea;p__Thaumarchaeota	0	0	0
k__Bacteria;p__Acidobacteria	14.90943	15.4180104	14.90943
k__Bacteria;p__Actinobacteria	2.0810288	1.5422145	2.0810288
k__Bacteria;p__Armatimonadetes	0.0138274	0.4424047	0.0138274
k__Bacteria;p__Bacteroidetes	16.921322	9.36905648	16.921322
k__Bacteria;p__BRC1	0.2177821	0	0.2177821
k__Bacteria;p__Caldiserica	0	0	0
k__Bacteria;p__Calditrichaeota	0.0138274	0	0.0138274
k__Bacteria;p__Chlamydiae	0	0.13644257	0
k__Bacteria;p__Chloroflexi	9.6238938	7.38030265	9.6238938
k__Bacteria;p__CK-2C2-2	0	0	0
k__Bacteria;p__Cloacimonetes	0	0	0
k__Bacteria;p__Cyanobacteria	0.4251936	0.37625072	0.4251936
k__Bacteria;p__Dadabacteria	0.0311117	0	0.0311117
k__Bacteria;p__Deinococcus-Thermus	0	0	0
k__Bacteria;p__Elusimicrobia	0.2661781	0.05788473	0.2661781
k__Bacteria;p__Epsilonbacteraeota	0	0.06201935	0
k__Bacteria;p__FCPU426	0.0345686	0.01653849	0.0345686
k__Bacteria;p__Fibrobacteres	0.0795077	0.03307699	0.0795077
k__Bacteria;p__Firmicutes	8.1409015	3.41106425	8.1409015
k__Bacteria;p__GAL15	0.0172843	0	0.0172843
k__Bacteria;p__Gemmatimonadetes	1.1891593	1.7902919	1.1891593
k__Bacteria;p__Halanaerobiaeota	0.2039546	0.02067312	0.2039546
k__Bacteria;p__Hydrogenedentes	0.4286504	0.16951956	0.4286504
k__Bacteria;p__Kiritimatiellaeota	0.0138274	0.04134623	0.0138274
k__Bacteria;p__Latescibacteria	0.2385232	0.50855867	0.2385232
k__Bacteria;p__Lentisphaerae	0	0.04134623	0
k__Bacteria;p__Margulisbacteria	0.0276549	0	0.0276549
k__Bacteria;p__Modulibacteria	0	0	0
k__Bacteria;p__Nitrospinae	0	0.16125031	0
k__Bacteria;p__Nitrospirae	0.9264381	4.54808567	0.9264381
k__Bacteria;p__Omnitrophicaeota	0.6014934	0.1943273	0.6014934
k__Bacteria;p__Patescibacteria	2.9037611	4.77548995	2.9037611
k__Bacteria;p__PAUC34f	0	0	0
k__Bacteria;p__Planctomycetes	6.3398783	4.53154718	6.3398783
k__Bacteria;p__Proteobacteria	27.941787	36.1986273	27.941787
k__Bacteria;p__Rokubacteria	0.3042035	0	0.3042035
k__Bacteria;p__Spirochaetes	0.3802544	1.87298437	0.3802544
k__Bacteria;p__Tenericutes	0	0	0
k__Bacteria;p__Verrucomicrobia	2.5235066	3.11337137	2.697330089
k__Bacteria;p__WOR-1	0	0	0
k__Bacteria;p__WPS-2	0	0	0
k__Bacteria;p__WS2	0	0.07442322	0.102820715
k__Bacteria;p__WS4	0	0	0
k__Bacteria;p__Zixibacteria	0.0138274	0.52923179	0.671762004

doi:10.6342/NTU202203744

Table A4 Relative abundance of phyla (%) of paddy PMFC-parallel, paddy PMFC-series Soil MFC-parallel and soil MFC-series

OTUs	PMFC-P (%)	PMFC-S (%)	Soil MFC-P (%)	Soil MFC-S (%)
k__Archaea;p__Crenarchaeota	1.687604056	0.279620004	0.927727069	0.38391281
k__Archaea;p__Diapherotrites	0.037838656	0	0	0
k__Archaea;p__Euryarchaeota	1.165430604	0.419430005	0.694298968	0.22291712
k__Archaea;p__Nanoarchaeaeota	0.533525049	0.154149489	0.709262307	0.34985603
p__Acidobacteria	17.48902679	26.02975444	24.25557384	24.3382148
p__Actinobacteria	0.537308915	0.881878473	0.571599581	1.10839345
p__Armatimonadetes	0.083245043	0.207922567	0.128684722	0.07740178
p__Bacteroidetes	16.24791887	9.119913963	14.50246895	12.1737515
p__Chloroflexi	4.707128803	6.183903925	7.924584767	5.16734264
p__Cyanobacteria	0.052974118	0.032263847	1.717791411	0.60373386
p__Elusimicrobia	0.332980173	0.118300771	0.371090827	0.32508746
p__Firmicutes	2.561677009	2.964689012	2.46895107	1.86383479
p__Gemmatimonadetes	1.505978508	2.495070801	3.034565315	5.03730766
p__Lentisphaerae	0.196761011	0.243771285	0.224450097	0.39939317
p__Nitrospirae	4.937944604	3.262233375	1.191081849	1.7771448
p__Omnitrophicaeota	0.280006054	0.541315648	1.101301811	0.6315985
p__Patescibacteria	3.783865597	5.337874171	4.40819991	7.4739156
p__Planctomycetes	3.859542909	4.122602617	7.125542421	6.42744357
p__Proteobacteria	32.0833964	30.24556372	21.28684722	25.4620886
p__Spirochaetes	1.778416831	0.58433411	0.335178812	0.42106567
p__Verrucomicrobia	4.707128803	5.592400072	5.874607212	4.69364377
Others	1.430301196	1.183007707	1.14619183	1.06195238

doi:10.6342/NTU202203744