DETERMINATION OF FORAGE GRASS CAPABILITY FOR ELECTRICITY PRODUCTION IN PLANT MICROBIAL FUEL CELL

BY

MS. NGUYEN THI VINH

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE
(ENGINEERING AND TECHNOLOGY)
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was approved as partial fulfillment of the requirements for the degree of
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ABSTRACT

Plant microbial fuel cell (PMFC) is a promising technology fascinating significant attention among researchers worldwide due to its sustainable and alternative approaches in bioelectricity generation. Because of the population explosion and the pressure on resource use, resources gradually be depleted, PMFC are viewed as a paramount and sustainable device for electricity production. The PMFC device serves a number of benefits, comprising of the wastewater treatment performance, the direct electricity production by utilizing a spontaneous combination of bio-electrochemical and physical processes. This thesis illustrates the discovery of forage grass capability for electricity generation under various operating conditions and evaluation of ambient parameters in PMFC. The outcomes interpret that PMFCs systems using Purple guinea grass are strongly influenced by configurations, water contents, defoliation, fertilizer and surrounding factors. In order to support the investigation, a series of experiments are performed in the following order: (i) Three PMFC systems using Purple guinea grass were installed including dry-soil, wet-soil, and waterlog PMFC under greenhouse condition. This experiment was carried out to determine the effects of soil water contents, temperature fluctuations, and circadian rhythm on current and power generations. Plants cultivation in waterlog condition were found to deliver better performance as compared to the others and the electricity of PMFCs increased for the high-temperature regime in the daytime. (ii) Purple guinea grass PMFC was grown in different configurations to maximize the effectiveness of PMFCs. In addition, a comparative analysis between defoliated plants and non-defoliated plants PMFCs on re-growth ability and re-bioelectricity generation was also carried out after completing configuration performance comparison. In this study, maximum power was examined through polarization curves and the output was calculated on the fundamental of the anodic area. Overall, the forage grass was able to perform in PMFC systems and might be an outstanding candidate for PMFC. It was observed that compared to single-chamber, double chamber equipped two cathodes, and air-cathode PMFC, the double chamber MFC is one of the most pertinent configurations for maximizing the efficiency of PMFC system using forage grass in terms of electricity production. Defoliation treatment carried out positive feedbacks, which generated a relative amount of electricity as non-defoliation and well fed for animals in long-term operation. In order to acquire tasty favor for feeding animals, it is considered to be harvested regularly and supported for both regrowth and electricity regeneration. On the other hand, PMFC systems in this study also were strongly affected by the variation of ambient factors. (iii) Considerations whether supplementing a huge amount of fertilizer for perennial purple guinea grass every year could be a right way for plant growth, electricity generation, and long-term soil properties operation, a comprehensive study with different fertilizer adjustments was determined. Four PMFCs including urea PMFC, a mixture of urea and compost PMFC, compost PMFC, and a control PMFC were instructed to examine the most suitable for the effectiveness of PMFC systems. The results showed that plants treated with only compost in PMFC obtained higher performance than others. The main reasons were attributed to changes in soil properties and bacteria activities in the systems.

To sum up, this study suggests that PMFC systems can be built up by discovering the potential of perennial plants and elucidating basic factors are essential in order to clarify influences on performances of PMFC systems. Hence, a certain plant PMFC can be assigned and identified the fundamental principle that can be proposed for further studies.

Keywords: Physical factors; treatments; power generation; nutrient feedbacks

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Ms. Nguyen Thi Vinh

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LIST OF SYMBOLS/ABBREVIATIONS

Symbols/Abbreviations	**Terms**
MFC	Microbial fuel cell
PMFC	Plant microbial fuel cell
EAB	Electroactive bacteria
IEM	Ion exchange membrane
ORR	Oxygen reduction reaction
SMFC	Sediment microbial fuel cell
P-SMFC	Plant-sediment microbial fuel cell
NA	Not available
EES	Ecological engineering system
CW-MFC	Constructed wetland microbial fuel cell
COD	Chemical oxygen demand
FESEM	Field emission scanning electron microscopy
OCV	Open circuit voltage
CCV	Closed circuit voltage
EC	Soil electrical conductivity
SD	Standard deviation
RSD	Relative standard deviation
D-PMFC	Dry-PMFC
W-PMFC	Wet-PMFC
WL-PMFC	Waterlog-PMFC
D-PMFC	Double-chamber PMFC
D-2C PMFC	Double-chamber two cathodes PMFC
S-PMFC	Single-chamber PMFC
U-PMFC	Urea-PMFC
CU-PMFC	Compost and Urea PMFC
C-PMFC	Compost-PMFC

CHAPTER 1

INTRODUCTION

1.1 Background

The generation of bio-electricity from living plants devotes a unique and lure concept known as plant microbial fuel cell (PMFC) (Can & Yakar, 2017; Lu, Xing, & Ren, 2015a). It is a fascinating approach for simultaneously biomass production, wastewater treatment, and electricity generation. The PMFC system converts solar power into bio-electricity through root exudates derived from the root system by a series of biochemical reactions (Strik, Bert, Snel, & Buisman, 2008). Exudates and organic compounds both high and low molecules are oxidized into electrons, protons and carbon dioxide by electroactive bacteria resided around the rhizosphere. Electrons at the anode are collected by providing an electron acceptor in the rhizosphere and transferred them into electricity (Nitisoravut & Regmi, 2017; Strik et al., 2011).

To create electron neutrality, protons that are generated from the oxidation of exudates pass through a membrane to the cathodic area where the final electron receptor couple with electrons and protons to accomplish the entire circuit. PMFC studies were performed in various directions. Numerous factors were reported to have a great impact on the PMFC performance such as choice of plants, light intensity, microbial communities, the distance of electrodes, salinity level, growing media, etc. (B. Liu, Ji, & Zhai, 2018; Md Khudzari, Kurian, Gariépy, Tartakovsky, & Raghavan, 2018; Sarma & Mohanty, 2018; Tapia, Rojas, Bonilla, & Vargas, 2017). PMFC systems are effectively determined under an oxidation-reduction potential balance between anode and cathode. A large number of protons, which are trapped in the anodic chamber can trigger an unbalanced potential of pH leading to the acidic pH in the anode and basic pH in the cathode. As a result, the performance of PMFC is jeopardized. To maximize power output, an unbalance of pH should be avoided. Promoting conductivity and mobility of protons is a compatible direction diminishing the unbalance of pH and improve electric generation in PMFC. Soil conductivity and water contents are substantial factors that could decelerate PMFC performance in the arid and semi-arid conditions (Domínguez-Garay, Berná, Ortiz-Bernad, & Esteve-Núñez, 2013). Soil water content has been described as a critical aspect, maintaining the anaerobic condition and the substrate consumption in PMFC. The low water level in the soil could strongly impact the proton mobility, which increases the internal resistances leading to a decrease in PMFC performance. In contrast, PMFC systems operated under high water content in soil typically increase proton transport, conditions for electroactive bacterial activities, and reduction of internal resistances (Chiranjeevi, Mohanakrishna, & Venkata Mohan, 2012). Effects of soil water content on electrical generation performance in PMFCs is, therefore, essential.

Plant microbial fuel cells have been operated under various natural physiochemical parameters including light intensity, ambient temperature, pH, humidity, and conductivity. Without clarifying the roles of the anodic area, an efficient PMFC would barely be obtained. As a key component in PMFCs, the anodic area must be situated and maintained under anaerobic condition and closely associated with anaerobic bacterial activities and substrates digestion (Larrosa-Guerrero et al., 2010). Temperature is a paramount parameter for anaerobic digestion. The operations of MFCs under various ranges of temperature have been studied and reported (Lianhua et al., 2010; Van Lier, Sanz Martin, & Lettinga, 1996). However, to the best of our knowledge, an evaluation of the effects of ambient temperature to forage plants and anaerobic bacterial activities in PMFCs has not been clearly demonstrated.

Photo-period and light intensities are critical physical factors that can strongly affect to the plant growth and the PMFC power output (L.Shirley, 2018; Md Khudzari et al., 2018; Sønsteby & Heide, 2009). Photosynthesis is the process of plants for capturing solar energy to generate organic compounds in the rhizosphere. The excretions from the root systems such as root exudates, gases and organic compounds under the soil are defined as rhizodeposition. Diurnal variation can limit the accumulation and transport of organic compounds to the rhizosphere and excretion of rhizodeposition and food sources for electroactive microbes, as well as causing reduction of electrons liberation.

In terms of configuration design, suitable configurations should be considered to eliminate physical barriers, maximize the power generations, and enhance the entire performance of PMFC systems. Various configuration designs were constructed in this study including single-chamber, double-chamber, air-cathode, double-chamber equipped two cathodes PMFCs to compare the most pertinent configurations for PMFC systems.

Fertilizers are paramount importance inputs for the plant growth, microorganism activities, and bioelectricity generation in the PMFC systems. The nutrient adjustments in the soil in PMFC systems might significantly change the electricity generation behavior as well as plant growth.

Furthermore, purple guinea grass is known to be forage and perennial plants that can be easily grown in a variety of moisture levels and harvested for feeding animals, such as cows and buffaloes. It can also withstand high light intensity (Khota, Pholsen, Higgs, & Cai, 2016). Therefore, purple guinea grass is a suitable object for PMFC systems in this study. Moreover, based on functional and perennial properties of purple guinea grass, a comparative study of defoliation treatment was also performed in order to clarify the regrowth capability and the bioelectricity regeneration after each batch of harvesting.

1.2 Problem statement

Numerous exertions have been carried out with PMFCs from lab-scale to field-scale works for the practical application of this technology. It is considered as one of the most sustainable and renewable bio-electrochemical technology for both wastewater treatment and electricity generation. This study focuses on discovering in detail a number of factors that affects the operations of PMFC systems using forage grass. Differ from the previous study, beyond basic and fundamental aspects for electricity generation, this study was embodied deeply numerous factors for one type of plant based on their practical characteristics involving soil properties, physical parameter, bacteria attachment, plant morphology, and plant physiology.

1.3 Research objectives

The general purpose of this research is to determine the performance of purple guinea grass in PMFC systems. Performances of forage PMFCs are followed by objectives:

✓ To evaluate the effects of water contents to PMFC systems.

✓ To determine the effects of physical parameters to forage PMFC performances**.**

- ✓ To clarify the influences of soil properties including soil nutrients, salinity, pH, conductivity, electrical resistivity, and moisture**.**
- ✓ To examine the relationships between factors to plant growth and power output**.**
- ✓ To explore the importance of PMFC configurations on the system performance of PMFCs.
- ✓ To evaluate the electricity regeneration ability based on the regrowth of purple guinea grass by a defoliation treatment.
- ✓ To evaluate the effects of urea and compost on soil properties, microbiological activities, and electricity production in PMFC systems.

1.4 Scope of study

This study focused on investigating various factors for electricity generation in plant microbial fuel cells using forage grass (purple guinea grass). This study revealed numerous influences of factors including negative and positive effects on the plant growth as well as electricity generation behavior. More importantly, the proposed study will help to understand deeply about using purple guinea grass for electricity generation in PMFCs and support for further studies.

CHAPTER 2
LITERATURE REVIEW

2.1 Theory background of plant microbial fuel cell

2.1.1 Originality of plant microbial fuel cell

In 2008, there were three reports published, which describe the presence of plants on MFCs calling PMFCs. The PMFC was first investigated out using reed mannagrass by strik et al. (Strik et al., 2008) coupled with declaring that electricity was produced without competing food sources, non-destructive, and bio-energy production. At the same time two other studies carried the study using the rice paddy plants. One of them was tested in laboratory pot culture systems while another one tested in a real rice paddy field. Over the period of time, PMFC technology has exploited numerous potentials in different types of plants such as semi-arid green roof ecosystem (Tapia et al., 2017), wetland plants (Y. Zhou, D. Xu, E. Xiao, D. Xu, P. Xu, X. Zhang, Q. Zhou, F. He, 2017), rice paddy (Kaku, Yonezawa, & Kodama, 2008), fresh marshy species and salt marsh species (M Helder et al., 2010), weeping alkali-grass (Md Khudzari et al., 2018), and floating MFCs (Schievano et al., 2016).

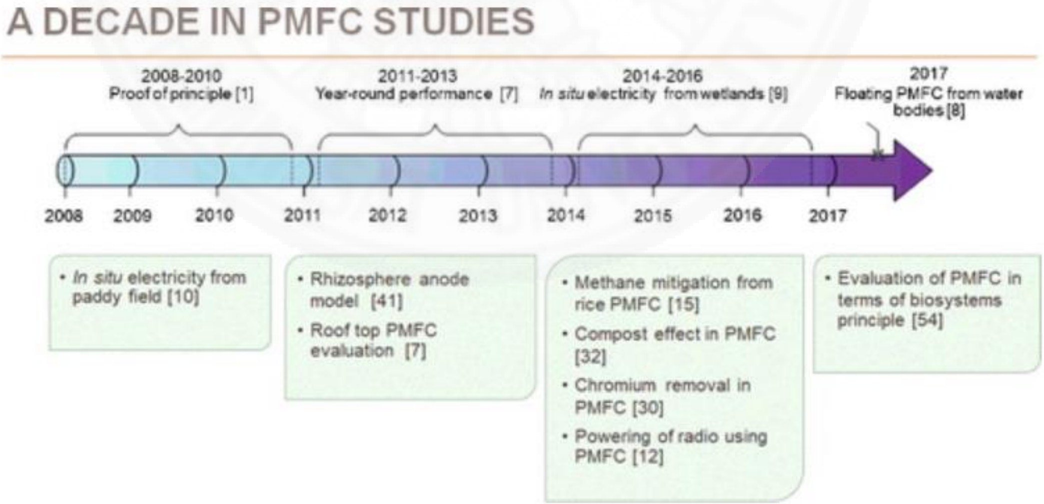


Figure 2.1 Progress of PMFC research following different stages (Regmi, Nitisoravut, & Ketchaimongkol, 2018).

Progress in the PMFC studies can be seen in Figure 2.1 which depicts the centrality of each phase over time. Accordingly, researchers focused on distinctive areas of the body in certain periods. During the period of 2008-2010, researchers mainly concentrated in clarifying the operating principles of the PMFC systems. In the later period (2011-2013), PMFCs were focused in the potentials of the rhizosphere in the anode chamber. PMFCs were more deeply into the direct effects that could influence the performance of PMFCs during the year 2014 to 2016. By 2017, the evaluation in terms of bio-system principal becomes more prominent. These changes show that PMFCs studies have noticeably changed chronologically. Researchers are constantly exerting to explore and deepen all aspects that may bring PMFC technology more and more practical in the future.

2.1.2 Electricity generation principal from plant microbial fuel cell

Plant microbial fuel cells are an appealing technology for sustainable bioelectricity generation and wastewater treatment. A PMFC is viewed as an important and sustainable device for electricity production. PMFC technology serves a number of benefits, comprising wastewater treatment and direct electricity production by utilizing a combination of bio-electrochemical and physical processes (Figure 2.2). A typical PMFC comprises an anodic area, a cathode, a membrane, and an external circuit. The anodic area contains indispensable components which are replenished with root-exudates, bacterial communities, and electron collectors. Exoelectrogenic bacteria near the rhizosphere in the anodic area oxidize organic compounds and discharge free electrons, protons, and other by-products. These electrons follow an external circuit to the cathode. Concurrently, protons pass through the separator to the cathode. At the cathode, electrons and protons react with the final electron acceptors to finish an entire circuit. Moreover, the PMFC systems embody a chain of bio-electrochemical interactions, which are:

- ✓ Physical parameters are factors that impact directly to the performance of plants in PMFCs such as pH, temperature, humidity, light intensity, rainfall.
- ✓ Chemical processes, where reactors occur for the electricity generation.
- ✓ Bio-systems showing significant interactions between plants and microorganism communities for PMFC performances.

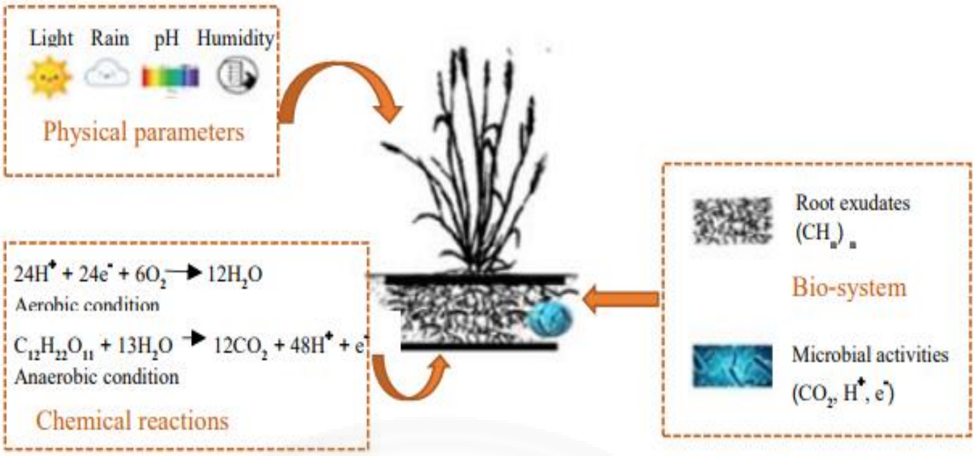


Figure 2.2 Series of biochemical interactions supporting for bioenergy generation in plant microbial fuel cells.

2.1.3 Plant microbial fuel cell for wastewater treatment

Plant-microbial fuel cells (PMFC) have been explored intensively for the bio-energy production from interplays between plants, microbial communities, and physical parameters. However, investigations for their applications are being widely utilized for wastewater treatment. PMFC technology concurrently generates electricity and decontaminates wastewater. Submerged aquatic plants were considered to significantly affect the nitrogen cycle in PMFCs. In the study of Peng xu et al. (Xu et al., 2019) *C. demersum* plant was used in PMFC systems to increase NO_3 through the measurement of ^{15}N abundance. Their outcome showed that the combination of sediment-MFC and aquatic plant *C.demersum* (P-SMFC) could remove 23 % NO_3 with the maximum power density of 9.82 mW/m^2.

P-SMFC also demonstrated its potential for remediating soil pollutants specifying Cr-polluted soil. *Pennisetum* and *I.aquatica* plants in P-SMFC studies interpreted their abilities on eliminating Cr in the soil which showed the reduction ratios of 99 Cr(VI) and 27.4 % total Cr with the high voltage output of 469.21 mV (Guan, Tseng, Tsang, Hu, & Yu, 2019). In another research, removal of Cr(VI) of *I.aquatica* P-SMFC reached up to 99 % while obtaining considerably power generation at 75.12 mW/m^2 (C. Cheng, Y. Hu, S. Shao, J. Yu, W. Zhou & Y. Chen, S. Chen, J. Chen, 2019). Those studies suggested that PMFCs are versatile functions encompassing bio-electricity generation, biomass production, and wastewater treatment. Several studies on wastewater treatment in PMFCs are shown in Table 2.1

Table 2.1 Plant microbial fuel cell for the purposes of electricity generation and wastewater treatment in single-chamber configuration.

Types of plant	**Treatment**	**Removal (%)**	**Maximum Voltage (mV)**	**Power Densities (mW/m^2)**	**Ref.**
H. Verticillata	NH_4^+-N	67.75	558.50	NA	(Shen, Zhang, Liu, Hu, & Liu, 2018)
Pennisetum	Cr(VI)	99	469.21	NA	(Guan, Tseng, et al., 2019)
	Total Cr	27.4			
C. Zizanioides ***T. Angustifolia***	Scarlet RR Dye	89	NA	76.9 NA	(Suhas K. Kadam, Anuprita D. Watharkar, Vishal V. Chandanshive, Khandare, Byong-Hun Jeon, Jyoti P. Jadhav, & Govindwar, 2018)
	Textile effluent	87			
I. Aquatica	Cr(VI)	99.76	NA	75.12 Cathode area	(C. Cheng, Y. Hu, S. Shao, J. Yu, W. Zhou & Y. Chen, S. Chen, J. Chen, 2019)
C. Demersum	NO_3^-	23.10	NA	9.82 NA	(Xu et al., 2019)
V. Zizaniodes	COD	99	NA	68 Anode ares	(Regmi, Nitisoravut, Charoenroongtavee, Yimkhaophong, & Phanthurat, 2018)

2.2 Factors influencing to PMFC performances

2.2.1 Circadian rhythm

Light intensity, quality and photosynthesis period are factors that significantly influence to plant growth and PMFC performance. A light cycle and illumination potential have been investigated in the photosynthetic pathways of PMFCs since they are directly linked to metabolic activities of bacteria (Sønsteby & Heide, 2009). On the other hand, light in the daytime is a potential energy source for photosynthesis resulting in carbohydrate formation and convey them into bio-electricity in PMFCs. Moreover, microorganism communities resided around rhizosphere should be received an amount of optimal light so as to maximize the decomposition of root's exudate for electricity generation. The influence of PMFC performance was reported in several studies (G. Kumar, D. Duc Nguyen, M. Huy, P. Sivagurunathan, P. Bakonyi, G. Zhen, T. Kobayashi, K.i Qin Xu, N. Nemestóthy, 2019; L.Shirley, 2018; Wagner, Besemer, Burns, Battin, & Bengtsson, 2015). For example, Strik et al. authenticated that the increase of light intensity could improve output voltage. In contrast, shading of plants declined the power output which was ascribed by hindering of the photosynthesis process and decreasing of rhizodeposits (Strik et al., 2011).

Four different photoperiods were investigated following order (daylight/darkness): 16/8 h (standard period), 24/0 h, 9/15h, and 0/24h. They found that power outputs declined under the period of 9/15h and 0/24h. In contrast, PMFCs systems which were controlled under photoperiods of 24/0 and 16/8 were differently performed. It was concluded that changes of photoperiods could affect to bio-electric generation due to light-related inhibition (Md Khudzari et al., 2018). Furthermore, photoperiod could bring significant impacts for PMFC systems such as consumption of organic compounds, efficiency of compounds transport to the rhizosphere, excretion of the rhizodeposition, oxidization exudates of electrochemical bacteria, and liberation of the electrons (Lea-smith et al., 2014). Both light and physiology are the limiting factors for bio-electricity production affecting the overall PMFC performance. Therefore, physiological plants can adapt photosynthesis in the root’s exudate production accompany with an efficient uptake by microorganisms that are also suitable for PMFCs as greater bio-energy productivity can be attained. Nevertheless, the determining functions of light intensity can maximize PMFCs performances.

2.2.2 Membrane in plant microbial fuel cell

Conventional ion exchange membranes (IEM) permit ions to pass through a conductive polymeric membrane. On the other hand, membrane plays important roles in PMFC systems which separate between two chambers as a separator, permits protons to pass through from the anode to the cathode, and keeps for the anode in the anaerobic condition (B. Logan, B. Hamelers, R. Rozendal, U. Schroder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, 2006). Because membrane plays a role as a separator, it prevents substances crossover including anolyte, catholyte between the cathodic chamber and the anodic chamber. To complete an entire circuit in PMFCs, electrons and protons must be moved to the cathode chamber to maintain oxygen reduction reaction (ORR). In this case, the IEM has functions allowing protons to pass through the membrane and other components of both chambers are retained, avoiding the disturbance between the two compartments.

Moreover, the anodic compartment should be always kept in the anaerobic condition ensuring anaerobic bacteria activities for attaining higher power output. Such aforementioned functions, ion exchange membrane has paramount importance roles to PMFC performance. Use of a conductive membrane can maximize the current and power densities by facilitating the transportation of proton (Mokhtarian et al., 2013). A conductive membrane in PMFCs is considered in various aspects including materials and structures
which have a major impact on cost-effectiveness and high power output (Zuo, Cheng, & Logan, 2008). In PMFCs, single-chamber which is membrane-less configuration whereas the anode and cathode chambers are directly connected though soil and soil plays as membrane. As compared to MFC, the PMFC for power production and waste remediation is therefore more cost-effective meanwhile MFCs usually invest more cost for PEMs.

2.2.3 Plant candidate for PMFCs

Plants are the main and indispensable component in PMFC systems. Numerous kinds of plants used in PMFCs are shown in Figure 2.2. However, there is a limited number of plant species that could be potential candidates for bio-energy production in

PMFC. Therefore, the application of plants to PMFC is set to certain standards. More specifically, in the mechanism of PMFCs operation, the anode area should be operated under anaerobic conditions to maximum efficiency**.** As a result, plants with anaerobic-withstand ability should be encouraged to be introduced into PMFC systems.

In research of Natalia et al. (Tapia et al., 2017) seven *Sedum* species in a semi-arid green roof were carried out to evaluate their ability for electricity generation. They applied the drip and weekly irrigation method for *Sedum* plants to observe dynamics in the current generation. The result came in different. Under the weekly irrigation, there was a sudden increase of current after watering due soil moisture made up 40 % v/v, while under drip irrigation current was decreased with moisture value around 5 % v/v. It was supported that the low water content in the soil could affect the mobility of proton transportation and increased the internal resistance of PMFC systems (Chiranjeevi et al., 2012). Their result also showed a closeD relationship between soil moisture content and power production. The higher moisture content was the higher was power output.

The power generation in PMFC systems is also very much depending on types of plant as it should be well selected. Numerous types of plant have been explored in the past studies including food crops, marsh plants, rooftop, succulent, and leguminous plant (M Helder et al., 2010; K. Takanewa, K. Nishio, S. Kato, K. Hashimoto, 2010; Sophia & Sreeja, 2017; Tapia et al., 2017). As aforementioned, the anode area should be guaranteed under anaerobic conditions to maximum efficiency. Based on the morphological and functional characteristics of the plants, purple guinea grass species are known to be forage grass that can be easily grown in variety of moisture levels and harvested for feeding animals such as cows and buffaloes. It can also withstand high light intensity (Khota et al., 2016). Therefore, purple guinea grass holds a suitable candidacy in PMFC systems.

Table 2.2 Various types of plants have been used in PMFCs.

Plant Types	Types	Research goal	Growth medium	Power density (mW/m^2)	Ref.
L. Serenne	C3	Chromium removal	Hoagland solution	55 Cathode area	(Habibul et al., 2016)
O. Sativa		Electrode distance	Soil / fertilizer	14.44 Anode area	(K. Takanewa, K. Nishio, S. Kato, K. Hashimoto, 2010)
		Methane gas mitigation	Vermiculite / Hoagland solution	72 Anode area	(Jan BAA, Jonas S, Evelyne B, Jo DV, Pascal B, Willy V, Korneel R, 2014)
		Anode microbe's analysis	Glucose/ acetate, bacto yeast/ Electrolyte solution	19 Anode area	(Kouzuma et al., 2013)
I. Squatica		Power generation	Anaerobic sludge from municipal wastewater	12.42 Anode area	(S. Liu, Song, Li, & Yang, 2013)
E. Srassieps		COD removal and electrode position	Domestic and fermented distillery wastewater	224.93 Anode area	(Venkata Mohan, Mohanakrishna, & Chiranjeevi, 2011)
C. Involucratus		Electricity generation and COD removal	Lotus soil and wastewater	5.9 Anode area	(Klaisongkram & Holasut, 2015)
G. Maxima		Electricity generation	Hoagland solution	67 Anode area	(Strik et al., 2008)
		Microbial community analysis	Hoagland solution	80 NA	(R. A. Timmers, M. Rothballer, D.P.B.T.B. Strik, M. Engel, S. Schulz, M.

					Schloter, A. Hartmann, B. Hamelers, 2012)
C. Indica	C4	Microbial community analysis	Tap water/ rumen microorganisms	18 Cathode area	(Lu, Xing, & Ren, 2015b)
A. Anomola		Bio-electricity and biomass production	Hoagland solution	222 Membrane area	(M Helder et al., 2010)

2.2.4 Medium solution and supporting matrix

The supporting matrix used in PMFC systems comprises graphite granules, vermiculite, and diverse soils (garden, wetland, sediment, and waterlog) (Cheng & Liu, 2014; F. T. Kabutey, Q. Zhao, L. Wei, J. Ding, P. Antwi, F.K. Quashie, 2019). In the construction of PMFCs, supporting matrix is important because it affects on the internal resistance and the distribution of root exudates to the anode (K. Takanewa, K. Nishio, S. Kato, K. Hashimoto, 2010). The PMFC performance is influenced by the exudate availability, the proliferation of microbial families, growth medium, soil properties, number of electrons and protons, operating conditions, configuration design, and physical parameters (F. T. Kabutey, Q. Zhao, L. Wei, J. Ding, P. Antwi, F.K. Quashie, 2019; Goto et al., 2015). However, the most influencing factor affecting plant and microbial behaviours is the medium solution or growth media in PMFCs. Therefore, beside the use of soils, several solutions are supplemented to the supporting matrix so as to enhance the power output. Study of rice *Oryza sativa spp. japonica* PMFC indicated that the amendment of graphene oxide to the soil improved the electricity generation with 49 mW/m^2 which was greater than graphene oxide-free PMFCs (Goto et al., 2015). Additionally, Acetate and modified Hoagland solution were added to *S. anglica* PMFC, the maximum output of 100 W/m^2 was achieved (Timmers, Strik, Hamelers, & Buisman, 2010). Similarly, with the support of the nitrate-less ammonium-rich medium solution in the *S. anglica* PMFCs the maximum current generation increased from 186 mA/m^2 to 469 mA/m^2 (M. Helder, Strik, Hamelers, Kuijken, & Buisman, 2012). When I.aquatica CW-MFC was fed with phosphate buffer solution and incubated with sludge, it could produce 12.42 W/m^2 which was 142 % greater as compared to 5.13 W/m^2 of unplanted CW-MFC (S. Liu et al., 2013). The additions for supporting matrix is suggested in PMFC systems so as to improve the bioelectricity production and overall PMFC performance.

On the other hand, purple guinea (Panicum maximum) is one of the most common cattle feed grass in Thailand (Hare, Phengphet, Songsiri, & Sutin, 2015; Pongtongkam, Nilratnisakorn, Piyachoknakul, & Thongpan, 2005). It is a perennial grass that is generally harvested with high yield and protein content sources for animal feed. Under regular cutting regimes (30-45 days), guinea grass usually produces dry matter yields of 33- 46 tons per hectare per year (Hare et al., 2015). Farmers applied 125-310 kg urea/hectare after each clipping and poultry manure at a rate of 2.8-5.6 tons/hectare in every 60-90 days. The purpose of urea supplement from farmers after each batch aims to foster the growing processes, branches, leaves, green color, strong photosynthetic leaves, and high yield. In recent years, the interest in using organic materials from agriculture residuals is being risen worldwide. Organic farming has been determined as an agriculture production systems which obviates the application of synthetic materials (Babalola, Adigun, & Abiola, 2018). Previous studies have shown positive impacts of organic in terms of environment, yield productivity, and soil fertility (Adugna, 2018; Lichtenberg, 1992). Although chemical fertilizers are substantial inputs to obtain higher crop productivities, the use of overloaded urea and chemical fertilizers can lead to the degeneration of the soil properties and the productivity of grass yield over time.

2.2.5 Defoliation of plants

Defoliation is the removal of grass parts, typically leaves or roots, by hand cutting or grazing. Numerous studies have shown that defoliation in different grass species can stimulate more root exudates in the form of carbon (Dyer & Bokhari, 1976; Patra AK, Abbadie L, Clays-Josserand A, Degrange V, Grayston SJ, Loiseau P, Louault F, Mahmood S, Nazaret S, Philippot L, Poly F, Prosser JI, Richaume A, Le R, XPatra AK, Abbadie L, Clays-Josserand A, 2005).

In the study of Bokhari et al. (Bokhari, 1977), they reported that grazing could increase the exudates of roots which contain mainly carbon. Compounds excreted from the root by grazing typically consist of amino acids, organic acids, sugars, etc., which generally spur rhizosphere processes with abundant nutrients for plants (Bokhari, 1977). A study with grazing on Poa pratensis grass in Yellowstone National Park showed the effects of herbivores on root carbon exudations, nitrogen contents, and responses in nutrient uptake of a plant (Hamilton, Frank, Hinchey, & Murray, 2008). The study showed that defoliated plants increased carbon excretion in the rhizosphere which was 1.5-fold higher than non-defoliated plants and simultaneously promoted the concentration of microbial biomass. Defoliation also facilitated rhizosphere processes leading to 2-fold and 1.5-fold increases for leaf and root nitrogen contents, respectively (Hamilton et al., 2008).

Typically, researchers assess the PMFC performance with plant growth for one life cycle (Liesje DS, Leen VDB, Hai SD, Monica H, Nico B, Korneel R, 2008; Shen et al., 2018; Tapia et al., 2017). There are still limited reports in the literature that have provided a systematic investigation of plant regrowth in association with electricity regeneration. A comprehensive analysis of defoliated-PMFC and non-defoliated PMFC is needed to be performed, to confirm whether defoliation brings positive or negative feedback for PMFC systems.

2.2.6 PMFC configurations

Another substantial key for electric generation in PMFC is the effective configuration. Designing an effective configuration should be focused in order to eliminate numerous bottlenecks including over-potential, Ohmic losses, internal resistance (Rabaey & Verstraete, 2005). These components should encompass of substrates, configuration design, inoculums, types of membranes, electrode materials, electrode distance and resistance (Cheng, Liu, & Logan, 2006). Moreover, an ideal configuration also can replace chemical catalysts and expensive proton exchange membrane.

Single-chamber PMFC illustrates for a membraneless configuration that the anode and cathode are placed on the either side of the bioreactor at a given distance. The single chamber PMFC construction based on the principle that the soil can act as membrane in PMFC systems and permit cations to pass through the soil matrix. Therefore, the anode is generally fixed at a pertinent distance near the rhizosphere where bacterial communities can utilize most nutrients excreted by root systems. While cathode is placed above soil surface and contacted directly to the air. Due to remarkable advantages such as simple operation, cost-effectiveness, and PEM is not required, the single-chamber PMFCs are used widely in numerous studies (Brunelli, Tosato, & Rossi, 2016; Lu et al., 2015a; Md Khudzari et al., 2018; Moqsud et al., 2015; Tapia et al., 2017). However, the biggest drawback of single-chamber is the large amount of oxygen diffusion into anode area reducing the activities of anaerobic bacteria and replaced by aerobic bacteria (Leong, Daud, Ghasemi, Liew, & Ismail, 2013). The single-chamber PMFC is illustrated in Figure 2.3.

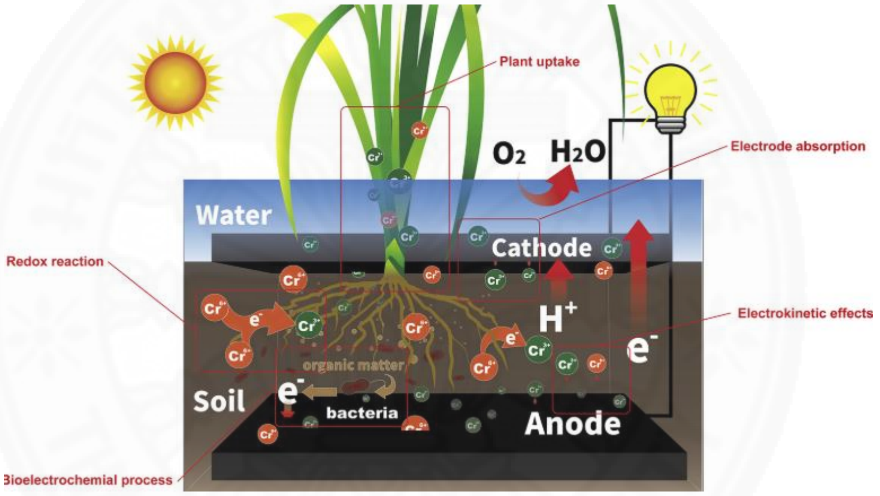


Figure 2.3 Single-chamber configuration of PMFC (Guan, Hu, & Yu, 2019).

In order to minimize disadvantages in terms of oxygen diffusion and internal resistance of single-chamber PMFCs, dual-chamber PMFC is developed that typically shows the separate chambers connecting to a PEM where protons pass through from the anode to cathode (W. W. Li, Sheng, Liu, & Yu, 2011). The dual-chamber PMFC is fabricated in different ways in previous works (Figure 2.4).

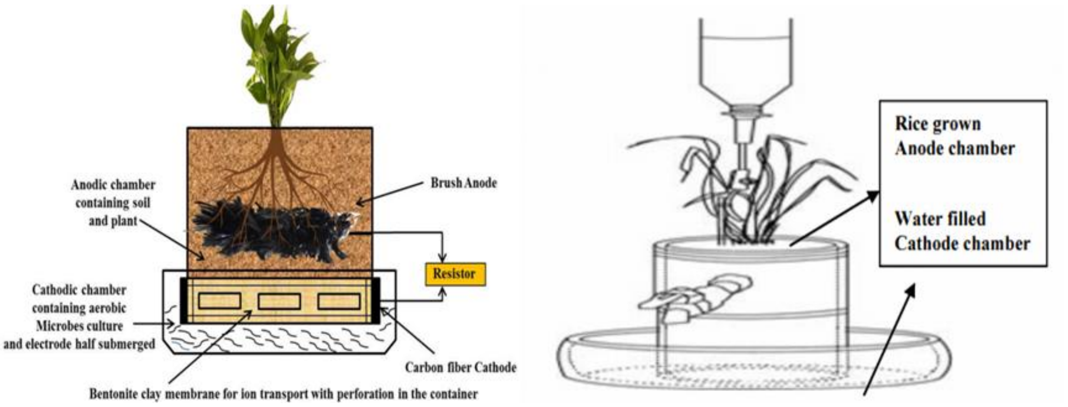


Figure 2.4 The dual-chamber in PMFC systems (Sarma & Mohanty, 2018)(Regmi & Nitisoravut, 2017).

A tuberous plant PMFC produced up to 222 mW/m^2 anode area from a single-chamber, which was constructed with the combination of clay and kaolinite and the clay wall was acted as a proton membrane (Sophia & Sreeja, 2017). Air-cathode PMFC with alkaligrass generated the maximum power density of 83.7 W/m^2 cathode area (Md Khudzari et al., 2018). In dual-chamber PMFC, *Spartina anglica* obtained the highest output of 222 mW/m^2 membrane surface area. These studies suggested that PMFCs can operate with various types of configuration with utmost productively electrical generation. However, a comparative study on the ability of the different configurations PMFC is still limited. It is necessary to consider whether configuration design literary effect to output generation.

2.2.7 Electrode materials

Electrode materials in PMFC systems are used to collect electrons released from the systems. Selecting effective electrode materials is essential to maximize the electrical generation as well as cost-effectiveness. Most of the electrodes usually made from the carbon in different types such as graphite granules, carbon cloths, carbon plate/rods, etc. Numerous studies revealed that the maximum power production and transfer of electrons were obtained when similar electrode materials for both cathodes and anodes were used in PMFCs (Chiranjeevi, Yeruva, Kumar, Mohan, & Varjani, 2019).

From Table 2.3, it is seemed that graphite felts were used in most PMFC studies due to its efficiency, cost-effectiveness, and durability. In most previous studies, the simple anode graphite is generally added with other electrode materials such as graphite granules, granular activated carbon to increase the effectiveness of PMFC (S. Liu, Song, Wei, Yang, & Li, 2014; Strik et al., 2008; Timmers, Strik, Hamelers, & Buisman, 2013).

2.2.8 Rhizosphere and microorganism in PMFCs

Rhizosphere which is directly related to the anodic areas in PMFCs represents an essential matrix which is filled with the carbon compounds, anaerobic bacteria, and electron acceptor. It facilitates the digestion of organic compounds and oxidizing them into electrons. Roots are utmost important for plant growth as well as metabolic activities. In PMFC, organic matters derived from plants both rhizodepositions and dead cell materials are often oxidized into electricity. Rhizodepositions comprise of exudates, lysates, and secretions. Exudates and organic compounds both high and low molecules are oxidized into electrons, protons and carbon dioxide by electroactive bacteria resided around rhizosphere (Nitisoravut & Regmi, 2017).

The proportion of net fixed carbon transferring to root systems is shown differently with annual and perennial plants. Among perennial plants, the proportion of 70-80 % of net fixed carbon generally is transported to roots, in which 8-65 % is delivered as rhizodepositions. While fixed carbon to roots of annual plants solely makes up 30-60 % (Howarth, 1984; Pabon, 2009). However, most of the PMFC works came from annual plants. There are limited reports about perennial plants in PMFC systems and it is appealing to determine their effectiveness in PMFC biotechnology.

On the other hand, the rhizosphere is the habitat for the microorganisms in the soil and supports for their activities and attachments in the root systems (Bakker, Berendsen, Doornbos, Wintermans, & Pieterse, 2013). EAB near the rhizospheres uses the root exudates as food sources and releases electrons to the anode by conductive wires or mediated electron transfer. Typically, the root systems support bacteria through providing essential substrates in form of carbon and transfer the elemental forms into ionic form aiding for the plant growth (Moulin, Muniue, & Dreyfus, 2001).

The presence of microbial communities is different in PMFC depending on plant types, soil matrix, growth media, and operating conditions (Cabezas, 2010;

Nitisoravut & Regmi, 2017). A study to determine the phylogenetic diversity in rice PMFC found that *Proteobacteria* was the most abundant population (38 %) in the total of detected species in closed circuit condition. The followed bacterial population was *Chloroflexi*, *Acidobacteria*, *Bacteroidetes*, and *Actinobacteria* (Liesje DS, Leen VDB, Hai SD, Monica H, Nico B, Korneel R, 2008). However, the presence of bacterial families showed differently in OCV when *Chloroflexi*, *Deltaproteobacteria*, *Firmicutes spp, Alphaproteobacteria, and Betaproteobacteria* were the most abundant species in rice field PMFC (Cabezas, Pommerenke, Boon, & Friedrich, 2015). In another work, Lu Lu et al showed differently whereas *Geobactor* species was the most dominant with 7.4% in *Canna indica* PMFC, followed by *Phylum Acidobacteria* (Schamphelaire, Boeckx, & Verstraete, 2010). The communities of 80 % *Ruminococcaceae and Clostridiaceae* were found in *G.maxica* PMFC (R. A. Timmers, M. Rothballer, D.P.B.T.B. Strik, M. Engel, S. Schulz, M. Schloter, A. Hartmann, B. Hamelers, 2012). It was reported that there were certain number of beneficial bacterial species for electricity generation in PMFC systems including *Geobacter sulfurreducens*, *Shewanella,, Rhodoferax ferrireducen*, *Bacillus*, *Geothrix, and Pseudomonas* (Jong et al., 2006; Logan, 2009).

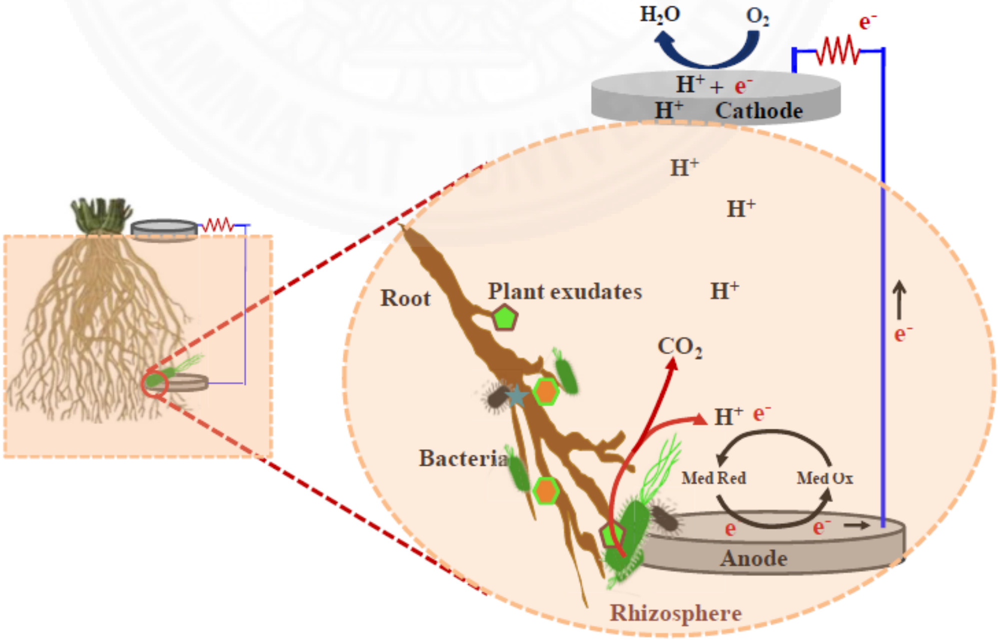


Figure 2.5 Rhizosphere operating mechanism in PMFCs (Chiranjeevi et al., 2019).

Table 2.3 Electrode materials used in PMFCs.

Types of plants	**Power output (mW/m^2)**	**Electron materials**		**Ref**
		Anode	**Cathode**	
G. maxima	67.0 Anode area	Graphite felt / graphite granules	Graphite felt	(Strik et al., 2008)
Rice Paddy	6.0 Anode area	Graphite felt	Graphite felt	(Kaku et al., 2008)
O. sativa	11.11 Cathode area	Manganese-based catalyzed carbon/ nickel mesh	Carbon felt	(S. Liu et al., 2014)
G. maxima	10/12 Membrane area	Graphite felt / graphite granules	Graphite felt	(Timmers et al., 2013)
T. latifolia	6.17 Anode area	Graphite	Magnesium	(Can & Yakar, 2017)
I.aquatica	55.05 Anode area	Carbon cloth/granular activated carbon/stainless steel mesh	Granular activated carbon	(Ueoka, Sese, Sue, Kouzuma, & Watanabe, 2016)
S. bicus ***C. dactylon***	23.0 10.07 NA	Nickel Graphite	Nickel Graphite	(Gilani, Yaseen, Zaidi, Zahra, & Mahmood, 2016)
S. anglica	88.0 NA	Graphite granules	Graphite-felt	(Marjolein Helder et al., 2013)
I. aquatica	75.12	Graphite felts	Graphite felts	(C. Cheng, Y.

	Anode area			Hu, S. Shao, J. Yu, W. Zhou & Y. Chen, S. Chen, J. Chen, 2019)
C. comosum, ***C. floribunda*** ***P. diffusus***	18.0 Anode area	Graphite	Graphite	(Azri, Tou, Sadi, & Benhabyles, 2018)

2.3 Applications of PMFCs

The PMFC biotechnology is available for diverse applications comprising environmental condition monitoring devices (Donovan, Dewan, Heo, & Beyenal, 2008), plant growth sensors (Brunelli et al., 2016), wastewater treatment (Venkata Mohan et al., 2011), pollution degradation, bioremediation, heavy metal recovery, electricity generation (C. Cheng, Y. Hu, S. Shao, J. Yu, W. Zhou & Y. Chen, S. Chen, J. Chen, 2019), and ecological engineering system (EES) (Can & Yakar, 2017; Lu et al., 2015a; Venkata Mohan et al., 2011). The applications of PMFC are discussed in detail below.

PMFC has been used for bioelectricity production from various types of wastewater and it has been interpreted as environment-friendliness, cost-effectiveness, high rate of organic removal, and sustainability (Habibul et al., 2016). The fuel cell using *Eichornia*-floating macrophyte ecosystem illustrated an effectual bioelectricity production and organics remediation with the current density of 224.93 mA/m^2, 86.67 % COD removal, and 72.32 % VFA removal, respectively (Venkata Mohan et al., 2011). The maximum power output of 9.4 mW/m^2 and 76 % COD removal were achieved from swine wastewater CW-MFC using aeration at cathode to improve the output of the systems (Zhao et al., 2013). In an up-flow *Typha latifolia* CW-MFC, the significant dedications were found with the high pollutant treatment rates including 100 % COD, 91 % NO_3^-, and 40 % NH_4^+ removal while the maximum power output and coulombic efficiency were obtained with 6.12 mW/m^2 and 8.6 %, respectively (y. L. Oon, S.A. Ong, L. H. Ho, Y. S. Wong, Y. S. Oon, H. K. Lehl, 2015). Similarly, up-flow CW-MFC coupled with cattail plant provided a maximum power output of 93 mW/m^2 while it delivered high pollutant removals of 99, 96, and 46 % for COD, NH_4^+, and NO_3^- removals respectively (y. L. Oon, S.A. Ong, L. H. Ho, Y. S. Wong, F. A. Dahalan, Y. S. Oon, H. K. Lehl, 2016). *Phragmites australis* was used in CW-MFC to generate electricity whiles removed nutrients from swine slurry wastewater. The maximum power density obtained was 0.268 mW/m^2 with the ammonium and reactive phosphorous removals of 75 and 85 to 86 5 %, respectively (Doherty, Zhao, Zhao, & Wang, 2015). In spite of low electricity generation in PMFC systems, it is a promising technology for wastewater treatment.

The wetland PMFC and soil rice paddy are enriched with various organic matters supporting for hydrophytes and bacterial activities that can be essential bioenergy production sources in PMFCs (Kaku et al., 2008). Experiments were carried out to mitigate gas emission and harvest electricity from wetlands and rice fields (Arends & Verstraete, 2012; Yan, Jiang, Cai, Zhou, & Krumholz, 2015). CW-MFC was assessed in a single chamber configuration which anode was placed in rhizosphere and cathode was placed on the surface to alleviate methane emission and produce electricity. The methane remediating rate was 71-82 % of the total methane fluxes with the maximum current density of 187 mA/m^2 (S. Liu, Feng, & Li, 2017). Macrophyte SMFC and a control SMFC without the plant were operated for 367 days to assess the degradation rate of pyrene and benzo compounds in the rhizosphere. It was observed that the combination of macrophyte SMFC obtained the complete degradation of 70 % as compared to control SMFC (Yan et al., 2015). Pot-culture MFCs cultivated with rice plants were compared, methane emissions in closed circuit condition MFCs showed a 20-fold reduction than unplanted PMFC (Cabezas, 2010). Jan B. A. Arends (Jan BAA, Jonas S, Evelyne B, Jo DV, Pascal B, Willy V, Korneel R, 2014) showed that inserting an electrode in the rhizosphere in a bioelectrochemical system resulted in current production before methane was emitted, the anoxic systems could postpone the methane emissions and about 50 % methane reduction was observed.

Moreover, the applications of PMFC-based technologies have been extended including bio-sensors. Bio-sensors are analytical facilities for detecting analytes which use biological elements as a physicochemical detector stickled to a physical transducer (J. Z. Sun, G. P. Kingori, R. W. Si, D. D. Zhai, Z. H. Liao, D. Z. Sun, T. Zheng, 2015; Labro, Craig, Wood, & Packer, 2017). A PMFC was carried out with the dual-purpose of biosensor monitoring plant health and power generation to provide a wireless electronic system to connect the environmental data and flora health status. The PMFC produced a maximum voltage and a maximum current of 502 mV and 590 μA respectively while serving as an energy source accompanied biosensor (Brunelli, Tosato, & Rossi, 2017). Davide Brunelli et al. (Brunelli et al., 2016) applied plant MFC to explore a sustainable wireless sensor node which was capable to monitor both health status and environmental parameters. Their results indicated that the power generated form PMFCs was adequate for obtaining energy- neutral smart sensor which samples and delivers data. Those experiments were observed that the rate of generated power was related to the health of plants living with symbiosis and bacterial colony. The applications of PMFCs are explicitly shown in Figure 2.6.

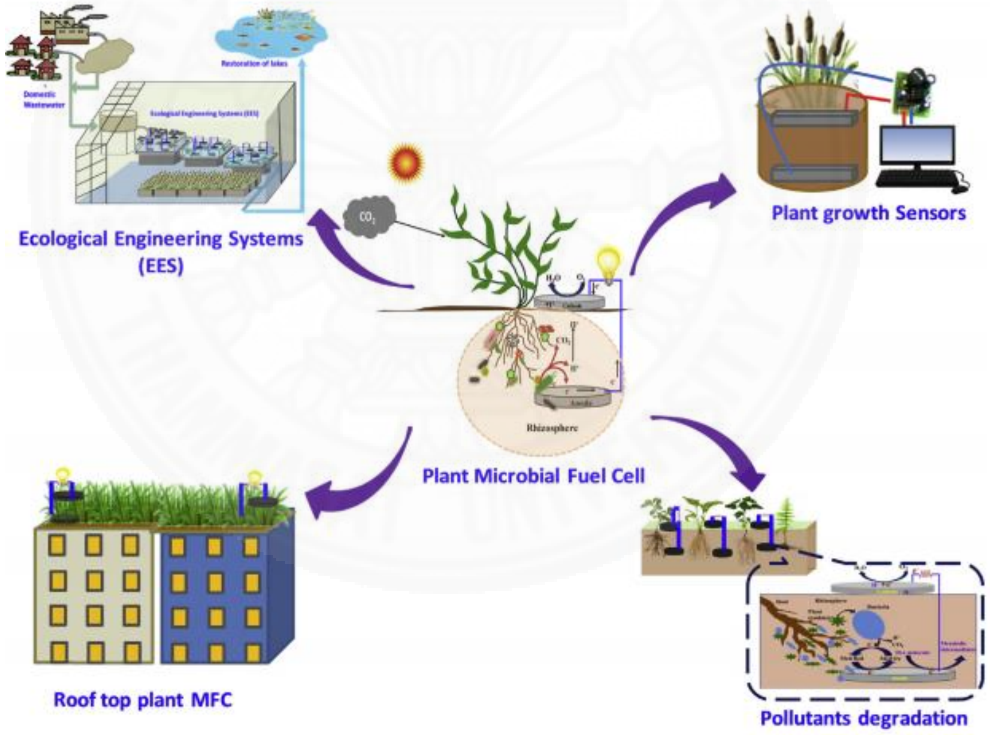


Figure 2.6 Basic applications of PMFC systems (Chiranjeevi et al., 2019).

2.4 Perspectives and challenges of PMFCs

Numerous exertions have been carried out with PMFCs from lab-scale to field-scale works for the practical application of this technology. It is considered as one of the most sustainable and renewable bio-electrochemical technology for both wastewater treatment and electricity generation. Plants and soil microorganisms are the core drivers of power production in PMFC systems. A variety of factors such as configuration design, electrode materials, plants, bacterial communities, electrodes position, photosynthesis pathway, and physical parameters have been intensively explored. This indicates that individual limitations of the PMFC technology are gradually solved and improved over time. The PMFC systems are cable to produce continuous and stable flow of electricity without any destruction to plant growth, it is therefore ensure abundant energy source for bio-sensors while treating the ecosystems (Regmi, Nitisoravut, & Ketchaimongkol, 2018). Diverse plants can be applied in PMFCs in many locations such as rooftop, indoor, food crops, marshy plants, forage grass for bioelectricity harvest while mitigating the gases emissions, restore the ecosystem, remediate wastewater as well as create a clean environment.

Beside significant contribution of PMFCs, challenges and drawbacks are inevitable. The biggest drawback of PMFC is the low yield of electricity from the systems. Further studies on configuration fabrication, electrode modifications, and improve operational conditions are recommended (Q. Zhao et al., 2017).

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Material and methods

3.1.1 Selection of plants

Based on the morphological and functional characteristics of the plants, guinea grass species (*Panicum maximum*) is nominated to investigate in PMFCs. In this study, the purple guinea was selected to observe their performable capacity to PMFCs.

Guinea grass is one of the most popular forage grass in Thailand that can be planted in a variety of soils and various conditions (Ratsamee et al., 2012). It is a perennial plant, easily harvests, and produces high yield for fermenting or dry provender. In addition, purple guinea has low agricultural chemical consumption and requires less intensive agricultural management. Guinea grass can withstand in harsh environmental conditions such as acidic soil, low fertility, etc. Although this plant prefers living in moisture conditions, it also can withstand in dry seasons. Life circle or root retention time of this plant can prolong up to three years or longer, thus its circle will contribute a significant in electricity regeneration by regular defoliation (leaf-cutting).

Choice of pertinent plants can help to maximize output efficiency in PMFC systems. Providing effectively exudates and good photosynthesis performance of plants are the ways to attain high power output in PMFC. Based on the photosynthetic pathway, plants are divided into three main types: C_3, C_4, and CAM. Each type of plant serves different efficiencies, the most efficient plants are C_4, which typically live in prolonged damp conditions, high temperature and light conditions (Nitisoravut & Regmi, 2017). Comparing C_4 plants with others, C_4 plants are better than C_3 and CAM plants in terms of higher intensity of photosynthesis, lower CO_2 offset, higher light saturation point, lower water demand, and higher yield. This means that C_4 plants have a higher solar energy conversion rate into electricity. On the other hand, *Panicum maximum* is C_4 plant and permanent species (Fladung, 1994). Therefore, with such characteristics, *Panicum maximum* might be a promising and exciting candidacy for plant microbial fuel cell.

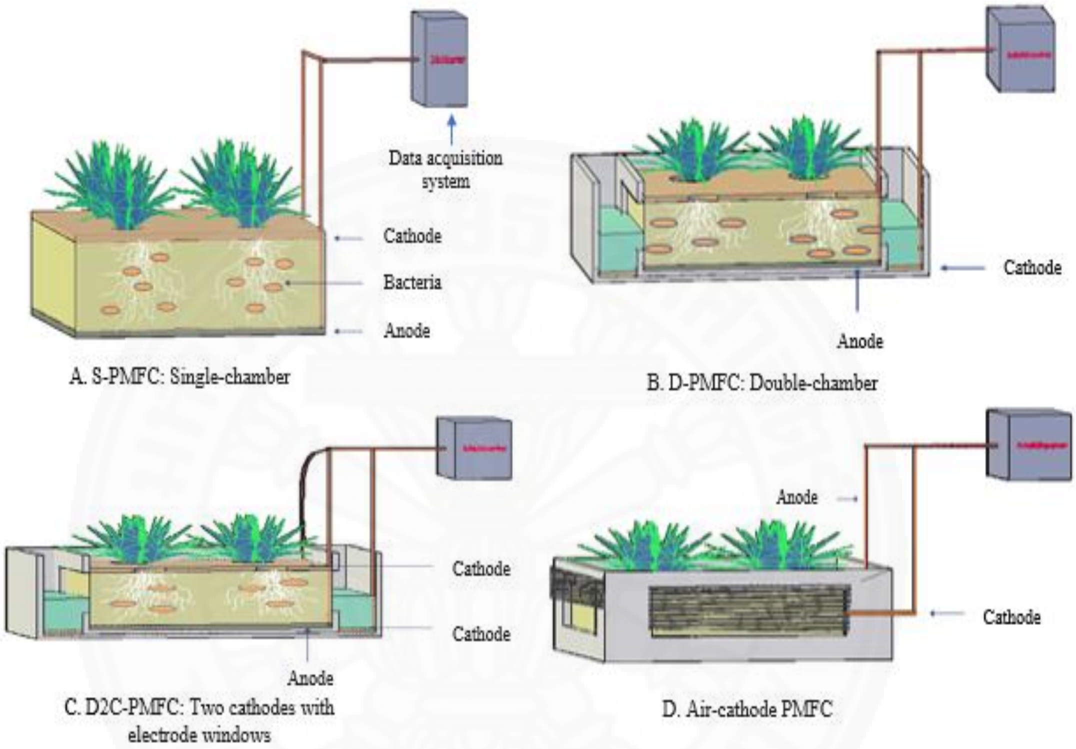
3.1.2 PMFC configurations and fabrications

Four PMFC configurations, including single-chamber (S-PMFC), double chamber (D-PMFC), double-chamber equipped with 2 cathodes (D-2CPMFC), and air-cathode, were constructed in this study using a rectangular styrofoam box (height 28 cm × length 37 cm × width 25 cm). Carbon cloth was selected as electrodes with dimensions of width 30 cm × length 20 cm, and thickness of 0.25 mm. Copper wire was adopted for the electric carrier between the anodic and cathodic chambers to complete the electrical circuit. Earthen sheets with a dimension of width 10 cm × length 20 cm were collected from tile-company (Thailand) and used as a membrane due to its high proton conductivity, cost-effectiveness, durability, and environmental friendliness. The carbon cloth anode was fixed approximately 14 cm below the soil surface in all PMFC systems. This was to assure anaerobic conditions for the oxidation process. In addition, placing the anodes at a pertinent distance around rhizospheres can utilize most exudates excreted from root systems to generate more electrons for PMFC systems. An anode area of 0.067 m^2 was used as the area for calculations in this study.

Figure 3.1A illustrates a single-chamber configuration where soil acts as a proton exchange membrane. The cathode was placed at the top-soil surface and attached with an electrical wire. In the double chamber (Figure 3.1B), four sides of the styrofoam containers were equipped with earthen membranes allowing proton transport to the cathode. Unlike a single-chamber PMFC, the carbon cathode in the D-PMFC was wrapped around the earthen separators to promote the effective collection of protons which were transferred through four sides of membranes to the cathodic chamber. In double chamber PMFCs, a styrofoam box was placed inside of a rectangular plastic box. The outer plastic box had dimensions of width 48 cm × length 75.5 cm × height 43 cm. This set up created a water-jacket circle around the inner box. A circulation pump was installed in order to promote water circulation.

Electrons in the anode area may pass spontaneously to the cathode area via two pathways. The first pathway is by the mobility of cations within the soil matrix as soil can be a membrane for cation transport. In the second pathway, protons can be transported through the electrode membranes which were wrapped around the soil matrix to the outer cathode. Based on these facts, cation transport was promoted by placing contemporary cathode material on the soil surface and around the membranes, which was called D2C-PMFC (Figure 3.1C).

Figure 3.1D shows an air-cathode PMFC configuration. The cathode was installed by wrapping carbon materials around membranes with direct contact to air. All of the PMFC configurations are indicated in Figure 3.1.



A. S-PMFC: Single-chamber

B. D-PMFC: Double-chamber

C. D2C-PMFC: Two cathodes with electrode windows

D. Air-cathode PMFC

Figure 3.1 PMFC configurations used in this study.

Figure 3.2 Purple guinea grass at different stages and construction of PMFC systems.

3.1.3 Growth media and experimental preparations

In cultivation, organic fertilizers are essential for soil fertility and plant growth. In order to enrich the fertility of soil and protect the environment, many farmers have utilized agriculture residuals as a cost-effective fertilizer, such as rice straw, hay, legumes, etc. In this study, rice straw and dry cow manure were used for the preparation of compost. Rice straw was chopped into pieces to facilitate decomposition and mixed with manure and water. This mixture was placed in an earthen pot and covered to ensure a temperature of 45 - 50 ^{0}C. After 15 days of composting, the mixture was checked to ensure suitable conditions for composting. After 30 days the mixture was decomposed into organic compost. Fifty grams of compost and 4.5 Kg of commercial soil were combined and put in styrofoam containers as a soil matrix for water content, defoliation, and configuration studies.

In fertiliser amendment studyabout 2.0 gram of urea and 35 grams of compost were applied in each PMFC pot. The PMFCs were completed with four treatments which are urea-PMFC (UPMFC), compost-urea PMFC (CU-PMFC), compost-PMFC (C-PMFC), and a control PMFC (without any urea and compost). All PMFC experimental systems were duplicated resulting in 8 pots. The purple guinea plant was selected for the experiments. Plants were cultivated in PMFCs including 2 bunches of total 30 cm tall and total biomass of 16 g. The experiments lasted for 50 days. Main functions of medium are illustrated in Table 3.1.

Table 3.1 Growth media used in this study.

Types of growth media	Purpose of uses
Organic matter (Cow manure couple with rice straw)	Biomass and electricity production
Phosphorus fertilizer	Rooting, flowering, exudates
Urea Fertilizer	Biomass and bacteria concentration
Potassium	Uptake water and stimulate exudate production

The properties of commercial soil were determined via ICP-OES method (Model: Optima 8300, PerkinElmer, Singapore), CHNS/O analyzer (Model: 628 series, Leco Corporation, USA), and microwave digestion (Model: Titan MPS, PerkinElmer, Germany). Several properties of soil are shown in Table 3.2.

Table 3.2 Chemical properties of soil in this study.

Chemical Properties	Concentration / value	Units
Ca	210.68	mg/L
K	33.9	mg/L
Mg	31.96	mg/L
Na	20.47	mg/L
pH	8.15	
Soil Conductivity	933	µs/cm
Carbon	10.7	%w/w
Hydrogen	1.46	%w/w
Nitrogen	9.32	%w/w
Sulphur	0.016	%w/w

3.1.4 Soil sampling and PMFC operation

All PMFC systems were placed in a greenhouse on the rooftop of the laboratory building of Sirindhorn International Institute of Technology, Thammasat University, Thailand. Open circuit conditions were initiated during the early stage of experiments.

The anode and cathode were connected with an external load of 100 Ω for soil water content, configuration, and defoliation experiments and 250 Ω for fertilizer treatment study. The external load was inserted after two weeks of operation to facilitate the adaptative capacity of bacterial communities in the new environment. The cell voltage and temperature were recorded at 30-minute intervals using a Wisco data acquisition system. A multimeter (UNI-T 30B) was occasionally used to assure the accuracy of the online data acquisition.Moreover, all PMFC systems were duplicated to assure the .accuracy the PMFC performance.

After completion of the comparative study on configurations, the D-2C PMFC was transformed into the D-PMFC by removing one cathode of the D-2C PMFC to aid the defoliation. After this transformation, systems were run under the open circuit voltage for five days to stabilize the operation. Plants in PMFC pots were clipped about 5 cm aboveground with a scissor and biomass were harvested for analysis. Soil organic and nitrogen contents were determined by taking soil samples at 12 cm deep after twenty-four hours and twenty days of treatment. These soil samples were measured by a LECO CHN328 elemental analyser (LECO Corporation; Saint Joseph, Michigan USA) at Thammasat University, Thailand.

The maximum power was determined via polarization curves by setting various external resistors of a resistor box (GAMMA CO 6DECADE). Series of external loads were connected in descending order (5000, 3000, 2000, 1000, 500, 250, 200, 100, 50, and 5 Ω) every 10 minutes.

3.1.5 Scanning electron microscopy (SEM)

Bacteria attachments in carbon cloth anode were observed by using the field emission scanning electroscope (FESEM). Small carbon pieces holding bacteria cells were removed carefully in anode by tweezers into anaerobic containers. The cells on surfaces were fixed with 2 % of glutaraldehyde in 0.1 M potassium phosphate buffer. After overnight fixation in 4 ^{0}C, cells were dehydrated with a graded series of ethanol (50%, 70 %, 85 %, 95 %, 100 %, and 100 %). They were dried using a critical point drying. The prepared samples were coated with gold to avoid destroying and protect sample surfaces. The final step was the observation of samples with a field emission scanning electron microscopy machine.

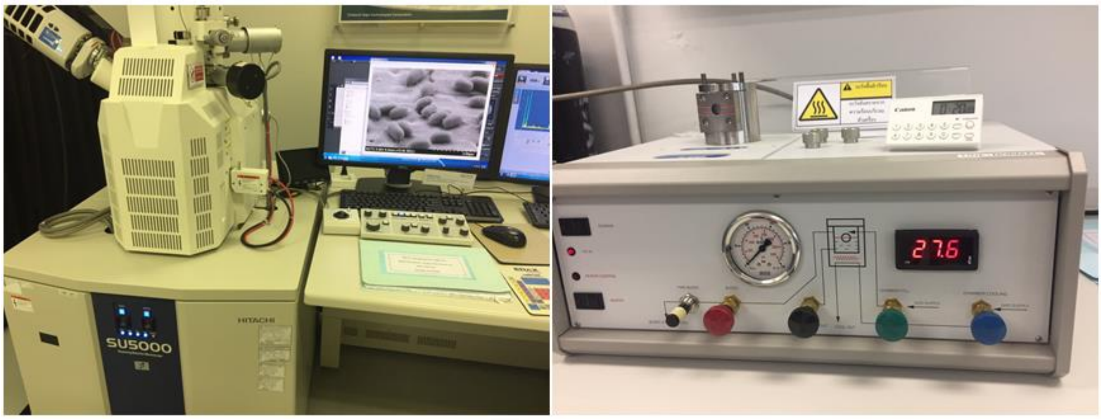


Figure 3.3 Field emission scanning electron microscopy and drying critical point facilities.

3.1.6 Soil measurements

a. Soil electrical conductivity, resistivity, and salinity

Because the electrical conductivity typically fluctuates along with the depth of soil and the nutrients are enriched at the vicinity of the rhizosphere. It is utmost paramount to take note which areas are the most pertinent place for selecting samples for analysis. In this study, the soil samples at a distance of 14 cm under the soil surface were selected for determining the soil electrical conductivity, resistivity, and salinity. A mixture of 1:3 soil and water was prepared by weighing 10-gram soil collecting from systems into 30 ml reverse osmosis water. The mixture was shaken in a 90 rpm shaking incubator at 25 ^{0}C for 1 hour. All aforementioned parameters were then measured by a conductivity meter (Mettler Toledo) (Figure 3.4). The used protocol was described in Kellogg soil survey laboratory methods manual (Kellogn, 2014).

b. pH

Soil-water (1:1) solution was prepared for pH measurement. A mixture of 20-gram soil and 20 ml of reverse osmosis water (Soil-water) was stirred in a 90 rpm shaking incubator at 25 ^{0}C for one hour. Subsequently, 20 ml reverse osmosic water containing 0.02 M $CaCl_2$ was added to the suspension of 1:1 ratio solution of soil and water, stirred for 30 seconds and measured. The pH measurement was supported by using OHAUS Starter 3100 pH Bench meter (Figure 3.4B). The used protocol was described as Kellogg soil survey laboratory methods manual (Kellogn, 2014).

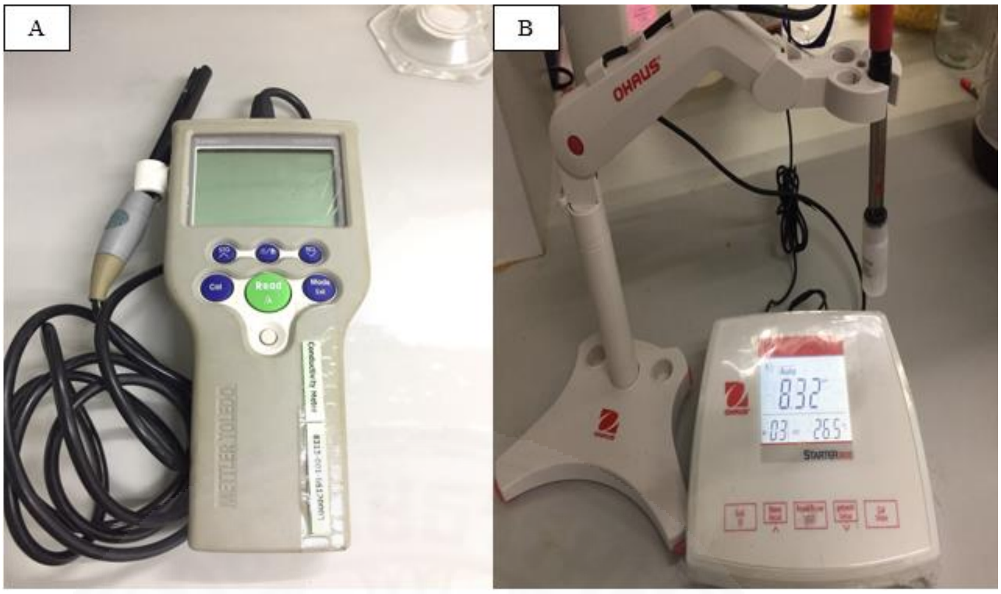


Figure 3.4 Conductivity meter (Mettler Toledo); B. OHAUS Starter 3100 pH Bench meter.

3.2 PMFC analysis

3.2.1 Bioelectricity generation

During early period of experiments, open circuit voltage (OCV) was monitored for all systems. The external resistors were installed to PMFC systems after two weeks of operation after acclimation of bacterial activities. Since then, voltage was recorded online using the Wisco data acquisition system at 30-minute interval and occasionally monitored by a multimeter (UNI-T 30B). The current and power densities were calculated based on anode area of 0.067 m^2. Maximum power was examined through polarization curves by applying various external loads on resistor box (GAMMA CO 6DECADE).

Cell voltage, current, power, and internal resistance are generally used as outputs to analyze and compare the performance of PMFC systems. Rhizosphere or anode area where is contained supporting matrix and natural biochemical reactions, anode is used as the projected area for output calculations. Alternatively, the cathode or membrane can also be projected areas depending on the purpose and concentration of studies.

3.2.2. Power density

Maximum power was examined by the Polarization curve by setting up various external loads using resistor box. So as to compare power generation between different systems, power density was standardized to the anode surface where series of biochemical reactor occurred. The power density was calculated on the fundamental of the anodic area.

Power: The performance of the PMFC system was evaluated through current and power outputs following the formula:

$$P = I \times E_{\mathrm{cell}} \qquad (2.1)$$

Where P is a power output achieved from PMFC systems (mW), E_{cell} is a voltage obtained from a closed circuit (mV), and I is a current computed by the Ohm's law.

Power density: In order to compare power generation between different systems, power density was standardized to the projected area. It was determined on the basis of the projected area (mW/m^2):

$$\mathrm{P} = E_{cell}^{2} / (\mathrm{A}_{n} \times \mathrm{R}_{\mathrm{ext}}) \qquad (2.2)$$

Where A_n is a projected surface (m^2) below the plant growth area, E_{cell} is a voltage obtained from a closed circuit (mV), and R_{ext} is an external resistor (Ω).

3.3.3. Biomass production

Plant growth was observed by help of digital camera, ruler, and scale. Any biological effects or organisms that are detrimental to the grass were monitored and analyzed. Besides, aboveground biomass, stem biomass, leaf area, and color of leaves were also observed.

3.3.4. Physical parameters

Ambient temperature was recorded with type K thermocouple connected to the Wisco data acquisition system at 30-minute interval. The light intensity was measured by a lux meter (GM1010) during 7 days of closed-circuit operation to assess their effects on the power production in PMFCs. The light intensities were recorded in the morning at 9:00 a.m. and in the afternoon at 15:30 p.m.

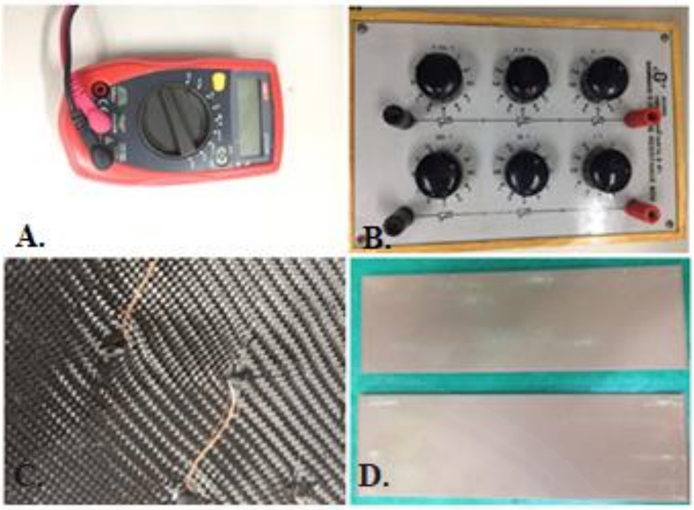


Figure 3.5 A. Multimeter; B. Resistor box; C. Carbon cloth; D. Earthen material.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Overall performance of purple guinea grass under different soil water contents in single chamber plant microbial fuel cell

4.1.1 Voltage fluctuation in open circuit condition PMFCs

The performance of forage grass PMFCs has been evaluated under open circuit condition. Soil water contents were manipulated to obtain three PMFC systems which were dry-soil (D-PMFC), wet-soil (W-PMFC), waterlog (WL-PMFC). Irrigation frequencies were made differently having one per week for D-PMFC, once per day for W-PMFC, and two times per day for WL-PMFC. As shown in Figure 4.1, all reactors obtained the maximum cell voltages at the different time reflecting the nature of bio-systems. In the case of D-PMFC, the cell voltage was stable at low power production due to the irregular irrigation regime. In the irregular irrigation regime, the PMFC sysmtems were not active when systems remained fixed status while the systems are recieved water regularly usually more fluctuative accompanied by water flow. The maximum voltage value of D-PMFC was 273 mV. While the significant fluctuations were observed with W-PMFC and WL-PMFC. The cell voltage generation in W-PMFC and WL-PMFC varied from the minimum voltage of 23 and 14 mV to the maximum voltage of 314 and 505 mV, respectively. The fluctuations of W-PMFC and WL-PMFC could be attributed to the frequent water irrigation. The negative values in days 5 and days 8 in were monitored to be affected by regular water supply when a short circuit phenomenon occurred leading negative cell voltage values. However, the voltages gradually increased to higher peaks after negative values. This showed the importance of water in the PMFC systems.

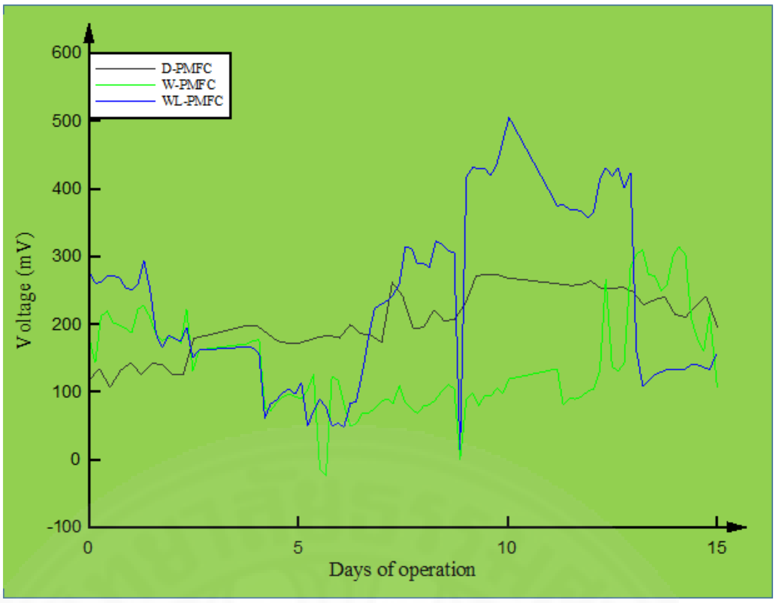


Figure 4.1 Cell voltage generated from PMFCs under open circuit voltage.

4.1.2 System power performance of PMFCs under different water contents

Figure 4.2 shows the power generations of the three PMFC systems operated at different water contents in the soil. The WL-PMFC produced a higher power output as compared to the W-PMFC and D-PMFC throughout the period of experiment with a maximum power of 10.13 mW/m^2 anode area. The maximum power outputs of the W-PMFC and D-PMFC were recorded at 6.7 and 2.3 mW/ m^2, respectively. The result revealed the importance of water content in the soil as it helped to promote a smooth flow of protons from the anode to the cathode, reducing the internal resistance of the system, and ensure the conductivity of the electrode surfaces. The soil moisture measurements showed that the WL-PMFC under regular irrigation obtained the soil moisture content with 57.6 % w/w, which was higher than that of 42 and 25 % w/w for W-PMFC and D-PMFC, respectively. This also reflected the suitability of plant for PMFCs which is preferable for high moisture plants. The outcomes in this study showed similarly as the research of Natalia et al. (Tapia et al., 2017) when the current increased sharply at high moisture of 40 % v/v while it tended to decrease with moisture value around 5 % v/v.

During the first week of operation under closed circuit, purple guinea grasses and anaerobic bacterial communities were introduced and allowed acclimatization to occur at which power density was gradually developed. The addition of 100-gram manure for each after 36 days provided essential nutrients for plant growth as well as carbon oxidation in the anodic chamber leading to improvement in performance. A decline of power output after 50 days was heavily affected by weather conditions on the rainy days leading to lower temperature and light intensity.

The anodic area in PMFCs represents an essential matrix which is filled with carbon compounds, anaerobic bacteria, and electron acceptor. It facilitates the digestion of organic compounds and oxidizing them into electrons. Thus, maintaining the anaerobic environment is utmost importance to maximize efficiency in PMFCs (M., Mokhtarian, Najafpour, Ghoreyshi, & Dahud, 2009). Oxygen diffusion could be critical to voltage losses as the presence of oxygen in the anodic area could diminish the anaerobic substrate consumption and compete for food sources (S. E. Oh, J. R. Kim, J. H. Joo, 2018). As a result, other end products will be released instead of electrons, causing voltage loss. Moreover, oxygen is the most propitious electron adopter. Oxygen-derived from cathode will compete with the anode to take out electrons, leading to a reduction of the Coulombic efficiency (S. B. Velasquez-Orta, I. M. Head, T. P. Curtis, K. Scott, & J. R. Lloyd, 2010). Hence, the better performance of waterlogged PMFC could attribute by the stronger activities of biomass degradation, anaerobic bacteria, and efficiency of blocking oxygen diffusion phenomenon.

These outcomes authenticated that the water contents of soil strongly affect to attain higher electrical generation. Figure 4.3 illustrates the maximum power generation which WL-PMFC provided much higher power output than that of W-PMFC and D-PMFC.

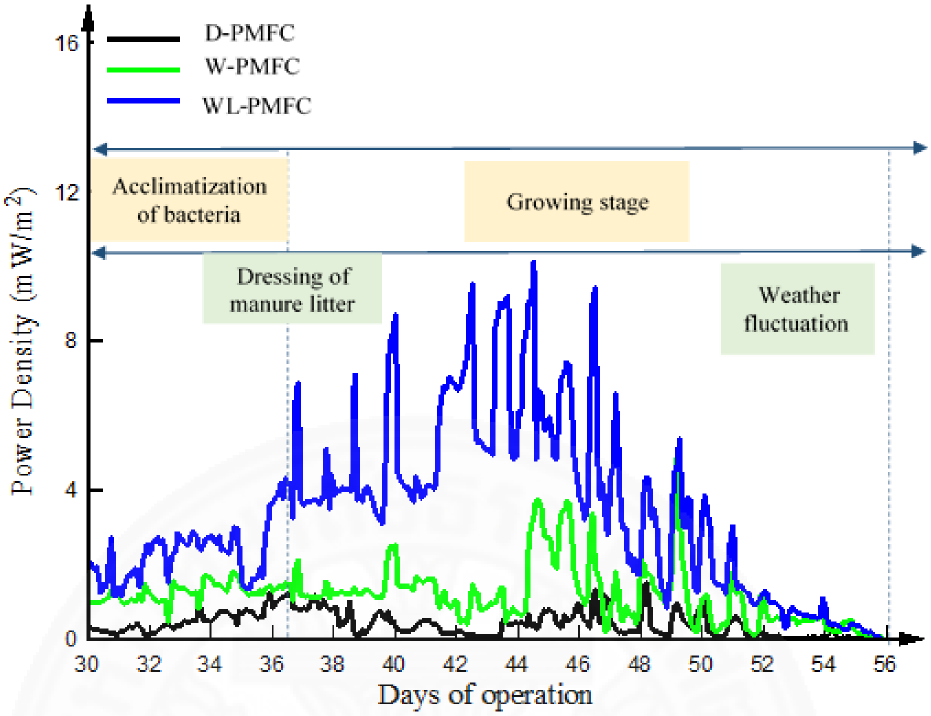


Figure 4.2 Variations of power density of the PMFC systems operated under different soil water contents (CCV-100 Ω).

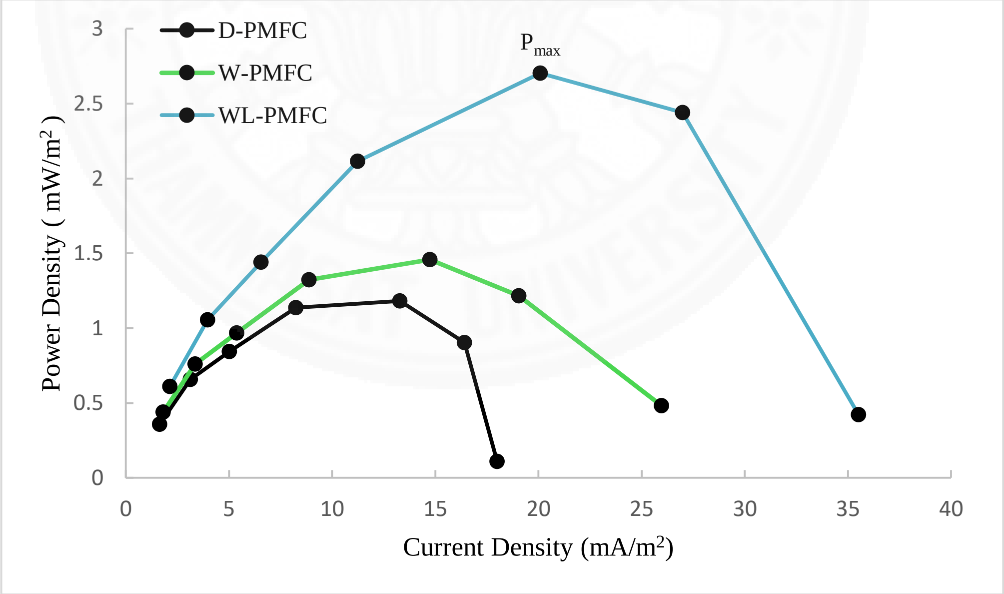


Figure 4.3 Polarization curves of the PMFC systems operated under different soil water contents.

4.1.3 Power production under ambient temperatures and circadian rhythm

To evaluate the effect of greenhouse temperatures on power generation of PMFCs, the generated voltage of PMFC and the temperature were monitored using online Wisco data acquisition. An external load of 100 Ω was connected and the temperature was recorded every 30 minutes. Fundamentally, 32 – 42 °C is known to be an optimum temperature interval for anaerobic digesters specifying by mesophilic microorganisms. A sharper one at 48-55 °C usually is a temperature interval for thermophilic microorganisms which are sensitive with a small variation of temperature (L. H. Li, Sun, Yuan, Kong, & Li, 2013). As soil matrix in PMFCs functions alike a typical anaerobic digester, it should also strongly affect by temperature variation. In this study, the temperature in greenhouse fluctuated from 27 °C to 47 °C. This facilitated an analysis of temperature in our systems for various temperature regimes identifying as low temperature range - LTR (27 °C – 34 °C), intermediate temperature range - ITR (34 °C – 41 °C), and high temperature range - HTR (41 °C – 47 °C).

In D-PMFC, the average power density at HTR was 4.4 mW/m^2, which was 77% and 93% greater than that of 1.0 and 0.3 mW/m^2 for ITR and LTR, respectively. Compared with 5.9 mW/m^2 for HTR in W-PMFC, power densities at ITR reduced by 71% (1.7 mW/m^2), and continued declining by 93% (0.4 mW/m^2) for LTR. Similarly, the average power density of waterlogged PMFC for HTR was 6.0 mW/m^2 while ITR and LTR delivered power outputs of 1.9 and 0.6 mW/m^2, respectively (Figure 4.4). These outcomes demonstrated that the higher temperature is, the higher power generates. However, there was no stable power exhibition when PMFCs reached up to 45 ^{0}C, this could cause by the presence of multi-bacterial communities being different in thermophilic conditions.

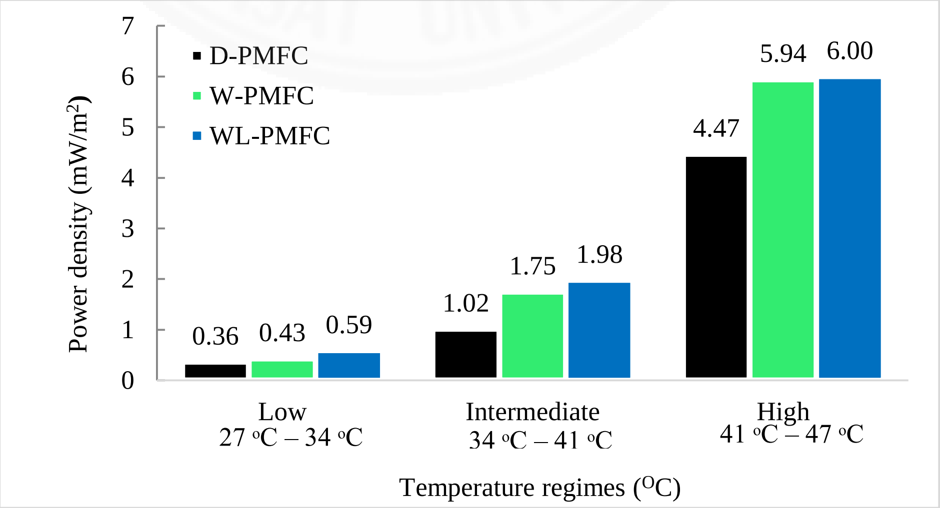


Figure 4.4 Average power density obtained under different ambient temperature range.

Additionally, the temperature might also affect the carbohydrate dynamics in plants and electroactive bacterial activities. The higher power obtained during the HTR might attribute to the accumulation of organic compounds including starch and carbohydrate concentrations, which were largely accelerated in the daytime temperature. The higher amount of organic matters could increase the food sources for the electroactive bacteria in rhizosphere and release more electrons.

Moreover, the lower temperature at night could slow down biochemical processes of starch synthesis as well as degradation (Eva-Theresa P et al., 2012; Ribeiro, Machado, Espinoza-Núñez, Ramos, & Machado, 2012). In connection with microbial activities, the mesophilic environment is usually the ideal habitat for the metabolism of exogenous microbes such as *Geobacter sulfurreducens, Shewanella, and Rhodoferax ferrireducen* (Jong et al., 2006; Logan, 2009). Therefore, the interaction between bacterial activities coupled with high carbon accumulation might resulted in achieving higher power output. Figure 4.5 illustrates the variation of power output along with circadian rhythm and ambient temperature. It was observed that power was higher during the daytime usually from 12:00 to 15:00 p.m. and reduced at night from 2:00 a.m. to 5:00 a.m. Improvement of electrical production during daytime was associated with rising photosynthesis activity. Furthermore, the photosynthesis of plants under the high temperature period could easily accumulate the carbohydrate concentration and promote the degradation of organic compounds in the soil.

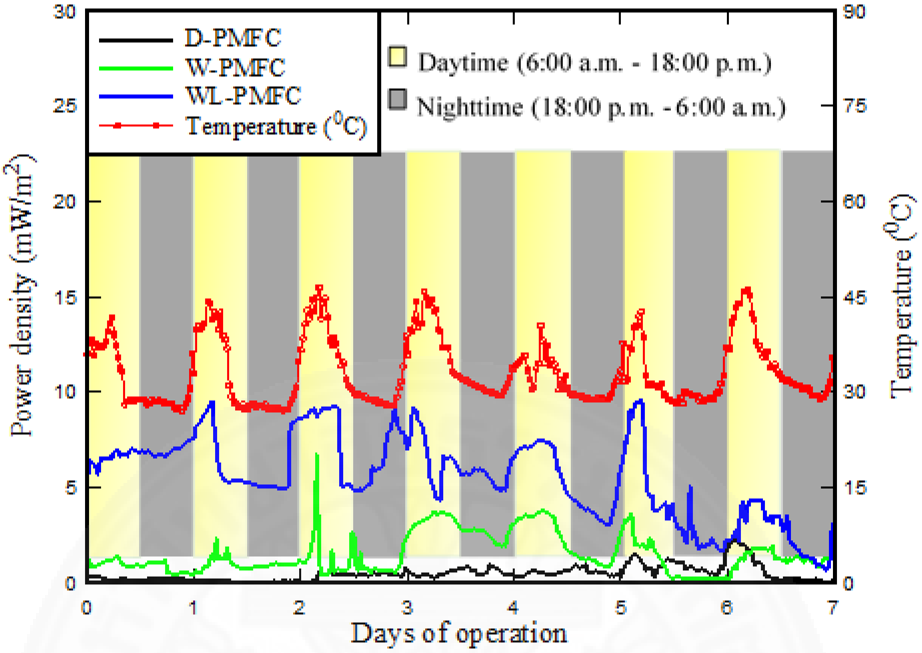


Figure 4.5 Power density of PMFCs under daily ambient temperature and circadian rhythm.

4.2 Comparison of system performance for the different operational configurations in forage grass plant microbial fuel cells

4.2.1 Voltage generation from open circuit condition of PMFCs

Figure 4.6 illustrates the average cell voltage generated from reactors under open circuit condition of PMFCs. The voltage increased rapidly in all PMFC systems during the first four days of operation. The higher voltage generated from D-PMFC and D-2C PMFC showed their important roles in enhancing the cell voltage of systems and eliminating drawbacks of configuration set-up. Therefore, setting up an effective configuration not only eliminated drawbacks of systems but also improved the output of PMFCs. The trend line of OCV was similarly as other studies (Kudke, Shinde, & Saptarshi, 2017; Regmi, 2017).

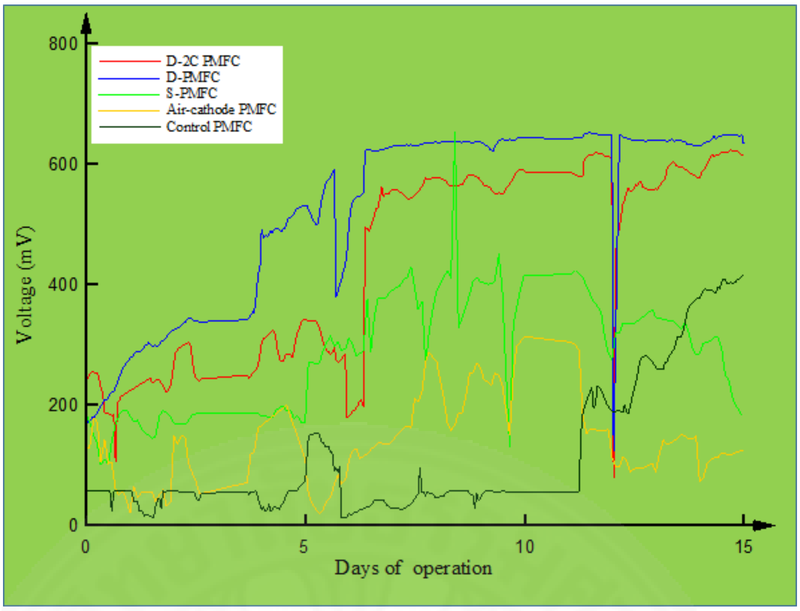


Figure 4.6 Cell voltage generated from different configurations PMFC under open circuit voltage (CCV-100 Ω).

4.2.2 Variation of power production under various configurations

Productive electrical generation and cost-effectiveness are two major concerns slowing the development of plant microbial fuel cells. Comparative analysis of various configurations under equilibrium conditions could help to improve the output generation. Overall, running different configurations in PMFCs defines the important roles in enhancing electricity generation. As shown in Figure 4.7, there was no significant difference in power generation in the first week of operation. However, after the first week, PMFC power output increased rapidly and differently in closed circuit conditions. This could be due to the adaption of bacterial communities and nutrient-enrichment phase of the PMFC operation. Later in the experimental period, the D-PMFC and D-2C PMFC had a greater performance in generating power. The D-PMFC obtained a maximum power density of 20.98 mW/m^2, which was a little higher than that of the D-2C PMFC with 18.87 mW/m^2. The D-PMFC obtained the maximum power density of 20.98 mW/m^2, which was higher than that of constructed wetland PMFC with 12.82 mW/m^2, A. donax CW-MFC in a 2017 study (Y. Zhou et al, 2017), and significantly lower than study of Liu et al. 2014 with 55.05 mW/m^2 (S. Liu et al., 2014).

PMFC systems were examined for the oxidation-reduction potential balance between the anode and the cathode (Leong et al., 2013). The additional transfer of protons via a supplemental cathode could cause redundant protons and a lack of proton acceptors, thereby, increasing the pH splitting and reducing the power performance. Hence, the lower performance of D-2C PMFC as compared with D-PMFC in this study may be due to an imbalance of output potential between the anode and the cathode.

The maximum power output of 20.98 mW/m^2 for the D-PMFC was 47.5%, 97%, and 99% greater than the 11.0, 0.5, and 0.1 mW/m^2 for the S-PMFC, Air-cathode PMFC, and control, respectively. As compared to D-PMFC, the values of Air-cathode and control were small which hardly showed in the Figure 4.7. Typically, electron production at the anode is important in PMFC systems, and electron acceptance at the cathode depends on the number of electrons coming to the cathode (Song, Zhu, & Li, 2015). The carbon-wrapped cathode membranes of the D-PMFC and D-2C PMFC in this study may increase the ratio of cathode surface area to volume, congregating the electrons and facilitating the transport of protons. The larger the area was, the better the PMFC performance. A large amount of oxygen usually diffused in the anode area of single and air cathode chamber than double chamber configuration slowing substrate consumption by anaerobic bacteria, and caused a low Coulombic efficiency (Leong et al., 2013). Although running the air-cathode PMFC and S-PMFC could reduce the cost, the lower performance of S-PMFC and air-cathode might induce by the rate of oxygen diffusion into the anode which was higher than the D-PMFC and D-2C PMFC.

Figure 4.8 shows the polarization curves obtained in this study. Typically, polarization curves are used to depict the maximum voltage obtained from various external loads. These polarization curves were executed at the stable operational period of the experiment. The trend s of polarization curves was alike as other literature in PMFCs (Can & Yakar, 2017; Ghadge, Jadhav, Pradhan, & Ghangrekar, 2015; Md Khudzari et al., 2018).

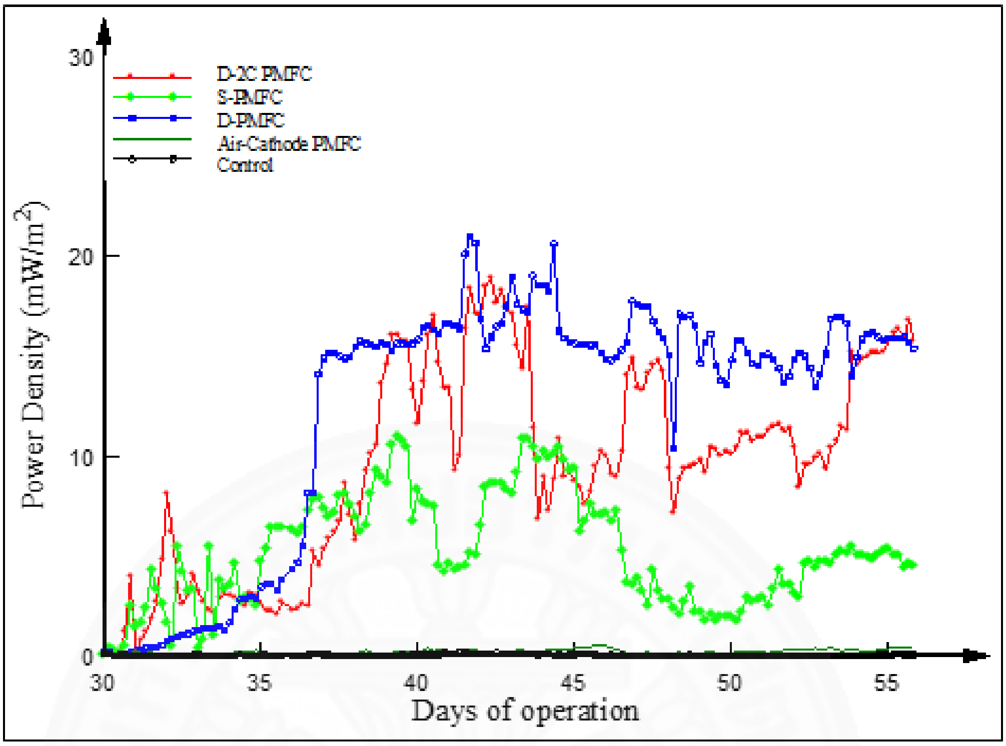


Figure 4.7 Performance comparison among different configurations under closed circuit.

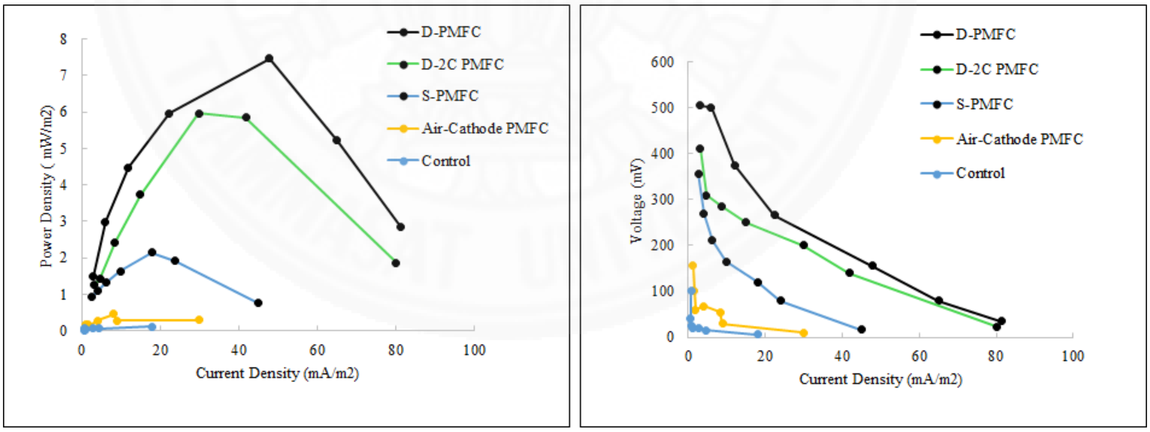


Figure 4.8 Polarization and power curves of PMFC systems.

4.2.3 Effects of defoliation on system performance

From Figure 4.9, in the short term or about two weeks after treatment, the power of the clipped-PMFC rapidly increased more than that of unclipped-PMFC. There was a slight decline in power output of the clipped-PMFC after day 77 while the non-defoliated PMFC still stably produced electricity. These results indicated that the unclipped-PMFC had more stable power generation in the long term than that of the clipped-PMFC. This was because PMFC systems without defoliation treatment had more falling leaves during the period of operation. This added organic matter from the decayed leaves, leading to the degradation of a food source for exoelectrogenic bacteria. Nevertheless, purple guinea grass grew in size (especially the stems) over time, causing a reduction in the number of leaves. Large stems with few leaves are incompatible animal feed. Although the output of the unclipped-PMFC was a little more stable than that of the clipped-PMFC, clipping regularly could produce quality biomass for animals while simultaneously maintaining the power production.

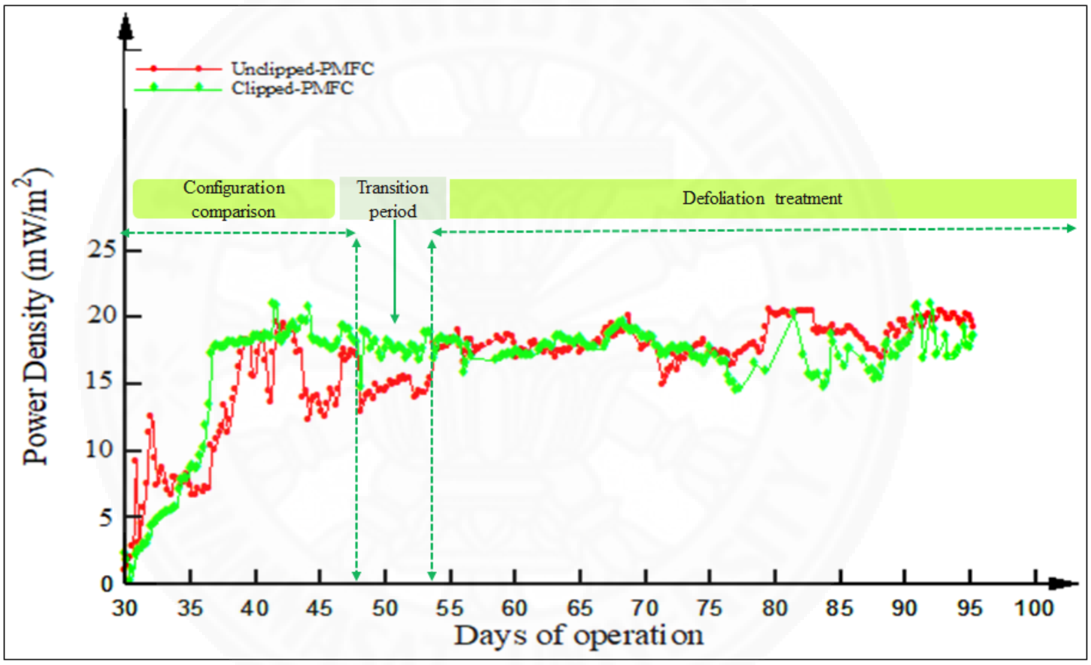


Figure 4.9 Power generation of unclipped and clipped PMFCs.

To understand the variation of power, the total amounts of carbon and nitrogen in the soil over a short term of twenty-four hours and a long term of twenty days were determined. Organic matter and nitrogen measurements were monitored during the rapid growth of stems after clipping and the increased voltage. One day after the defoliation treatment, the voltage sharply increased from 325.64 mV for the unclipped-PMFC to 365.3 mV for the defoliated plant PMFC. The soil carbon content of the clipped-PMFC was 15 % greater than the unclipped-PMFC with a significance of $p < 0.05$ (Figure 4.10A). For the nitrogen availability after twenty-four hours, the soil nitrogen content for the clipped-PMFC was not affected by defoliation ($p > 0.05$) (Figure 4.10B).

These results showed that purple guinea after defoliation could induce root exudation leading to a higher amount of carbon content in the soil, which provided more food for the anaerobic bacteria in the PMFCs. In the study of Frank (Frank & Groffman, 1998), grazing by herbivores triggered a carbon flow to the root systems, concurrently increasing the size of the associated microbial community, and helping with nitrogen mineralization. Numerous studies have investigated the effects of defoliation on various carbon pathways, including crown carbon, root exudation, microbial carbon, tissue carbon, etc. [21, 4]. Henry et al. (Henry et al., 2008) demonstrated that microbial biomass carbon of defoliated plants increased significantly within 1.5 days after treatment as compared to non-defoliated plants. Moreover, the nitrogen in soil was not affected by clipping within one day, which is in agreement with the result of E. W. Hamilton et al. (Hamilton et al., 2008). However, several studies showed that clipped plants could stimulate rhizosphere N mineralization, aboveground N, and potential net N mineralization (Hamilton et al., 2008; Hilbert, Swift, Detling, & Dyer, 1981). Defoliation significantly induced the root exudation and the growth of microbial communities, leading to larger microbial nitrogen mineralization. All of these factors contributed to the rapid increase in voltage after twenty-four hours of defoliation.

For long-term effects, the power generation and rhizosphere feedback of defoliation after twenty days of experiment were assessed, to compare the output of the PMFC systems. A power output of 19.81 mW/m^2 for the unclipped-PMFC was obtained, which was greater than that of the clipped-PMFC (17.46 mW/m^2) in twenty days after treatment. From Figure 4.9, the power of the unclipped-PMFC gradually increased after day 73 while the clipped-PMFC tended to decrease during the remaining period of the experiment. Analysis of soil carbon content showed that the higher power from the unclipped plants was supported by a greater amount of soil carbon, which was 1.3 times greater than that of the clipped-PMFC (Figure 4.10A). Degradation of leaves falling from non-defoliated plants leads to the accumulation of carbon on the soil surface of the PMFCs. There was no significant difference in nitrogen content for the clipped-PMFC and the unclipped-PMFC ($p > 0.05$).

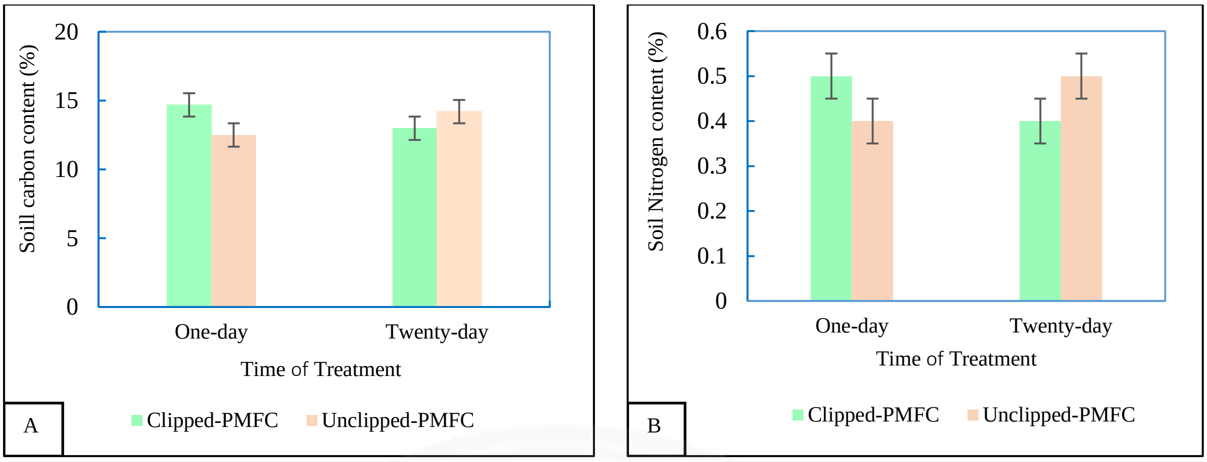


Figure 4.10 A. Soil carbon content and B. Soil nitrogen content in clipped and unclipped-PMFC after a one-day and twenty-day treatment.

4.2.4 Biomass production of PMFCs with and without defoliation

Purple guinea regrew well after defoliation with high aboveground biomass output of 6.4 g/m^2 anode area for the first and second clippings. Figure 4.11 shows that the total biomass production after clipping was less than 5 % of the non-defoliated PMFC (6.7 g/ m^2). Although the non-defoliated PMFC had a higher aboveground biomass production for each batch, the amount of biomass produced in the unclipped-PMFC was mainly from the larger stem size of plants (with fewer leaves). A leaf is the main photosynthetic component that produces nutrients for the growth and development of most plants. Increasing the size of the stem could lessen opportunities for photosynthesis of purple guinea and hinder the accumulation of carbon. It was recommend that purple guinea grass should be harvested every 50 days for animal feed. Non-defoliation is applied when farmers need to harvest seeds.

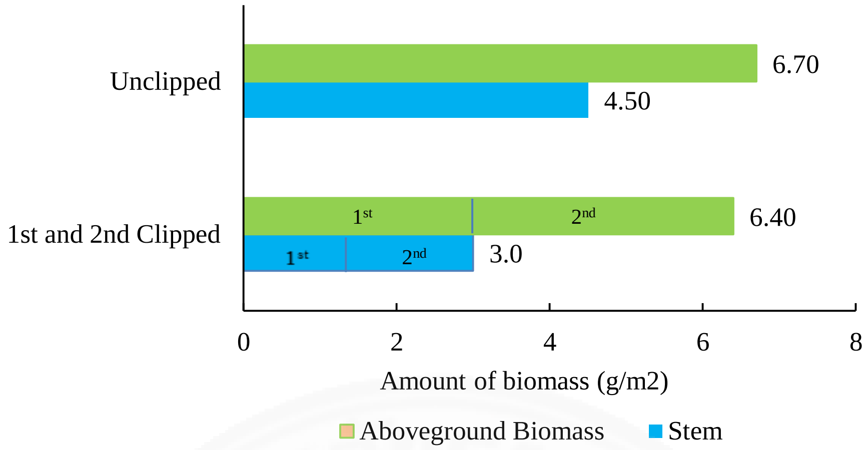


Figure 4.11 Biomass production in terms of stem weight and aboveground biomass in PMFC systems.

According to Aahlrichs and Baue (Ahlrichs & Bauer, 1983), the leaf area index determines the photosynthesis capacity of plants. It is used as a reference parameter for plant growth. A larger leaf area index can accelerate light absorption with nutrient intensification. In this study, the leaf area was largest (0.16 cm^2) after the first defoliation treatment. This affirmed the higher photosynthesis capacity and stimulated higher power in the clipped-PMFC during the first two weeks after treatment (Figure 4.12). The aforementioned long-term output of the unclipped-PMFC was a little more stable than that of the clipped-PMFC. However, the increase of stem size and the decrease of leaf area (0.1 cm^2) for the unclipped-PMFC created unfavorable conditions for animal feeding and power generation. Based on the results of this study, purple guinea can be harvested for animal feed while maintaining reasonable electrical production.

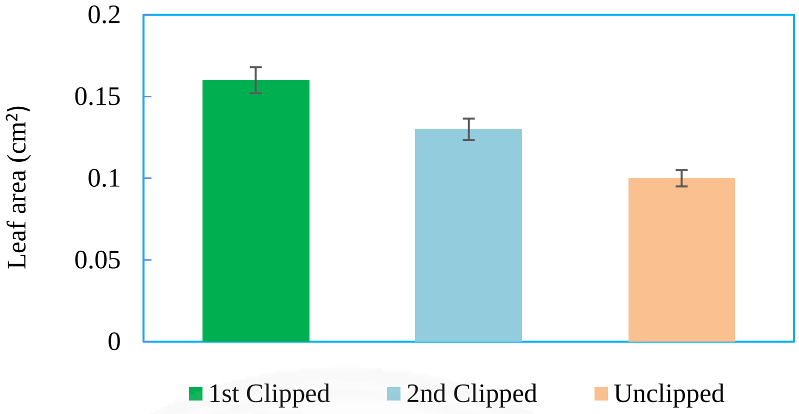


Figure 4.12 Leaf area of clipped and unclipped PMFCs.

4.2.5 Dynamic performance of PMFCs under ambient temperature variation

The average ambient temperature was recorded during 7 days of stable operation from day 60 to day 66 to evaluate the effects of temperature on PMFCs performance (Table 4.1). The average power densities of the unclipped-PMFCs in a temperature range of 32-36 ^{0}C were 14.79-17.51 mW/m^2. Compared to the maximum temperature recorded (36 ^{0}C), a lower temperature leaded to a lower power generation (15.50 % lower at 32 ^{0}C, 14.0 % lower at 33 ^{0}C, and 9.5 % lower at 34 ^{0}C). Similarly, the highest average power density of the clipped-PMFC was 16.53 mW/m^2 at 36 ^{0}C while at a lower average temperature, PMFCs delivered lower performance with 14.45, 14.50, and 15.23 mW/m^2, at 32 ^{0}C, 33 ^{0}C, and 34 ^{0}C, respectively.

These results demonstrated that a higher temperature could contribute positive improvements in terms of power generation. This could be attributed to the carbon dynamics and the activities of the bacterial communities at different temperature regimes. The higher power gained during the high-temperature regime during daytime was ascribed to the congregation of organic matter, comprised of starch and carbohydrate concentrations. Gathering a large amount of carbon compounds could be a huge food source for anaerobic bacteria to liberate more electrons. In addition, the biochemical processes of starch synthesis and decomposition could be slowed by a lower temperature at night (Eva-Theresa P et al, 2012; Ribeiro et al., 2012). In relation to bacterial activities, several types of anaerobic bacteria such as *Geobacter* *sulfurreducens, Shewanella, and Rhodoferax ferrireducen* are normally dominant in a moderate temperature environment (Jong et al., 2006; Logan, 2009). The cooperation among microbial communities accompanied by a large amount of carbon contributed to higher system performance.

Table 4.1 Effects of temperature on unclipped and clipped PMFCs, for power generation.

Day	**Average ambient temperature (^{0}C)**	**Average power output (mW/m^2)**	
		Unclipped-PMFC	**Clipped-PMFC**
60	32	14.79	14.45
61	34	15.84	15.23
62	36	15.29	15.29
63	36	16.28	16.53
64	36	17.51	16.43
65	33	15.05	14.50
66	33	15.79	15.40

4.3 Variation of power generation under different fertilizer additions in plant microbial fuel cells

4.3.1 Power generation in PMFCs with fertilizer adjustments

Four different fertilizer treatments were constructed in this study comprising urea PMFC (U-PMFC) which urea was the main fertilizer component in soil, the mixture of compost and urea was added in soil matrix called CU-PMFC, and C-PMFC represented for soil containing solely compost. The overall performance of PMFCs was assessed via the power output normalized to the projected anode area. It was observed that the power performance of the C-PMFC ranged from 0 – 19.76 mW/m^2 which was higher than others with the lowest internal resistance of 188 Ω during 8-week of the experiment (Figure 4.13). While the lower power generations were found with power densities from 0.009 - 2.85, 0.001 - 3.4, and 0 - 0.14 mW/m^2 for U-PMFC, CU-PMFC, and control PMFC, respectively. The internal resistances of U-PMFC, CU-PMFC, and control PMFC were found significantly higher than that of C-PMFC with 386, 256, and 357 Ω, respectively. This revealed that amending solely compost in PMFCs could reduce the internal resistance while producing high power output. The lower internal resistance was induced by soil moisture, soil salinity, soil pH, and the bacterial attachments which are explicitly explained in subsequent parts.

Figure 4.13 illustrates the trends of the power generation of all PMFCs adjusting different fertilizer regimes. During the closed-circuit condition, the power generation of C-PMFC increased rapidly throughout the experimental period. The power output of U-PMFC seemed stable for the whole period of study and increased slightly from day 48. The striking fluctuations in electricity generation of C-PMFC and U-PMFC showed well adaptations and active activities of microorganisms in new environments. While there was a minor variation in the power production of CU-PMFC until days 40 and days 50.

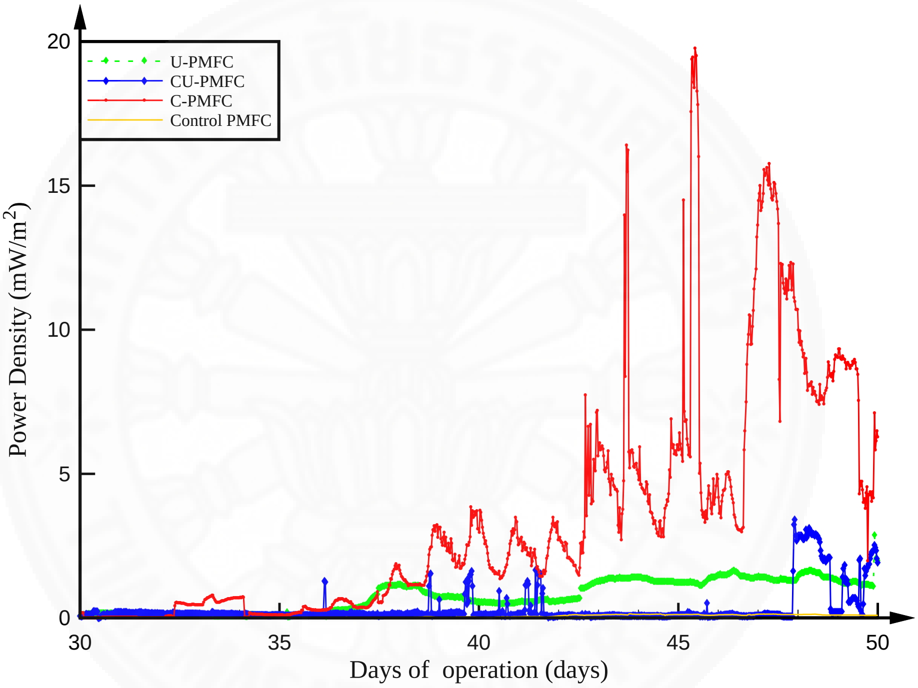


Figure 4.13 PMFCs comparison under different fertilizer treatments (CCV-250 Ω).

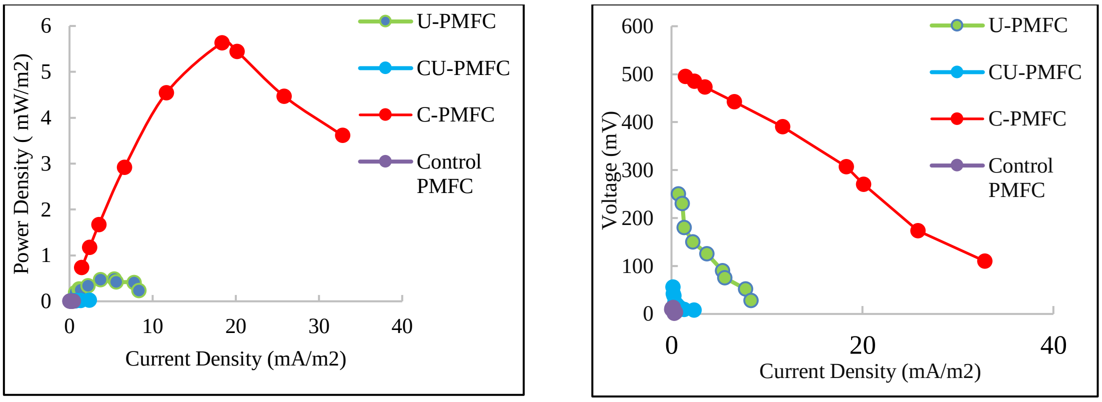


Figure 4.14 Polarization curves obtained from three PMFCs and the control PMFC in this study.

4.3.2 Effects of soil pH on power generations

In order to verify the hypothesis that the PMFC systems were affected by soil chemical parameters, soil pH, electrical conductivity (EC), salinity level, resistivity, and soil moisture were measured. From Figure 4.15, soil pH shows no difference between U-PMFC, CU-PMFC, and C-PMFC ($p > 0.05$) and significant lower than a control PMFC with $p < 0.05$. The decrease of pH in PMFCs with fertilizers applications might supposedly induced by the mineralization of organic compounds from fertilizers as well as root biomass (Hanč, Tlustoš, Száková, Habart, & Gondek, 2008). Study of Moreno at al. (Moreno, García, Hernández, & Pascual, 1996) also confirmed that soil pH of Barley plants in calcareous soil containing sewage-sludge composts was gradually decreased overtime. However, the soil pH has been reported to increase in some manure-added soils (Bickelhaupt, 1989; Whalen, Chang, Clayton, & Carefoot, 2010). The results in this study was in line with the study of rice paddy PMFCs (Moqsud et al., 2015), adding compost from kitchen and yard waste declined the pH value and the voltage increased up to 700 mV which was 3 times greater than without compost PMFCs. Amendments of individual fertilizers to PMFCs resulted in changes of soil pH and created an active operational threshold for forage PMFC systems as compared to the control PMFC.

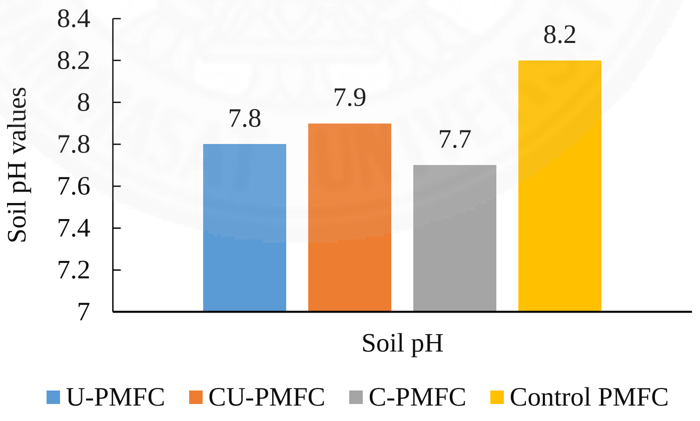


Figure 4.15 Soil pH and soil resistivity values in this study.

4.3.3 Influences of soil properties

Salinity is one of the main factors leading to the higher performance of C-PMFC in this study. It was observed that the PMFCs generated the best output with a maximum power density of 19.76 mW/m^2 C-PMFC at the lowest salinity value of 0.4 ppt (Figure 4.16a). The salinity values of C-PMFC were found significantly lower than U-PMFC and CU-PMFC with $p < 0.05$ and no significant difference with the control PMFC (t-test $p > 0.05$).

The decrease of power output accompanied by the increase of soil salinity value in U-PMFC and CU-PMFC was explicitly indicated in this study. In general, the responses of plants to nitrogen fertilizer are shown at different soil salinity levels (Esmaili, Kapourchal, Malakouti, & Homaee, 2002). The saline soil might hinder plant growth via reduction of water absorbance, nutrient uptake by ionic interferences, and reduction of metabolic activity because of the salt toxicity (Irshad et al., 2002). Hence, the lower power production in U-PMFC and CU-PMFC were attributed to the higher salinity values in PMFC systems inhibiting plant growth as well as microorganism activities.

The effects of salinity values on electricity generation behavior of this study showed similar outcomes to those evaluated in previous sediment-MFC and MFC studies (Lefebvre, Tan, Kharkwal, & Ng, 2012; Schamphelaire et al., 2010). The negative effects including high osmotic pressure and prohibition of bacterial activities at higher salinity level caused the lower power generation in weeping alkaligrass PMFC (Md Khudzari et al., 2018). The study of O. Lefebvre (Lefebvre et al., 2012) also indicated that the addition of 20 g L$^-$ NaCl to MFC systems produced a maximum power of 35 W/m^3 which was greater than that of 30 % and 50 % as compared to without (27 W/m^3) and a 40 g L$^-$ NaCl-added MFCs (18 W/m^3), respectively.

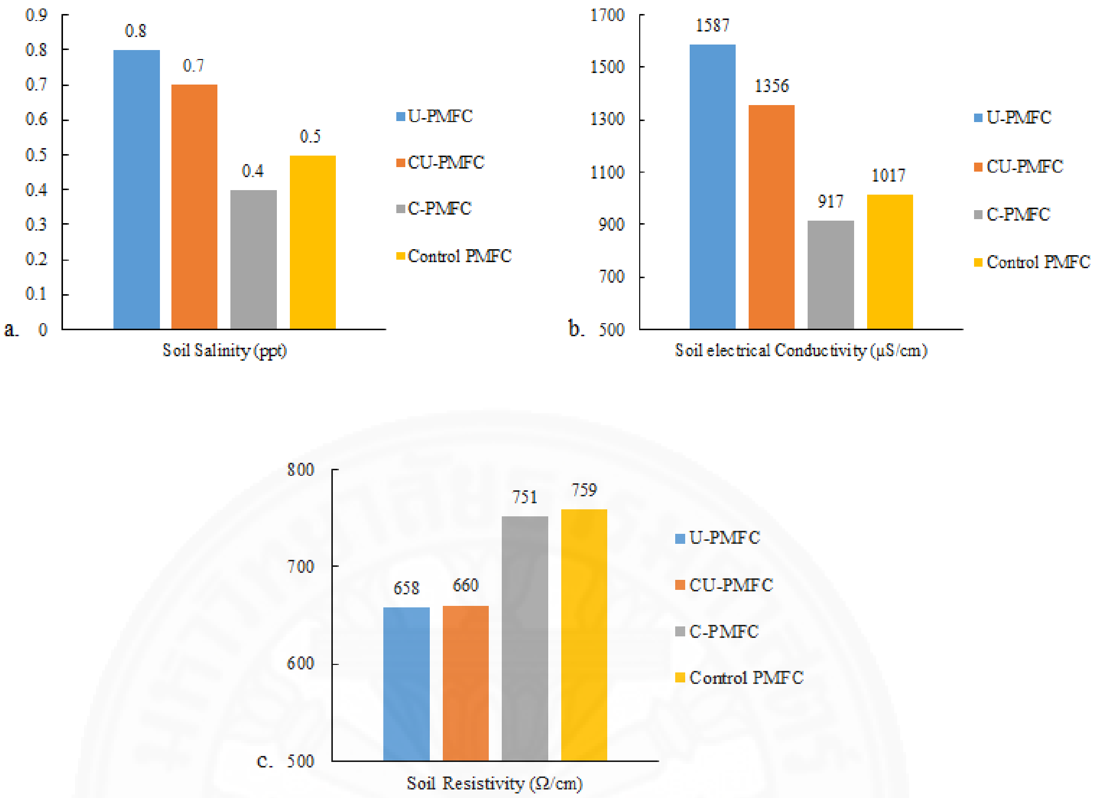


Figure 4.16 Soil salinity, soil electrical conductivity, and soil resistivity values in this study.

Soil electrical conductivity was measured in PMFCs (Figure 4.16b). The EC values of U-PMFC and CU-PMFC were significantly higher than C-PMFC and control PMFC ($p < 0.05$) and increased when the soil salinity increased. Typically, the higher EC values are expected to promote the ease mobility of cations including protons in PMFC systems leading to the higher power production. However, the hypothesis was uncertain in this study whereas the lowest EC value of 917 µS/ cm C-PMFC was detected for the highest output production. The obtained results was in agreement with the study of J. Md Khudzari (Md Khudzari et al., 2018), which indicated obscure relationship between electricity production and EC values. There was no significance difference in soil resistivity in all PMFC systems ($p > 0.05$) (Figure 4.16).

4.3.4 Anode morphology and bacterial attachment

The bacterial attachment, structure, and morphology on anodic electrodes were observed by a field emission scanning electron microscope (FESEM) (Figure 4.17). The image of FESEM showed bacteria colonized on all tested anode electrodes. The image and observations from FESEM showed bacterial communities were denser at C-PMFC anode surface than others. The results showed that bacteria more preferable and stronger in the soil matrix containing mainly compost.

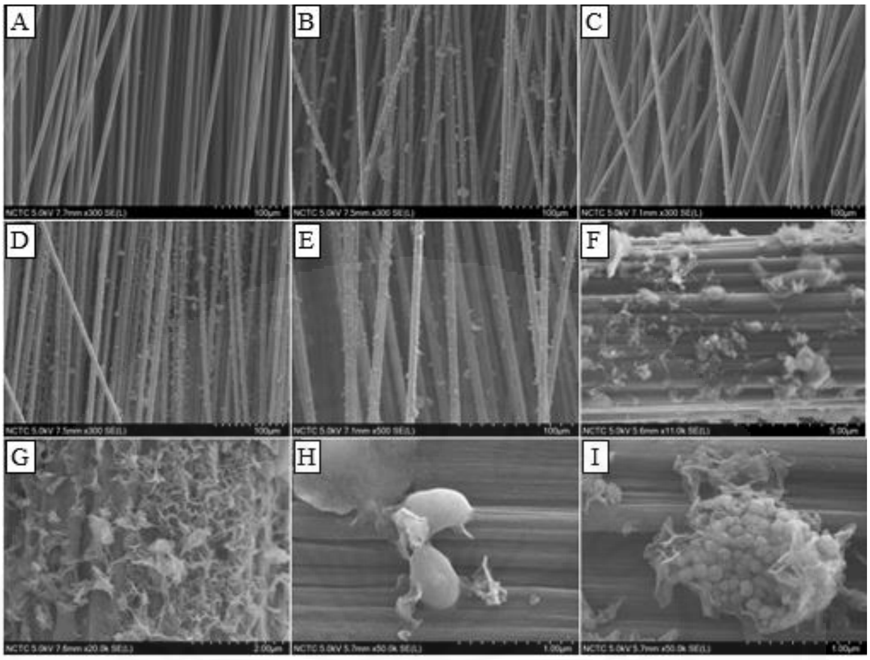


Figure 4.17 (A) FESEM image of original carbon cloth; (B) U-PMFC anode; (C) CU-PMFC anode; (D) C-PMFC anode; (E) Control-PMFC anode; (F) Bacterial attachment on carbon surface (G) Bacterial debris on carbon cloth anode; (H) Bacterial shape; (I) Bacterial clusters.

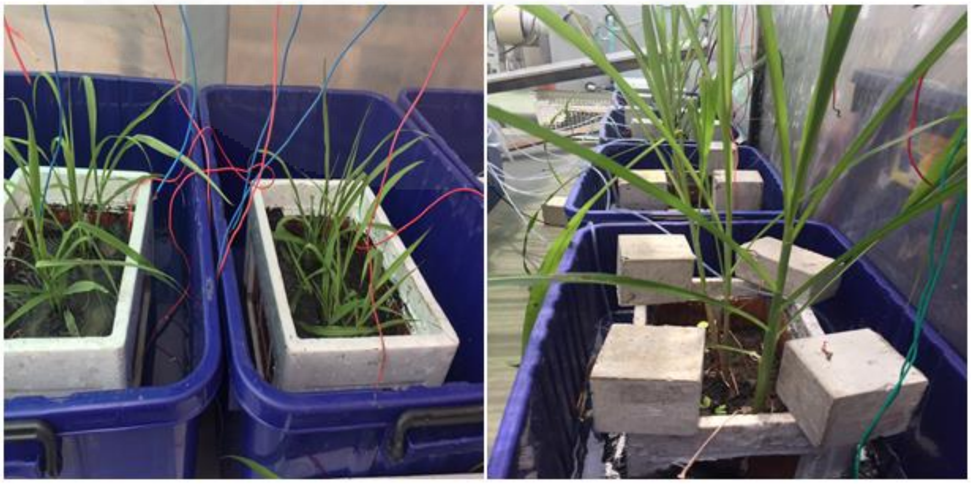


Figure 4.18 Purple guinea forage grass plant microbial fuel cell in lab operation.

4.3.5 Effects of soil moisture on output productivity in PMFC systems

Soil conductivity and water contents are substantial factors that could decelerate

PMFC performance in arid and semi-arid conditions (Domínguez-Garay et al., 2013). The water content has been described as a critical aspect, maintaining the anaerobic condition and substrate consumption in PMFC. The low water level in soil could strongly impact to proton mobility, which increases the internal resistances and reduces PMFC performance. In contrast, PMFC systems operated under high water content typically facilitate proton transport, conditions for electroactive bacterial activities, and mitigation of internal resistances (Chiranjeevi et al., 2012).

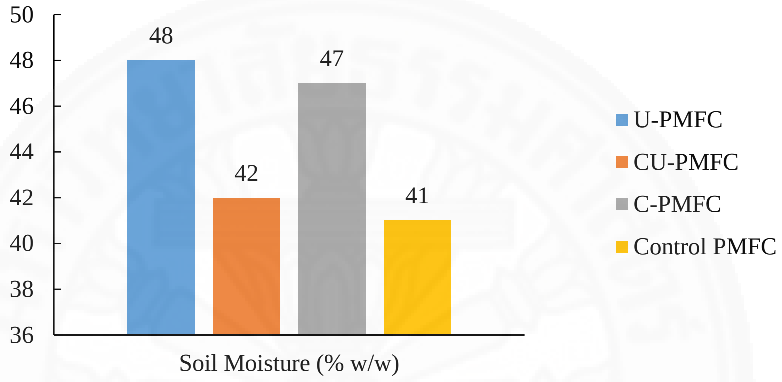


Figure 4.19 The measured soil moisture values

Soil moisture values were measured by a method as described in the report of Alpana Shukla et al (A. Shukla et al, 2014) to evaluate its impacts on the internal resistance and power generation. The soil moisture value of C-PMFC was 2 % lower than U-PMFC and 11 and 13 % greater than CU-PMFC and control PMFC, respectively (Figure 4.19). Based on results, the C-PMFC and U-PMFC performed better throughout the experimental period. Nonetheless, the higher moisture values of C-PMFC and U-PMFC increased their overall performance. In research of Natalia et al. (Tapia et al., 2017) seven *Sedum* species in a semi-arid green roof were carried out to evaluate their ability for electricity generation. Under the weekly irrigation, there was a sudden increase of current after watering due to the soil moisture made up 40 % v/v, while under drip irrigation current was decreased with moisture value around 5 % v/v. Therefore, the higher soil moisture also contributed to the higher power generation and decreased the internal resistance of compost PMFCs. The higher moisture might correlate to the leakage of water from the anode to cathode via pores of earthen membrane.

4.3.6 Effects of light intensity to PMFC performance

The light intensities were measured by a lux meter during 7 days of closed circuit operation to assess its effects on the power production in PMFCs. The light intensities were recorded in the morning at 9:00 a.m. and in the afternoon at15:30 p.m. The average light intensity of 25187 lux in the morning was 72 % lower than in the afternoon (90750 lux). The power output was observed in PMFCs, where power density increased as the light intensity increased. As shown in Figure 4.20, purple guinea forage grass was characterized by the strength of light intensity with clear variations, the average power density of C-PMFC of 2.13 mW/m^2 in the afternoon was 50 % greater than that of in the morning (1.06 mW/m^2). Similarly, the average power generation in the morning of 0.02 mW/m^2 CU-PMFC was 81 % lower than that of in the afternoon (0.13 mW/m^2). U-PMFC was observed to be less affected by light intensity than others with a minor difference between two periods (7.14 %).

The higher power output obtained at higher light intensities was ascribed to the increase of the organic compounds comprising of protein and carbohydrate concentrations. Congregating a large amount of carbon compound could be a copious food source for electron active bacteria to liberate more electrons. In addition, the thicker biofilms are usually formed under high light availability resulting in the diversity of microbial communities (G. Kuma et al., 2019; Wagner et al., 2015). The attained results in this study well correlated with reports in other studies (S. Liu et al., 2013; Md Khudzari et al., 2018; Moqsud et al., 2015) and showed reversely as compared to the study of P. Jyoti Sarma and K. Mohanty (Sarma & Mohanty, 2018) when constant voltages were generated irrespective the night time or day time.

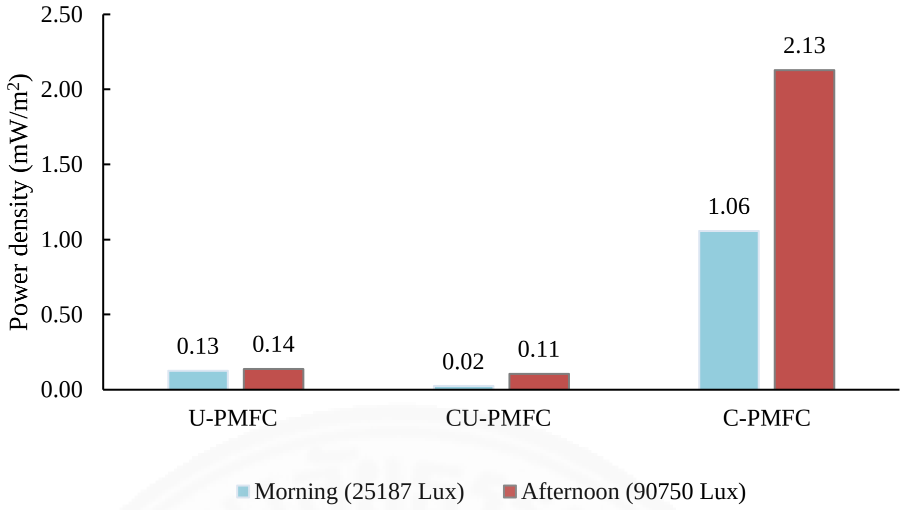


Figure 4.20 Effects of light intensity to power generation in PMFCs.

4.3.7 Soil nutrient contents under urea and compost amendments

The soil nutrients including carbon, nitrogen, sulphur, and hydrogen were measured, and the collected data were analyzed by statistical analysis (Figure 4.21). The t-test results showed that there was no significant difference for all treatments in soil carbon, hydrogen, and nitrogen contents in PMFCs ($p > 0.05$). The sulphur contents of C-PMFC showed no difference with U-PMFC ($p > 0.05$) and significantly higher than CU-PMFC and control PMFC ($p < 0.05$). The result was in line with the study of O. A. Babalola when it was observed that there was no significant difference on nitrogen content for all soils applying urea and compost in maize (Babalola et al., 2018).

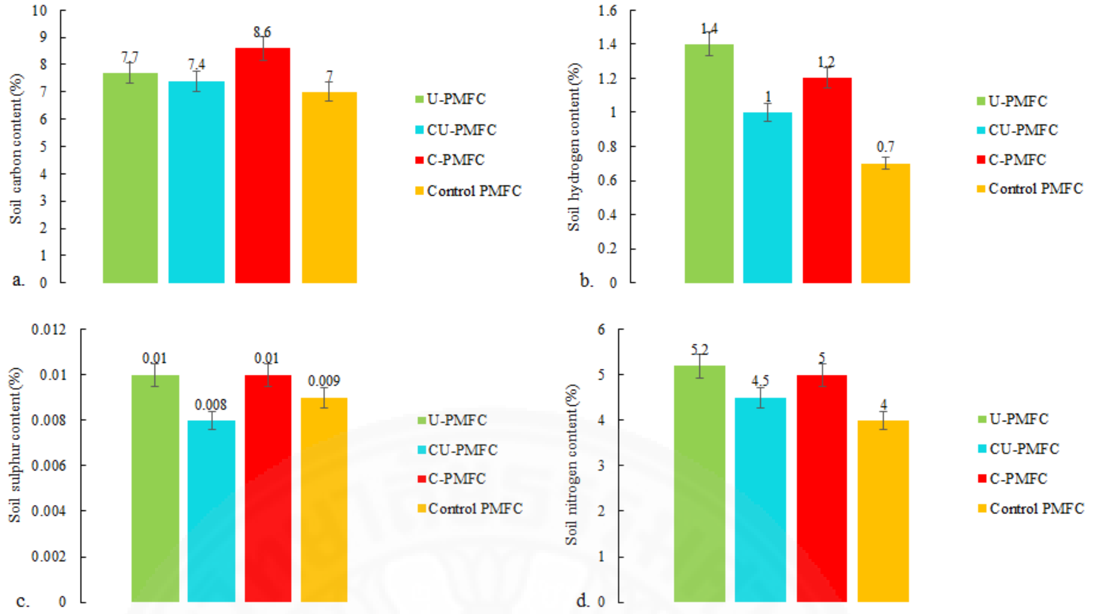


Figure 4.21 Carbon, hydrogen, sulfur, and nitrogen content values in PMFC systems.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

This research demonstrated that PMFCs growing with purple guinea grass generated higher power output when operated under waterlog soil as compared to wet-soil and dry-soil. The power output in waterlogged PMFC was 1.4 times and 4 times better than that of wet-soil and dry-soil PMFCs, respectively. The better performance during waterlog condition demonstrated the success of blocking oxygen diffusion as well as maintaining anaerobic condition. From our results, it is confirmed that plants are capable of living in wet or submerged soil conditions. Thus, it can be the top candidates for the bioelectricity generation in PMFC systems. It was shown that the power output of PMFCs was significantly affected by the greenhouse temperature and circadian rhythm. The electricity of PMFCs increased with a high temperature regime up to 47 ^{0}C. The effects of circadian and temperature on PMFCs were considered as limitations on the bacterial activities in the soil, carbohydrate accumulation, and biochemical processes.

Based on PMFC configuration investigated which are single-chamber, double chamber equipped with 2 cathodes, and air-cathode configurations, the double chamber is one of the most pertinent configurations for maximizing the efficiency of a PMFC system in terms of electricity production. Air-cathode and single-chamber PMFCs failed to deliver electricity due to the presence of oxygen, leading to a decrease in anaerobic bacteria. A defoliation treatment carried positive feedbacks, which generated more electricity than non-defoliation. To acquire biomass for feeding animals while maintaining the production of electricity, it is necessary to harvest regularly. Soil organic carbon was significantly influenced by defoliation, which increased 15% after treatment. Moreover, PMFC systems were strongly affected by ambient factors, including diurnal rhythm and temperature. A higher temperature and high light intensity in the daytime led to higher performance of PMFC systems.

Moreover, this study explored intensively the roles and impacts of fertilizer additions to the bioelectricity generation as well as soil properties in PMFCs. Four model lab-scale PMFCs including urea PMFC, compost-urea PMFC, urea-PMFC, and a control PMFC without fertilizer were built and observed. The purple guinea forage grass PMFC with compost was more beneficial for soil properties in terms of organic enrichment and soil salinity regarding high power production. The higher moisture might correlate to the leakage of water from the anode to cathode via pores of earthen membrane. High light intensity also positively affected to PMFCs resulting in accumulation of carbon compounds and thick biofilm formation, hence improved the overall performance of the systems.

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APPENDICES

APPENDIX A

TEST RESULTS: % CHN IN STUDY OF FERTILISER AMENDMENTS

	Method	**Mass**	**Mass (mg)**	**Carbon %**	**Nitrogen (%)**	**Hydrogen (%)**
U-PMFC						
	Macro CHN	0.05410	54.10000	6.7804	4.7362	1.4364
		0.04500	45.0000	8.7038	5.6876	1.4147
Average		0.04955	49.55000	7.7443	5.2199	1.4256
SD		0.006	6.0	1.36326	0.67275	0.01536
RSD		12.99	12.99	17.60	12.91	1.078
T-test results compared to C-PMFC				0.2277 p > 0.05	0.283 p > 0.05	0.0798 p > 0.05
CU-PMFC						
	Macro CHN	0.06500	65.00000	6.9073	3.9201	1.0692
		0.04840	48.40000	8.0320	5.2276	1.0719
Average		0.05670	56.70000	7.4696	4.5738	1.0705
SD		0.01	12	0.79525	0.92453	0.00191
RSD		20.07	20.70	10.65	20.21	0.178
T-test results compared to C-PMFC				0.2033 p > 0.05	0.271 p > 0.05	0.1331 p > 0.05
Control-PMFC						
	Macro CHN	0.04800	48.00000	7.5708	3.1280	0.84017
		0.03240	32.40000	6.4360	5.0254	0.71097
Average		0.04020	40.20000	7.0034	4.0767	0.77557
SD		0.01	11	1.08954	0.94841	0.091375
RSD		27.44	27.44	15.55	23.26	11.78

T-test results compared to C-PMFC				0.063 $p > 0.05$	0.2937 $p > 0.05$	0.0881 $p > 0.05$
C-PMFC						
	Macro CHN	0.05280	52.80000	9.0332	4.7914	1.1390
		0.04860	48.60000	8.3469	5.1721	1.2329
Average		0.05070	50.70000	8.6901	4.9818	1.1859
SD		0.003	3	0.48526	0.26919	0.06637
RSD		3.858	5.858	5.584	5.404	5.597

APPENDIX B

TEST RESULTS: % S CHN IN STUDY OF FERTILISER AMENDMENTS

	Method	Mass	Mass (mg)	Sulfur (mg)	Sulfur (%)
U-PMFC					
	Furnace Temperature 1350 ^{0}C	0.1023	102.3000	0.01588	0.01470
		0.0626	62.6000	0.00786	0.01189
Average		0.0824	82.4500	0.01187	0.01329
SD		0.03	28	0.005671	0.001988
RSD		34.05	34.05	47.87	14.95
T-test results compared to C-PMFC		0.46529 $p > 0.05$			
CU-PMFC					
	Furnace Temperature 1350 ^{0}C	0.1083	108.3000	0.01042	0.00911
		0.0519	51.9000	0.00451	0.00823
Average		0.0801	80.1000	0.00747	0.00867
SD		0.04	40	0.004183	0.000628
RSD		49.79	49.79	56.03	7.247
T-test results compared to C-PMFC		0.0242 $p < 0.05$			
Control-PMFC					
	Furnace Temperature 1350 ^{0}C	0.0875	87.5	0.00787	0.00852
		0.0493	49.3	0.00499	0.00958
Average		0.0684	68.4	0.00643	0.00905
SD		0.03	27	0.00204	0.00075
RSD		39.49	39.49	31.69	8.325

T-test results compared to C-PMFC		0.03015 $p < 0.05$			
C-PMFC					
	Furnace Temperature 1350 ^{0}C	0.0998	99.8000	0.01313	0.01246
		0.0559	55.9000	0.00854	0.01447
Average		0.0779	77.8500	0.01083	0.01346
SD		0.03	31	0.003244	0.001421
RSD		39.78	39.87	29.94	10.56

APPENDIX C

TEST RESULTS OF SOIL PROPERTIES IN STUDY OF FERTILISER AMENDMENTS

	Salinity (ppt)	**Electrical Conductivity (µS/cm)**	**pH**	**Resistivity (Ω/cm)**
U-PMFC				
	0.7	1488	7.7	696
	0.8	1536	7.9	594
	0.9	1896	8.0	648
	0.8	1430	7.9	697
Average	0.8	1587.5	7.9	658.8
SD	0.0816	210.1833	0.1258	48.8493
RSD	10.20	13.24	1.59	7.41
T-test results compared to C-PMFC	0.0006 $p < 0.05$	0.0026 $p < 0.05$	0.1264 $p >0.05$	0.0949 $p > 0.05$
CU-PMFC				
	0.5	1054	8.0	820
	0.8	1570	7.9	623
	0.7	1293	7.9	540
	0.8	1507	7.8	660
Average	0.7	1356	7.9	660.75
SD	0.14142	233.64503	0.08165	117.42622
RSD	20.20	17.23	1.03	17.77
T-test results compared to C-PMFC	0.008345 $p < 0.05$	0.018907 $p < 0.05$	0.06 $p > 0.05$	0.10055 $p > 0.05$
Control-PMFC				
	0.6	1058	8.3	711

	0.5	892	8.3	955
	0.3	1134	8.4	732
	0.6	985	8.1	606
Average	0.5	1017.25	8.275	751
SD	0.1414	103.3098	0.1258	146.7447
RSD	28.28	10.1557	1.5202	19.5399
T-test results compared to C-PMFC	0.1576 $p > 0.05$	0.2319 $p > 0.05$	0.0003 $p < 0.05$	0.1650 $p > 0.05$
C-PMFC				
	0.3	596	7.7	1700
	0.5	1146	7.8	880
	0.3	917	7.9	543
	0.5	1010	7.7	960
Average	0.4	917.25	7.775	751.5
SD	0.1155	233.9022	0.0957	294.8635
RSD	28.875	25.5003	1.2308	39.2367

BIOGRAPHY

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Publications

Nguyen V & Nitisoravut R (2019) Bioelectricity generation in plant microbial fuel cell using forage grass under variations of circadian rhythm , ambient temperature , and soil water contents. Proceedings of *Asia Power Energy Eng Conference* (240–244). China: IEEE Publishing.