EXAMINING DIFFERENT CLASSES OF PLANTS UNDER VARIOUS OPERATING CONDITIONS FOR BIOELECTRICITY PRODUCTION IN PLANT MICROBIAL FUEL CELL

BY

ROSHAN REGMI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIRMENTS FOR THE DEGREE OF MASTER OF SCIENCE
(ENGINEERING AND TECHNOLOGY)
SIRINDHORN INTERNATIONAL INSTITUTE OF TECHNOLOGY
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A Thesis Presented

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ROSHAN REGMI

Submitted to

Sirindhorn International Institute of Technology

Thammasat University

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (ENGINEERING AND TECHNOLOGY)

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NOVEMBER 2017

Abstract

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Bachelor of Science in Agriculture (First Division with Honors), Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad India, 2014.
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Climate change and food security are burning topics being discussed nowadays. To mitigate impact of fossil fuels, different kinds of alternative sources of energy have been researched and applied in the last decade. Generation of electricity from living plants has been evolved as a new kind of renewable source of energy known as plant microbial fuel cell (PMFC). This thesis embodies investigation of four different types of plants varied in their morphological and physiological traits accompanied with various operating conditions in PMFC system. The result reveals that plant types, support medium, configuration, and physical parameters are the drivers for electricity generation in PMFC. In the first experiment, while comparing the double chamber paddy microbial fuel cell with earthen material as a separator with traditional sediment MFC one, the former one outnumbered the performances of the latter one in terms of power generation. The main favorable conditions identified in this experiment were growth stages and volume of catholyte. In the second experiment, paddy was grown in single chamber mimicking the real field condition in terms of design and soil used. The nature of electric signal from sediments and plants were compared. Moreover, to know the effect of substrate addition, reactors were amended with *Azolla* which generated almost three times current than non-amended reactors. In the third experiment, hydroponic plant morning glory was assessed in stackable double chamber PMFC to determine the role of plant at anode vs cathode chamber. In the fourth experiment, long term ability of power generation from two plants, tomato and vetiver were investigated. Nature of *in-situ* electricity signal were determined. In this study, plant growth nature and vitality were carefully observed since it altered power output. Moreover, two types of plant performed differently under the same design. Taking account of all factors, maximum power depicted from polarization curve was normalized to anode area which was used to compare system performances. Overall, the results revealed that marshy grasses like vetiver is an ideal plant for long term performances which is devoid of reproductive phase, food crops like rice is influenced greatly by its growth stages in bioenergy harvest, and hydroponics plant like water spinach is limited by root exudates and oxygen released through roots. Double chamber enhances the performances efficiently as compared to a single chamber configuration. This study concluded that each system should be designed based upon physiological and morphological traits of plants. To interpret all the factors underlying for PMFC performance, biosystem principle is proposed. A new paradigm of trios' assessment of plant, soil and microbes' health has been proposed for future research.

Keywords: Biosystems, Bioelectricity, MFC, PMFC, Power density

Acknowledgements

It gives me immense pleasure to acknowledge Assoc. Prof. Dr. Rachnarin Nitisoravut, my thesis advisor, who guided me throughout the experiment. It would not be possible for me to gather mountain of knowledge without him. I express my deep gratitude to my thesis committee members, Dr. Yongsak Kachonpadungkitti and Dr. Korakot Sombatmankhong for their valuable suggestions.

I am thankful to EFS scholarship program. I would take this opportunity to thank lab technician, Mr. Prasitchai Chaiamarit and graduate student Mr. Jaranaboon Katechaimongkol, the school of Bio-Chemical Engineering and Technology for helping me in logistic. I convey my deep appreciation towards the Center of Scientific Equipment for Advanced Research, Thammasat University for the support in analyzing the samples.

I dedicate this work to my wife, Ranjita Sapkota, for her untiring efforts and motivation to do research.

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Nomenclatures

A + P = *Azolla* amendment + paddy planted

COD = Chemical Oxygen Demand

CW-MFC = Constructed wetland microbial fuel cell

EDS = Energy dispersive spectroscopy

I-V = Current- Voltage

MFC = Microbial fuel cell

NA +NP = No *Azolla* + No paddy

NA + P = No Azolla + paddy planted

OCV = Open circuit voltage

OM = Organic matter

PMFC = Plant microbial fuel cell

PMFC1, PMFC2, PMFC3 = Different double chamber paddy microbial fuel cell

R1, R2, R3, R4, R5, R6 = Different reactors

RP= Reproductive phase for paddy microbial fuel cell

SEM= Scanning electron microscopy

SMFC = Sediment microbial fuel cell

SPMFC= Single chamber paddy microbial fuel cell

VP = Vegetative phase for paddy microbial fuel cell

Chapter 1

Introduction

1.1 Background theory

Depletion of energy reserves combined with climate change are two major challenges being incited due to excessive reliance on conventional fossil fuels (Stern, 2007). In such circumstances, alternative source of energy has gained increased attentions among researchers and stakeholders in past few decades. With a view to diminish CO_2 footprint in an environment many clean sources of energy like wind, solar, hydro, and geothermal establish themselves as a suitable candidate for future green energy. However, accompanied with energy intensive process, land scape transformation, geographical constrains, these technologies adoption rate is still not substantial. Increased global population demands safe water and enough food. On the same tone, one quarter of world population are living under dark, mostly in rural areas of developing countries. Currently, combustion of coal shares a large part of electricity source in global scenario (World Bank). Appended to such situations, there is an urgent call to maintain balance in energy consumption at all regions of world. Human activities are in a center to mitigate climate change and establish climate resilient society. This leads towards the development of technology that can address the key issues the global energy is facing viz electricity generation, mitigate environmental footprints, and ensure safe water and food. Microbial Fuel cells (MFCs) that are capable of deriving energy from organics enriched medium under mild operating conditions probably offers a possible solution. Mother technology MFC gives rises to other many technologies accompanied with same principle of generating electricity with an aid of microbial metabolism decomposing organic matters.

Rhizosphere facilitated electricity generation has gained an increased attention with an account of concurrent biomass and bioelectricity production. A promising progeny of MFC technology, Plant MFC (PMFC) produces micropower in clean and sustainable way without any ecological footprints. Electricity generation in a PMFC employs the use of rhizodeposition as substrates at root- soil interface (Strik et al., 2008) (Nitisoravut & Regmi, 2017). Rhizodeposition are classified as low molecular weight compounds like organic acids, amino acids and high molecular weight compounds mostly cellulose, dead tissue, slough of cells, root cap etc. Microbes can utilize any substrates to yield electrons for the current generation (Timmers et al., 2012). Anaerobic region accompanied with an electrode receives the electrons, thereof provided by microbes and pass through the circuit, which when reach another electrode called cathode placed in aerobic region produce a small amount of current. Therefore, electricity produced via a redox ramp between anaerobic and aerobic regions. Mild in operation, devoid of geographic constrains, and no scarification of land make this technology an upper hand than currently offered sustainable source of energy like solar energy, wind energy, and geothermal energy (Nitisoravut & Regmi, 2017). As a neonate technology, researchers are currently working in extemporizing the technical difficulties, and finding the science of relationship affecting the system performances.

With a history of a decade, PMFC demands rigorous research in terms of designing effective configurations, and finding the effects of different factors for system performance. Three classes of plants are currently functional in a PMFC system viz wetland grasses (Helder et al., 2010), hydrophytes (Venkata et al., 2011), and paddy . The first one is mostly applied to generate electricity from marshy land. The second one is mostly used for wastewater treatment couple with electricity generation called Constructed Wetland MFC (CW-MFC). The latter one, paddy has been the favorite for researchers to be used in PMFC. It has engrossed the attention of the researchers because of waterlogged condition around rhizosphere zone which is suitable for anaerobic oxidation of organics. Furthermore, population growth at geometric mean and cultivated land remaining static incite pressure on sufficient production of food. Moreover, rice is the stable food of south Asia and consumed all over the word (Khush, 1997). Simultaneous harvest of biomass and bioelectricity from paddy PMFC supply a small amount of electricity in a rural area along with biomass harvest (Moqsud et al., 2015). Moreover, mitigation of methane gas from paddy with a deployment of PMFC further adds a beauty of this technology given the climate change is the most discussed issue in a today's society (Arends et al., 2014). Therefore, in comparison to other class of plant, growing rice and harvesting bioelectricity offers dual advantage of green energy and food security.

Cost of reactor fabrication affects the real-world use of technology. To apply the system in a real scenario emphasis should be made on the cost effective, no additives, devoid of high cost membrane (Nafion) and chemical catalysts free designs. Those chemicals and membranes further may harm the soil properties and can act as a secondary pollutant (Nitisoravut et al., 2017), and may have residual effect on the harvested final product. Thus, sustainable way to address these scenarios is to use locally available materials to improve the system performance and most importantly focus should be given in altering configuration rather than adding those additives. Earthen material has been researched in the field of MFC with promising ability for proton exchange thereof improving the performance as comparable as Nafion membrane (Behera & Ghangrekar, 2011; Jimenez et al., 2017; Thanh & Nitisoravut, 2015; Winfield et al., 2013).

In terms of design, paddy PMFC has been practiced in a sediment system, where anode is buried in the root arena and cathode is placed on the overlying water. Both *in-vitro* (Moqsud et al., 2015) and *in- vivo* application of paddy PMFC has been witnessed. Leakage of organics from anode area to the cathode and oxygen from cathode to anode can decrease the performances of the system while operating as a single chamber sediment system (Arends et al., 2014; Timmers et al., 2012).

From PMFC perspective, plant types via root morphology, photosynthetic activity, and root exudations affect bioenergy harvesting. Effectiveness of plants to achieve higher system performance is attended with robust character, high root biomass, and adaptability. Marshy and robust grass like vetiver because of its dense roots, adaptability with extreme environmental conditions, sparse of reproductive growth, and resistance to biotic stress (insects and diseases) (Danh et al., 2009), vetiver grass can be an ideal plant for plant microbial fuel cells. Moreover, this grass is widely used for phytoremediation and nutrients removal from contaminated water (Chen et al., 2004).

Furthermore, for dual harvest of biomass and bioelectricity, investigations of edible plants that are grown easily with minimum care need to be done. Apart from growing plants in PMFC system, low cost easily scalable reactors are utmost for its pragmatic use. Finally, there are many operating parameters that need to be optimized for maximum power output. This work designs different low cost PMFC to facilitate different types of plants for bioenergy production by deploying microbial fuel cell concepts and investigates taking into all those factors in consideration to clarify the factors affecting power generation with biosystems principle.

1.2 How plant microbial fuel cell (PMFC) is well described with the help of biosystems principles

Biosystems refers to biotic and abiotic components, interrelated to each other for definite purpose. The photosynthetic method of converting solar energy into sugars and finally biomass is a sequence of interrelated changes (Alocilja, 2000) and an orthodox example of biosystems. In alike manner, a PMFC captures the root exudates outcomes from photosynthesis and converts to bioelectricity accompanied with microbial metabolism (De Schamphelaire, Van den Bossche, et al., 2008) . A PMFC can be hypothesized as an open loop type of biosystems comprising of many factors underlying to produce bioelectricity. Figure 1.1 depicts schematic of biosystems principle (Nitisoravut & Regmi, 2017) for interpretation of PMFC mechanism. The biocontrol structure (plant) absorbs the solar energy and undergo photosynthesis resulting in the production of carbohydrates. Unused carbohydrate is secreted to rhizosphere region in the form of exudates or rhizodeposition. Bioprocess structure (Microbes) oxidizes those exudates releasing electrons and protons under favorable condition. Under redox gradient of two different types of electrode (anode and cathode) the system produces voltage. As per the principle of open loop system, the output voltage is dependent to input signal and other factors.

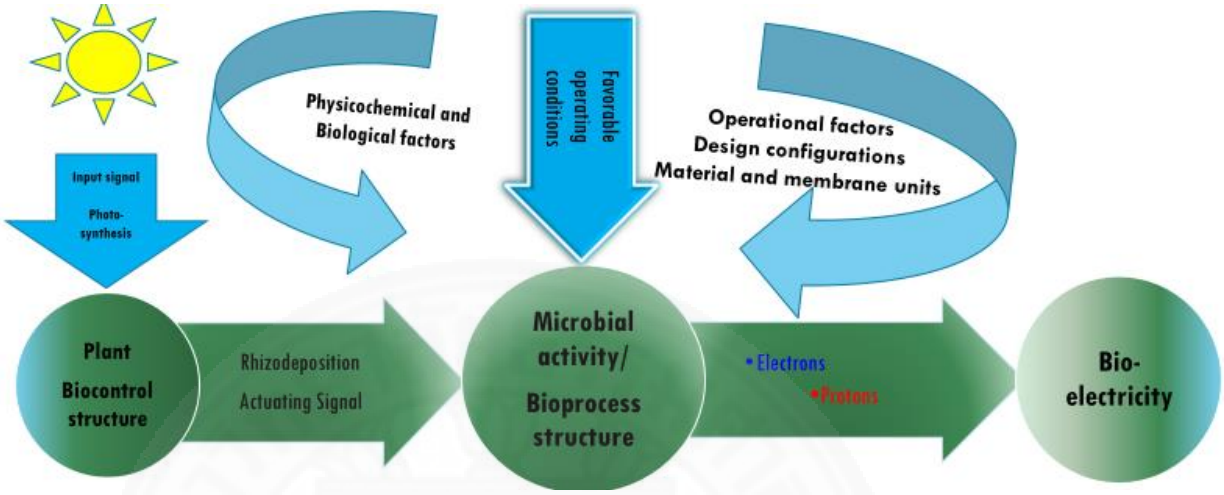


Figure 1.1 Interpretation of PMFC in terms of biosystems principle

1.3 Problem statement

PMFC study is still at very neonate stage and many questions are still unanswered. This study will investigate various factors that might affect voltage generation from PMFC. More focus would be given its plant science aspects. Economic crops like tomatoes and water spinach will be investigated for the first time in this study. It is aimed to identify those variables that differ significantly between different system configurations subject to various modifications. This research investigates four different classes of plants for bioelectricity production based upon their morphology and physiology.

1.4 Research hypothesis

This research is designed to assess the hypothesis that PMFC mimics the classical biosystems principle. Being involved the multidisciplinary field it is quite hard to predict the efficacy of systems in one factor. Thus, it is hypothesized that PMFCs system can act as an open loop biosystems which accommodates input signal (Sunlight/

photosynthetic pathway), biocontrol structure (plants), actuating signal (rhizosphere world), bioprocess structure (microbial population), activation energy (operating parameters), and outputs (power output). The latter one is subject to change with change in any former parameters.

- ✓ Trends of voltage generation differs based upon plant morphology and physiology
- ✓ Same plant can perform differently in different designed system.
- ✓ Operating parameters has an influential effect in power output of the system

1. 5 Research objectives

This study is conducted to achieve the following objectives.

- ✓ To clarify the effect of plant morphology and physiology in PMFC.
- ✓ To study the effect of configuration and electrode materials for power output.
- ✓ To provide the robust data about various factors and analyze boosting and declining factors for PMFC efficacy.
- ✓ To lay foundation for interpretation of PMFC in coming days within biosystems principles.

1.6 Scope and significance of study

Effect of plant type is neglected aspect in PMFCs' research. Moreover, there is an urge to illuminate the effect of different operating parameters. The present study will extend existing knowledge in PMFCs by unravelling different factors affecting the system performance. Most importantly, results on different mode of operation will be helpful for further improvement of PMFC research. The future focal points and application of PMFC can be directed based upon these results.

Chapter 2

Literature review

2.1 Historical advancement of PMFC

In the year 2008, while scholars from the Netherlands provided the principle for green electricity from living plants from marshy grass (Strik et al., 2008), researchers in Japan attempted to acclimatize the paddy field for bioenergy harvest in terms of biomass and bioelectricity (Kaku et al., 2008). Over a period of the time, PMFC technology has witnessed green roof top (Helder et al., 2013), floating water body (Schievano et al., 2017), marshy wetlands (Wetser et al., 2015), and paddy field (Takanezawa et al., 2010). Lighting the garden streetlights (Khush, 1997) to powering radio (Bombelli et al., 2016), the technology makes itself stand as a tough candidate for future green energy. Researchers are actively working in PMFC research with focus in electricity generation in natural conditions. Figure 2.1 depicts the historical advancement in PMFC studies. Moreover, MFC integrated constructed wetland is rapidly evolving mostly engrossed on wastewater treatment rather than electricity generation. As of October 9, 2017, Scopus search for key word “Plant Microbial Fuel Cell’’ depicted 670 documents. Furthermore, limiting with the key word “bioelectricity’’ yielded 162 papers. While filtering with root exudate as a key word only 32 documents are available. According to subject area, environmental sciences was the highest followed by chemical engineering.

2.2 Mechanism of electricity generation from plant microbial fuel cell

Autotrophic organisms like plant utilize carbon dioxide and water in presence of Solar radiation to yield biomass in the green part (chlorophyll) of its leaves. Depending upon the type of plant species up to 50 percent of photosynthates is utilized by plants for conducting various metabolism excreting remaining to rhizosphere in the form of rhizodeposition or and exudates which comprises different kinds of organic acids, high molecular weight compounds like cellulose, dead tissues, debris and slough of cell wall. Bacteria and other microbes around rhizosphere decomposes these organic compounds under an anaerobic region releasing electrons and protons. Electrons are received at anode and passed through a wire and reach a cathode completing the circuit, electricity is produced, so called “bioelectricity”. Electricity is thus generated by the redox gradient between two electrodes (Bennetto, 1990). The main difference between MFC and PMFC is the latter one is accommodated with plant as a supplement of substrates for microbial metabolism (Strik et al., 2008) . The key in PMFC is therefore, plant-microbe harmony at the soil interface, driven by rhizodeposition coupled with efficient engineering. Effect of plant, soil microbial characteristics, and design configuration are the most probable factors affecting the overall performances of PMFCs. Figure 2.2 illustrates the mechanism of PMFC. Table 1.2 illustrates the different methodologies may deploy plants for electricity generation.

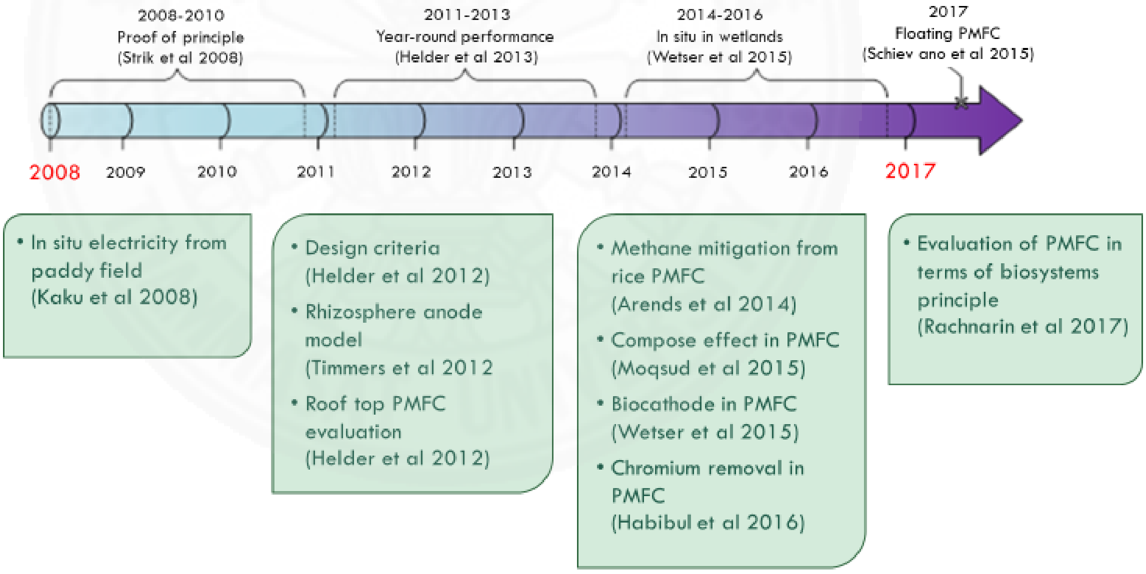


Figure 2.1 Historical advancement of PMFC research

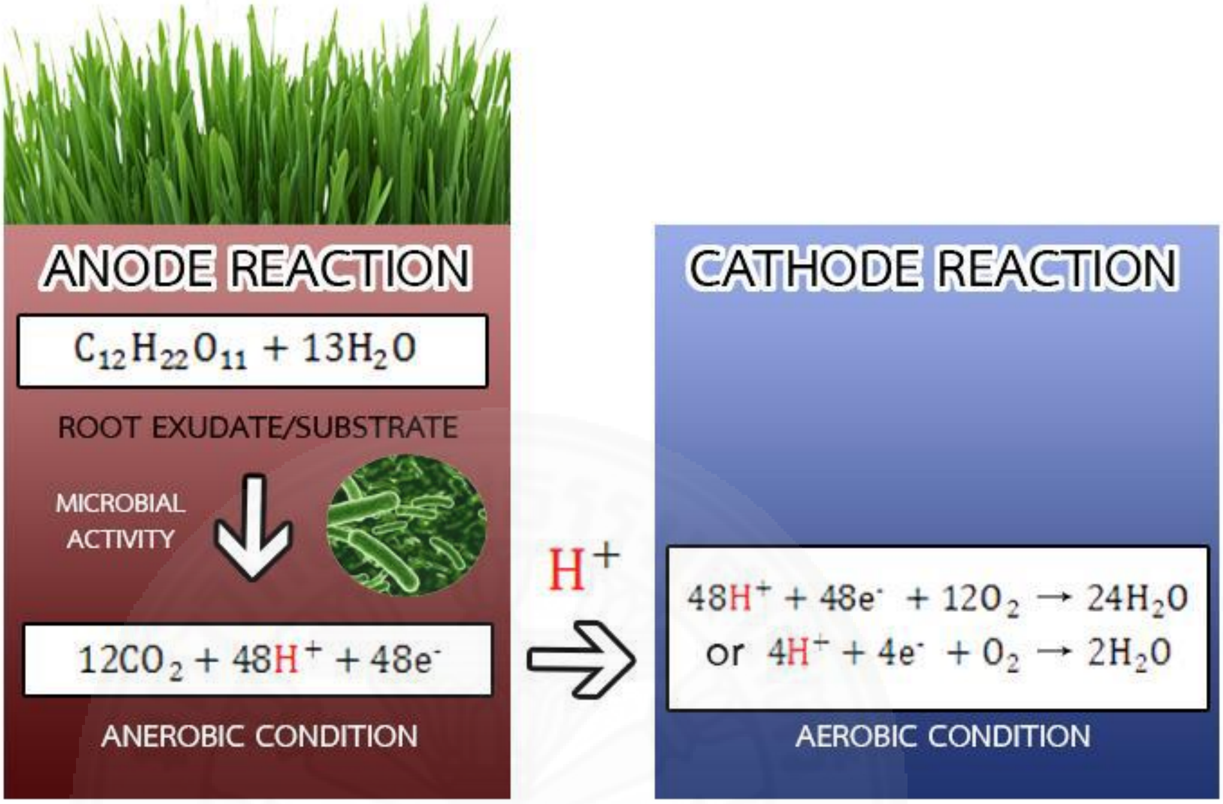


Figure 2.2 Mechanism of electricity generation in PMFC

2.3 Analysis of system performances

Power density, long term operation, Coulombic efficiency, internal resistances, etc. are the parameters being considered for analyzing the system performance of P/MFC. Researchers use power density to compare the power output of different systems. Since the biological reaction occurs at the anode, power output is found to be normalized to the projected anode surface area. However, cathode reaction limits the overall power generation in most of the cases and difficulty of expression of anode area (granular form) make researchers use cathode area to normalize the system performance. In terms of PMFC, plant growth area, anode cross section area, membrane areas are also used to normalize the power generation from the system. Besides this, Coulombic efficiency represents the fraction of the total coulombs transferred from the substrate to the anode.

Maximum power density is obtained by constructing polarization curve, and internal resistance is calculated by a slope of I-V curve (Figure 2.3). Equations for various calculations are depicted in equations as below. Moreover, biomass production is also key parameter for PMFC performance.

Electricity production is accompanied with substrates decomposition by the bacterial population, thereby decreasing the organic loading. Unlike MFC, in PMFC, organic matter might be added *in-situ* through rhizodeposition, therefore measurement of organic content signifies the organic degradation and organic matter (COD removal) addition in the system. There should be strong correlations between organic degradation and the power density. As yet there are no perfect explanations about mechanisms of relationship between COD removal (Equation 3) and the power density for PMFCs. This might be due to many other variables that can affect the power output of the system. For example, types of microbial populations, electrode materials, types of the substrates, pH (Deepika et al., 2015; Xu et al., 2015). Hence, the systematic comparison of efficacy of different reactors in uniform basis is quite difficult. It can be concluded that there are multiple boosting and declining factors for the system performance which need rigorous research to provide more scientific facts about the mechanism of relationship. Most importantly, the technology would be applicable if it is cost effective and easy to upscale. For these, understanding of the attempts made in improving efficiency of the system is an important with the methods of the operation.

$$P = E^2 / ARext$$

$$CE = M \int Idt / Fbq\Delta COD$$

$$COD\ removal = \frac{\Delta COD}{influentCOD}$$

Where, P = Power density

E= Voltage generation

A = Area (anode, cathode or plant growth area)

R_{ext} = External resistance

CE= Columbic efficiency

M = Molecular weight of Oxygen

I= Current generation within time frame t

ΔCOD = Initial COD – Final COD within time frame t

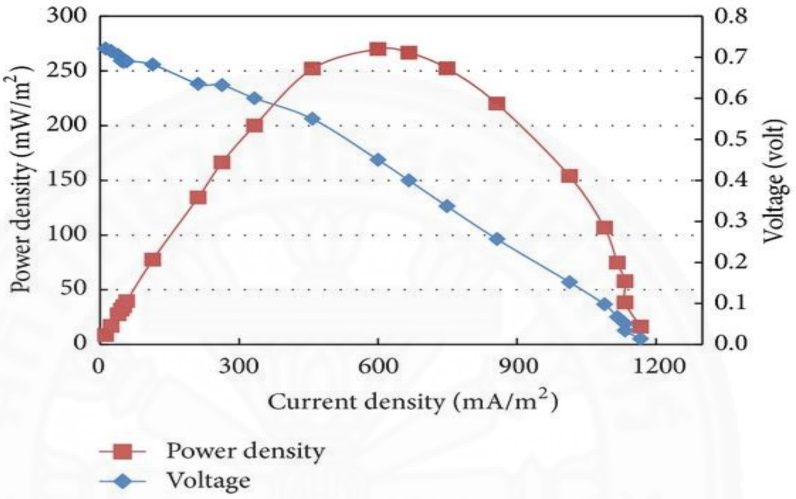


Figure 2.3 Typical polarization curve

2.4 Factors affecting system performance of PMFC

Following factors directly or indirectly affect system performance of PMFC.

2.4.1 Light

Light intensity (Shirley, 1929), quality (Shin et al., 2008), and photoperiodism (Junttilla, 1980) are the input signal that can affect the growth of plant and system performace of PMFCs. Effect of an illumination as a light cycle (Wu et al., 2013) and a power (Juang et al., 2012) has been studied in the photosynthehtic microbial fuel cell since it is directly connected to the metabolic action of microorganisms. It is required to have an optimal light intensity for the microbial species and the efficient operation of a system (Xiao & He, 2014). Moreover, light is potent factor for photosynthesis to form carobhydrates and thereafter bioelectricity in PMFC. Light should be optimal for the diverse microbial community in the rhizopshere so that maximum of root exudates can be decomposed for electricity generation. The effect of light in the PMFCs' performance was well documented in quite number of studies. For instance, augmentation in light intensity could increase output voltage (Strik et al., 2008). Similarly, shading of plants decreased the electric output which was attributed to inhibition of the photosynthesis resulting with decline in rhizodeposition. Other way around, addition of external substrate in the form of acetate increased the electric output in dark which elucidates the role of light in triggering of root exudates. In one of the study two different types of plants viz *O. sativa* and *E. glabrescens* unveiled dissimilar periods in attaining the maximum power in light phase i.e., 3-4 h and 6-8 h, respectively. Alterations in time for accomplishment in the maximum power was accounted for the physiology of plant such as synthesis of the organic compounds, transportation of compounds to the root, release of the exudates and absorption of the exudates by bacteria and release of the electrons (Bombelli et al., 2013). Therefore, light is not only the limiting factor for power generation while plant physiology also affects the overall performances. Thus, plants having the physiology that can adapt the photosynthetic matters in root exudates with the concurrent absorption by the microorganisms are well suitable to PMFCs since greater bioenergy yield can be attained. However, identification of the ideal light intensity for an efficient photosynthesis, optimal microbial activity, and developed rhizodeposition are the issues that are indispensable to be researched intensively.

2.4.2 Selection of plants

Choice of plants is mostly directed by its availability in the local vicinity rather than depth knowledge in their physiology and morphology. Nevertheless, there are some fundamentals being set up for it. Marshy grasses, paddy, and macrophytes (hydrophytes) are three general classification of plants being investigated. The former one is adopted for its saline tolerance, widely spread in Europe, and non-competition for land (Helder et al., 2010). Paddy is opted for its anaerobic condition developed in rhizosphere region that favor the oxidation reaction of exudates by microbes. Out competition of methanogens by electrogens in PMFC thereby reducing methane emission from paddy further add the motivation for researchers to conduct researches in paddy PMFC (Arends et al., 2014). Macrophytes or hydrophytes are mostly used in constructed wetland-microbial fuel cell (CW-MFC) (Liu et al., 2013) since these classes of plants are well known for its phytoremediation ability since long in CW technology. In this condition, plants are either incorporated at anode region or at cathode region. Inclusion at anode enhances the rhizodeposition/ substrates supporting microbial activity while at cathode, radial oxygen released by plant roots augment the oxygen reduction which is one of the bottle neck in MFC technology.

Use of marshy grasses is directed by their adaption to the system, high biomass production, and salinity tolerance (Timmers et al., 2010). Helder et al., studied fresh water species *Arundinella anomola,* along with marshy species *Spartina anglica* and *Arundo donax,* to compare their performance with the result reported in the earlier study. The maximum power reported for a PMFC using *S. anglica* was 222 mW/m^2, twice that of the result obtained using the same plant earlier (Helder et al., 2010). This may be due to the difference in electrode materials. Thus, the same plant can perform differently under varied operating conditions. Optimization of the operating parameters is therefore an important aspect for an. Table 2 depicts an overview of PMFC studies so far.

2.4.3 Photosynthetic pathways of plants

Rhizodeposition and photosynthetic pathway (Helder et al., 2010) are two possible effects in terms of selection of suitable plant in PMFCs. According to photosynthetic activity, plants are classified into three classes, i.e., C3, C4, and CAM. Plants in each class vary from one another in their mode of biochemical transformations. It has been well documented that C4 plants possess higher photosynthetic activity than that of other classes of plants (Wang et al., 2012). Therefore, from PMFC view, selection of C4 plants might have higher system performance. Nevertheless, some species of C3 plants, like *A. donax* as reported in the study (Helder et al., 2010), exhibit higher efficiency than that of C4. Therefore, investigating of photosynthetic pathways of plants is vital for choosing suitable plants. Less photosynthetic activity of C3 plants is ascribed to photorespiration respiration. In contrast, C4 plants overcome these wastages of energy with their unique photosynthetic pathway. Moreover, C4 plants never get saturated with light and outnumber the C3 plants in extreme hot and dry conditions in fixing the available carbon for carbohydrate formation (Brown, 1978). Therefore, C4 plants inclusion in PMFC technology have the following advantages over C3 plants (Deng et al., 2012) .

A. C4 plants exhibit the theoretical maximum limit photosynthetic efficiency (Pe), 6.0 % against C3 plants of 4.6 %.

B. Rhizodeposition is directly proportional to photosynthates formed (Rp). Therefore, more of the rhizodeposition in C4 plants, as compared to C3 plants, is subsequently available for microbes and fuel production, (Ra) 30 % and (Er) 9 %, respectively.

C. Moreover, C4 plants thrive well in hot and dry conditions.

Following equation explains the conversion efficiency.

$$\text{Conversion efficiency (CE)} = \mathrm{Pe} \times \mathrm{Rp} \times \mathrm{Ra} \times \mathrm{Er}$$

On the other hand, CAM plants dwell in dry and arid regions and differ from C3 and C4 because of their capability to uptake CO_2 at night time leading to water preservation in their tissues. CAM plants grow sluggishly resulting in fewer biomass production at a given time than that of other categories of plants (Hartsock & Nobel, 1976). Dissimilar morphology and make-up of these classes of plants offers challenges and prospects at a same time to do more studies.

S. anglica and *G. maxima* were pioneer plants and repetitively explored grasses in PMFC. In addition to this, *O. sativa* is the food crop being used in most of the studies. While comparing the power density, *S. anglica* outclassed the latter two in terms of maximum power in most of the studies being conducted. More bioenergy was harvested using this grass (Helder et al., 2012; Helder et al., 2010) than *G. maxima* (Strik et al., 2008) (Timmers et al., 2012) and *O. sativa* (Ueoka et al., 2016) aided PMFCs. Even though these studies were carried at varied working conditions, it is apparent that the effect of the plant is noticeable in the system performance. The improved performance of *S. anglica* might be because of its C4 pathway, a vigorous morphological character as compared to other plants. Except for a couple of studies, maximum yield from *O. sativa* is around or lower than 20 mW/m^2. This might be attributed to the consequence of growth phase, which is less robust in nature than grasses, and requires careful cultivation techniques. But, the average power density generated was less by tenfold than maximum power in these grasses functioned PMFC, posing exertion for long term steady power generation (Helder et al., 2010).

Sparse or negligible reports are available to conclude about the effect of photosynthetic activity on power generation so far. Apart from this, the efficiency of the system is affected by influences like electrode constituents and types, support/ medium for plant growth, and other operational parameters (Timmers et al., 2012). Hence, it is hard to link a system grounded upon merely on photosynthetic pathways. To have a strong insight, potentiality of different classes of plants must be investigated in an identical system and operation. Nevertheless, compiling of previous studies indicates that the power output from C4 plants is higher than C3 in most of cases. It is undoubtedly true that long term steadiness of a system requires longevity/vitality of plants that survive with severe ecological settings. In this respect, C4 (operated for 703 days) (Helder et al., 2012) and CAM plants are better than C3 plants.

2.4.4 Rhizodeposition

The voltage generation depends the quantity of root exudates (Bacilio et al., 2003), root morphology (Chiranjeevi et al., 2012), plant-microbe relation and photosynthetic efficiency (Takanezawa et al., 2010). Thus, performance of a PMFC can be enhanced with better rhizodeposition at optimal conditions directed by choice of suitable plants. Exploiting the maximum rhizodeposition for electricity production is obligatory for sustainable and extended operation (Strik et al., 2008).

Rhizodeposition provides substrates for bioelectricity production which comprise of root deposits, included of low molecular compounds (LMW) like organic acids, carbohydrates which are effortlessly degraded by microbes, and high molecular complexes like cellulose, dead cells, and slough of root which takes lengthy time for voltage generation. The performances of a PMFC therefore depends upon these exudations and the nature of microbial decomposition. For example, reactors employing hydrolysis of cellulose and dead cells did not display 24-hour fluctuation (Timmers et al., 2012) while oscillatory performance was showed by the biological cells that depend upon consumption of LMW exudates continuously supplied by photosynthesis in the light phases (Takanezawa et al., 2010). After insightful knowledge of the mechanism of plant-microbes-rhizosphere cumulative and antagonistic effects (Bais et al., 2006), it is possible to engineer an efficient system. The role of the rhizodeposition is understood but merely studied at micro-level in PMFCs. Nevertheless, some attempts were made on its quantification (Kuijken et al., 2011). Timmers et al. (2012) elucidated the hydrolysis of root exudates in current generation and claimed that current was limited by oxygen loss in the anodic section. Besides, plants with high root biomass were suggested for PMFCs. (Timmers et al., 2012). Mechanisms of rhizodeposition have been explored over many decades by researchers working in plant sciences. PMFC researchers need to apply those already understood mechanisms for long term and maximum bioenergy harvest from the system.

Rhizodeposition in plants are governed by many factors. Plant ecophysiology significantly regulates the quantity of carbon release through roots. Total root morphology has significant effect on exudation. For crop ecophysiology, the essential factors are plant age, types and number of microorganisms, nitrogen content in soil, and atmospheric CO_2 concentration. Besides these influences, there are many additional physical parameters that modify the deposits. Light intensity, photoperiod, soil pH, anoxia, and defoliation are the important ones (Kuzyakov, 2002). Defoliation of roots can change the trend of the root exudates (Henry et al., 2008; Murray et al., 2004; Paterson & Sim, 1999, 2000; Paterson et al., 2005). Thus, suitable settings for the intensification of exudates through the defoliation could be a fascinating area of investigation. So far, no reports are available that study the effect of defoliation in PMFC.

When plants become older, rhizodeposition declines**.** Hence, it can be assumed that power yield of a PMFC drops near the termination of the life cycle. It is of curiosity to see in what way plant produces current at different stages of its growth. The highest currents were recorded at the seedling and tillering stages in the paddy PMFC, which was operated for 98 days through five different stages: seedling, tillering, midseason aeration, filling and ripening (Deng et al., 2016). A likely description could be high microbial actions and more exudates at initial stages (Bacilio et al., 2003) or higher photosynthetic products exploited by the plants for fruit development, rendering fewer to the root at latter stages (Moqsud et al., 2015). Thus, plants can produce additional current at asexual stage rather than a reproductive stage. Perhaps the reason grasses tend to accomplish higher currents in a PMFC system is that most species are devoid of reproductive phases. Decrease in power output in the marshy grass operated PMFC was attributed to the vitality of plants rather than effect of growth stages (Strik et al., 2008).

All exudates cannot be employed for electricity generation if decayed by the non-electricity generating microbes. For example, Columbic efficiency in a glucose nourished MFC is lesser than an acetate fed, butyrate fed, and propionate fed MFC because of the breakdown of the glucose by the non-electricity generating bacteria (Deng et al., 2012). Moreover, for the identical microbial association, power density differs with diverse types of fuel (Du et al., 2007). Apart from root exudates, the power output in a PMFC is augmented with an organic amendment. To cite instances, addition of compost at the rhizosphere of a paddy plant had a considerable enhancement in power density as compared to the control (Moqsud et al., 2015). Besides using the wastewater from different industry rich in organic loading (Behera et al., 2010; Bermek et al., 2014; Durruty et al., 2012; Guo et al., 2013; Huang & Logan, 2008; Sakai & Yagishita, 2007; Sciarria et al., 2013; Zhang et al., 2013b), organic wastes were explored like kitchen and bamboo wastes, and various food industry wastes (Cercado-Quezada et al., 2010; Guo et al., 2013; Li & Ni, 2011; H. Wang et al., 2012) in MFCs. Additionally, harvested plants and their by-products in the form of crushed materials (Zang et al., 2010) and straw (Hassan et al., 2014) were used as substrates for MFCs operation. These studies entail that enrichment of the external substrates in PMFCs could enhance the bioelectricity production. However, care should be taken to optimize the designed system since a higher rate of fuel sometimes fortified the fermented bacteria, thereby decreasing the power output (Du et al., 2007) by outcompeting the electrogens. Exploring the effects of different kind of substrates is essential to get clear picture of functioning of well PMFC system.

2.4.5 Microbial world

Microbes are the initiator of the electrons in PMFC while it does by utilizing the substrates of the rhizosphere zone via two mechanisms either by direct electron transfer or mediated electron transfer. The later one require the mediators to aid in the electricity production (Logan & Regan, 2006). There is a unique relationship exists between the root zone of the plants and the microbial population. The classical manifestation of this relationship is the availability of feed by rhizosphere to the microbes and the role of microbes for better uptake of nutrition to the plants (Moulin et al., 2001). Better the microbial community adapted for the system, higher the chance of improved system performances. Understanding the microbial population helps to understand the competition among the electrons donors in PMFC.

Wide range of bacteria prevail in MFC like δ-Proteobacteria to the communities composed of α-, β-, γ- or δ-Proteobacteria, Firmicutes and many unknown classes (Logan & Regan, 2006). Similarly, δ-Proteobacteria, β-Proteobacteria, and Firmicutes predominated the anodic region while the waste sludge with different electron donors were fed to the MFC system. Furthermore, types of substrates affect the time to achieve the stable current in the MFC, 300 h for communities enriched with lactate, acetate and formate while 700 h for succinate, NAG and uridine (Kan et al., 2010). However, one of the study proposed that predominance of *Geobacter spp*. in the acetate-fed systems was consistent with good MFC performance and independent of the inoculum sources (Yates et al., 2012). When these understandings are linked to the PMFC system, it is very important to know the types of root exudates pertaining to the strain of microbes at the anodic rhizosphere of various plants.

From one gram of soil, 4600 distinct genomes of prokaryotes were estimated while studying the phylogenetic diversity and DNA heterogeneity in the soil bacteria (Torsvik et al., 1990). However, the microbial community shaping depend upon the phylogeny and the species of the plant (Berg & Smalla, 2009). The complex nature of the rhizosphere environment often makes difficult to quantify the microbial populations in the root system (Bais et al., 2006). Nevertheless, attempts have been made to elucidate the microbial population from the anodic region in the PMFC (De Schamphelaire et al., 2010; Lu et al., 2015; Timmers et al., 2012). *Geobacteracea* family has been detected in the best performing reactors than that of low current producing reactors. Geobacteracea are an obligate anaerobes associated with the power generation in the sediment MFC (Lowy et al., 2006). However, the degree of appearance of these strains in two different studies using *G.maxima* (Timmers et al., 2012) and *O. sativa* (De Schamphelaire et al., 2010) was different. It might be because of the different types of electrodes used and the plant species itself. In the later study, the range of effect of the plant in the bacterial community was influenced by the types of support like vermiculite and soil, this suggest that types of soil used in the PMFC also affects the power performances via the bacterial community. Unlike other studies, Lu et al., 2015 studied the potentiality of the current production in the PMFC system using *C. indica* plant in oligotrophic conditions without any external substrates. Furthermore, their study revealed the relationship between fermentative bacteria and electrochemically active bacteria. This study suggested the improvement in the current production devoid of external supply of nutrients by limiting the competition of the denitrifying bacteria (Lu et al., 2015) . Apart from donating electrons to the anode, microbial population at cathode region was reported to assist the electricity generation so called bio cathode (He & Angenent, 2006; Rothballer et al., 2015). Thus, it is equally important to unlock the microbe's role at the cathode region in the PMFC.

2.4.6 Soil characteristics

The soil-root consortium is a vicinity supporting microbes and maintains the relationship between microbes and plants (Gobat et al., 2004). Without understanding the role of soil, an efficient PMFC would hardly be achieved. Soil inoculum bacterium generated higher voltage but lower columbic efficiency than pure culture of *Geobacter* sulfurreducens indicating the presence of the non electrogen bacteria in soil (Jiang et al., 2010). In the same study, performance of three different types of soil in a soil MFC were compared and electrogenic activities of the soil were reported. Interestingly, 60 percent of the isolated microbial communities from the anode represented the strains that were capable of electricity generation and had certain common inherent community traits. Different strains were obtained in MFCs with agricultural soil as inoculum, which produced 17 times higher power than that of forest soil-based MFCs. This study showed the agricultural soil fed MFCs had lower C to N ratios, polyphenol content, and acetate concentrations than forest soil MFCs. A less diverse microbial community was observed, e.g., *Deltaproteobacteria*, *Geobacter*, in the best performing reactors while low-power MFC anode communities were dominated by *Clostridia*. Therefore, soil physicochemical and biological properties affect the power performance in PMFCs. Power increases were related to lower C/N ratios in the treated anode (Feng et al., 2010). The lower C/N ratios of agricultural soil might be another reason for system performance. Another reason might be the forest soil has a more diverse bacterial community and higher degree of non-electrogens that decreases the power output than in the agricultural soil since bacterial diversity of forest soil was more phylum rich whereas the agricultural soils were more species rich (Roesch et al., 2007).

Soil structure (Wakelin et al., 2008), soil texture (Sessitsch et al., 2001), nitrogen availability (Frey et al., 2004), and soil pH (Lauber et al., 2009) are the drivers to shape the bacterial community. Apart from the organic decomposition, inorganic matter present in the soil can affect the redox potential (Patrick Jr, 1981). Soil can yield electrons via chemical decomposition, such as sulphur species, humic acid, (De Schamphelaire, Rabaey, et al., 2008), and iron (II) (Meek & Chesworth, 2008). Soil continuously undergoes redox reactions (Vepraskas & Faulkner, 2001). Soil MFC has been included in school and college projects in the recent years (Jude & Jude, 2015; Root et al., 2011). These findings concluded that the nature of soil, with its microbial world, plays a pivotal role for electricity production in PMFCs. To engineer the best PMFC system, therefore, demands good understanding of soil roles unless it is applied as a hydroponics system.

2.4.7 Designing efficient configuration

Pragmatic usage of any technology is determined by factors like its economic competence, long period operation, ease of handling and environment friendliness (Logan et al., 2006). Fabrication cost, instable performance and fouling of materials poses difficulties for the real-world usage of an MFC. For instance, for system cost, the reactor alone accounts for 68.5%, anode and cathode 8.2%, membrane 11%, mediator 1.4%, and collector 2.7% (Deng et al., 2012). On the contrary, a PMFC can be functioned by installing anode and cathode materials *in-situ* without obligation of highly expensive proton exchange material (PEM). Many studies already investigated that sediment type MFC can produce substantial power, but relies on the marshy land (De Schamphelaire et al., 2010; Dominguez-Garay et al., 2013; Jeon et al., 2012; Timmers et al., 2012). Extension of this knowledge and technology to further classes of plants is yet to be done to derive energy ubiquitously.

The uniqueness of a PMFC lies in generating *in-situ* bioelectricity from rhizodeposition of the living plants (De Schamphelaire et al., 2008). Instead of using chemical catalysts and expensive PEM, consideration should be focused to designing an effective configuration. Upscaling of this technology need to sort out numerous bottlenecks such as an increase of internal resistance, over- potential during activation, Ohmic and concentration losses, insufficient electrical contact between bacteria and anode, etc. (Rabaey & Verstraete, 2005). Therefore, different facets should be carefully explored for enhanced performances. These investigations should comprise inoculums, substrate (fuel), type of PEM (and the absence of this material), cell internal and external resistance, solution ionic strength, electrode materials, and electrode spacing (Cheng et al., 2006). Increment of power generation could be achieved with decline in internal resistances. Quantification and characterization of internal resistances in an MFC (Fan et al., 2008) and a PMFC (Timmers et al., 2012) were done to comprehend the preventive factors for maximum derivation of energy from the system. Designs and operation that promote readily transportation of ions towards cathode region is essential to reduce internal resistances thus improved power generation can be achieved (Sleutels et al., 2009). To cite an instance, PEM-less configuration offered less internal resistance when the electrode distance was kept larger than the thickness of the inter-diffusion zone in a PEM-less microfluidic fuel cells (Kjeang et al., 2009).

There are two major types of congifuration being designed and studied under PMFC viz sediment type (Single chamber) and Double chamber. Depth of anode sections (Takanezawa et al., 2010), size of electrodes (Nattawut & Kanyarat, 2014), and relative position of anode and cathode placement (Oon et al., 2015) are the factors being considered while modelling a single chamber PMFC. Paddy PMFC are mostly studies under sediment type single chamber PMFC. In a paddy PMFC, the anode was usually found to be dipped 2-5 cm under the sediment and the cathode was left on the border of water- sedmient surface (Helder et al., 2010; Kaku et al., 2008; Moqsud et al., 2015; Takanezawa et al., 2010). However, placement of cathode in soil around rhizospehere was also reported in one of the studies to harmess the oxygen released by roots for reduction reaction and offering less distance between the two electrodes (Chen et al., 2012). While evaluating the factors affecting the electrical ouptut from paddy PMFCs, it was reported that the power from a 5cm dipped anode was almost three times than that of a 2 cm depth anode (Takanezawa et al., 2010). Likewise, higher performance was attained when the anode was positioned 5 cm in soil (Deng et al., 2014) for a soil MFC. From these outcomes it can be decided that identification of the appropriate anodic zone is crucial for providing the anoxic conditions and utilization of the released carbon by microorganisms in PMFCs (Takanezawa et al., 2010). Similarly, changing the distance of anode from the root region change the power output (Chiranjeevi et al., 2012). A recent study revealed the effect of anode and cathode size in the performances of the paddy type PMFC. This study suggested that the anode is the limiting factor until the microbial community has acclimatized, while in the long run, decrease in the cathode performance limited the efficacy of the system (Ueoka et al., 2016).

In independent studies of two different types of configurations for flat plate (Helder et al., 2012) and tubular PMFC in a double chambered system (Timmers et al., 2013), the former was claimed to have lower internal resistance than the latter. Wide-ranging of internal resistance and output current at different anodic regions affect the power output as a function of root morphology. Therefore, understanding and connecting PMFC design to achieve maximum returns from root growth could substitute expensive membranes. Zhang et al. (2013) while introducing spiral spargers in a tubular MFC, reported accomplishment of higher COD elimination from wastewater (Zhang et al., 2013a). Such engineering methods can be useful to a PMFC for improving energy effectiveness. Combined technology is attractive these days, generally to modify the configuration for improved efficiency (Xu et al., 2015). Such a hybrid method of the application would be similarly appropriate to a PMFC system based upon the objective of the research.

2.5 Other similar technologies

Potter (1911) inculcated the idea of employing the potential of microbes for electricity generation (Potter, 1911). Based upon this concept, MFC technology has been evolved. The core operation of an MFC relies on the differences of redox gradient during microorganisms' metabolism when digesting substrates. Over a decade different modification in terms of design and application have been done in order to generate electricity with other applied applications. Some of technologies have been described briefly.

2.5.1 Sediment MFC (SMFC)

Sediments MFC converts the organic rich sediments into electricity via microbiological process. The underlying idea of sediment MFC is the redox gradient developed vertically where the underneath of the system act as an anode and underlying water surface behaved as a cathode. This concept could be applied promisingly in remote water bodies to extract electricity from organic-rich aquatic sediments (He et al., 2007). Soil structure (Wakelin et al., 2008), soil texture (Sessitsch et al., 2001), nitrogen availability (Frey et al., 2004), and soil pH (Lauber et al., 2009) are potential factors affecting sediment MFC. Apart from the organic decomposition, inorganic matter present in soil can affect the redox potential (Patrick Jr, 1981). Soil can yield electrons via chemical decomposition, such as Sulphur species, humic acid, (De Schamphelaire et al., 2008), and iron (II) (Meek & Chesworth, 2008). Therefore, soil continuously undergoes redox reactions (Vepraskas & Faulkner, 2001). Sediment MFC has been included in school and college projects in the recent years (Jude & Jude, 2015; Root et al., 2011). Sediment MFC is referred by different terms based upon its mode of operation, nevertheless the principle behind it is same. When applied to river bed and ocean it is coined as fresh water SMFC and benthic SMFC respectively, and small lab scale soil and mud operated MFCs are termed as a soil MFC and a mud MFC respectively. The performance of sediment MFC is greatly affected by microbial community shaping in an anode region, and conductivity of solution in underlying water.

2.5.2 Photosynthetic MFC (algal based)

Algae generates biochemical energy via conversion of solar energy. Excessive growth rate, and high CO_2 fixation rates make microalgae being taken in serious account to combat the CO_2 impact. Microalgae can be used either at anode or cathode in MFCs, the former one is to provide substrates for bacterial metabolisms, while the latter one is practiced enhancing the oxygen reduction rate at a cathode chamber. Switching of substrates between two chambers is also practiced enhancing the power performances. When applied as a cathode catalyst, these types of MFC is popularly known by biocathodes**.** The underlying principle of these kinds of MFCs is same as PMFC (photosynthesis), however the major difference is PMFC operates with higher plants at an anode region to provide rhizodeposition, but algae is incubated in a cathode chamber to utilize the oxygen production for aerobic process. Thus, during day time, algae carry out photosynthesis by utilizing CO_2 to generate biomass, simultaneously consumed O_2 during night time to obtain energy via oxidation of produced organic matters (Pandit & Das, 2015).

2.5.3 Constructed wetland MFC (CW MFC)

CW has been well known as an environmental technology for wastewater treatment. Stratification of zones into anaerobic and aerobic based upon redox reactions is the underlying principles of CW MFC. These kinds of MFCs are mostly practiced for wastewater treatment whilst incorporating plant either in anodic or cathodic chamber (Doherty et al., 2015). Overview of constructed wetland is provided in review article. Table 1.1 summarizes the maximum power generation from CW MFC modified from Doherty et al. (2015) study.

Table 2.1 System performance of plant assisted constructed wetland MFC adapted from (Doherty et al., 2015)

Influent (mg/l)	Power density (mW.m^{-2})	COD removal (%)
8000	7.44	75
180	5.62	85.7
560	20.76	95
1058	12.37	76.5
200	12.42	94.8
250	44.63	95
583	10.51	64
135	1.84	85.7

Table 2.2 Different methodologies of generating electricity implying MFC principle

Types of MFC	Operating principle	Anode set up	Cathode set up	Application
Sediment MFC	Redox gradient developed vertically on marine and river sediments	Anode is buried in the reduced region at the bottom of body	Cathode is placed over the underlying water body	Generation of electricity from ocean or river bed
Plant MFC	Rhizodeposition of plant acts as a substrate for electricity generation	Anode is placed at rhizosphere area of plant	Cathode either at water-soil interface (as in sediment) or separate chamber (double chamber	Generation of electricity from living plants
Photosynthetic MFC (algal based)	Algae biomass based upon photosynthesis is incorporated in MFCs	If anode is filled with algae, it is to provide substrates	Mostly inoculating cathode with algae for enhancing oxygen reduction, also called biocathode	Production of algal biomass enhancing MFC performances in terms of electricity generation and wastewater treatment
CW MFC	Incorporating plant either at anode or cathode coupled with traditionally practiced constructed wetland.	Plant role into anode to provide extra substrates for enhanced MFC performances	Plant can be incorporated at cathode to utilize oxygen released through roots to enhance MFC performances	Wastewater treatment
Integrated MFCs	Combining MFCs with other technology			Upscaling and practical application

2.6 Major challenges on P/MFCs

Higher internal resistance, over potential during activation, Ohmic and concentration losses, insufficient electrical contact between bacteria and anode, etc. (Rabaey & Verstraete, 2005) are major bottlenecks for efficient operation of fuel cell system. These aspects need to be considered to improve its efficiency. These comprise several inoculums, chemical substrate (fuel), type of proton exchange material (and the absence of this material), cell internal and external resistance, solution ionic strength, electrode materials, and electrode spacing (Cheng et al., 2006). Apart from these factors, detail understanding of rhizosphere world, long term performances of plant in the system and influences of physico-chemical and biological parameters are key for further improvement of PMFC system (Nitisoravut & Regmi, 2017).

2.7 Application of PMFCs

Table 2.3 provides the overview of the PMFC studies along with their application. Although MFC has been initially proposed to generate the electricity via organic degradation in wastewater, many diversifying products and applications have been emerged. Plant incorporated MFC technology can have useful applications as mentioned below.

- Floating PMFC in water body to supply bioelectricity
- Green roof top PMFC
- Electricity generation from paddy field and wetlands
- Methane gas mitigation from paddy field and wetlands
- CW- MFC for simultaneous wastewater treatment and electricity generation

Table 2.3 Overview of PMFC studies

Plant Types	Types	Research goal	MFC fabrication		Growth medium/ Substrate	Operating condition	Power density $mW\cdot m^{-2}$	Ref
			Anode	Cathode				
A. anomola	C4	Bio-electricity and biomass production	Graphite rod in graphite grains	Graphite felt	Hoagland solution	Climate chamber	22	(Helder et al., 2010)
A. calamus		Pyrene and Benzo pyrene degradation	Graphite felt	Graphite felt	Pyrene and benzo pyrene rich water	Climate chamber	-	(Yan et al., 2015)
C.indica	C4	Microbial community analysis	Graphite disk	Carbon cloth	Tap water/ rumen microorganisms	Ambient	400	(Lu et al., 2015)
C. involucratus	C3	Electricity generation and COD removal	Graphite felt	Graphite felt	Lotus soil and wastewater	Ambient ζ	5.9	(Nattawut & Kanyarat, 2014)
E. crassieps	C3	COD removal, electrode position	Graphite discs	Graphite discs	Domestic and fermented distillery wastewater	Miniature benthic system	224.93	(Venkata et al., 2011)

E. glabrescens	C4	Bio-photo voltaic cell	Carbon fiber	Stainless steel	Professional medium	Climate chamber	0.088 GJ·ha-1year-1	(Bombelli et al., 2013)
G. maxima	C3	Electricity production	Graphite granules	Graphite felt	Hoagland solution	Climate chamber	67	(Strik et al., 2008)
		Microbial community analysis	Graphite granules	Graphite felt	Hoagland solution	Climate chamber	80	(Timmers, Rothballer, et al., 2012)
		Design configuration	Graphite felt / Graphite granules	Graphite felt	Ammonium rich ½ Hoagland solution	Climate chamber	12- 18 Membrane area	[117]
I. aquatica	C3	Power generation	Granular Activated Carbon(GAC)	Stainless steel with GAC	Anaerobic sludge from municipal wastewater	Constructed wetland	12.42	(Liu et al., 2013)
L. perenne	C3	Chromium removal	Graphite granules	Carbon felt	Hoagland solution,	Green house	55	(Habibul et al., 2016)

					wastewater with chromium			
O. sativa	C3	Plant-microbe interaction	Graphite felt	Graphite felt	NPK fertilizer/ acetate solution	Rice field	6	(Kaku et al., 2008)
		Anode microbe's analysis	Graphite felt	Carbon/ polytetrafluorethylene coated	Glucose/ acetate, bacto yeast/ electrolyte solution	Rice field	19 ± 3.2	(Kouzuma et al., 2013)
		Bio photovoltaics cell	Carbon fiber	Stainless steel	professional growing medium	Climate chamber	980 GJ·ha-1year-1	(Bombelli et al., 2013)
		Methane gas mitigation	Graphite granules	Graphite felt	Vermiculite with Hoagland solution	Climate chamber	72	(Arends et al., 2014)
		Electrode placement and size effect	Graphite felt	Graphite felt	Soil / fertilizer	Rice field	14.44	(Takanezawa et al., 2010)

		Electricity production	Graphite granules	Graphite granules	Hoagland solution	Green house	33	(Schamphelaire et al., 2008)
		Effect of electrode size	Graphite felt	Graphite felt	Soil	Rice field	80	(Ueoka et al., 2016)
		Effect of compost	Carbon fiber	Carbon fiber	Onada soil	Ambient	23	(Moqsud et al., 2015)
P. setaceum	C4	Anode placement	Graphite plate	Graphite plate	Red soil	Ambient	163	(Chiranjeevi et al., 2012)
S. anglica	C4	Bioelectricity and biomass production	Graphite rod in graphite grains	Graphite felt	Hoagland solution	Climate chamber	222	(Helder et al., 2010)
		Long term performance evaluation	Graphite granules	Graphite felt	Hoagland solution	Climate chamber	110	(Timmers et al., 2010)

		Bio-cathode application	Graphite felt multiple layers	Graphite felt Single layer	Nitrate less ammonium rich medium	Climate chamber	679 (PGA υ)	(Wetser, Sudirjo, et al., 2015)
		Design configuration	Graphite felt	Graphite felt	Growth medium	Climate chamber	240	(Helder et al., 2012)
T. latifolia	C3	Simultaneous electricity and wastewater treatment	Carbon felt	Carbon felt, porous air spargers	Sludge from glove manufacturing company/gravel for support	Constructed wetland	6.12	(Oon et al., 2015)

Chapter 3

Methodology of research

3.1 Research frame work

Figure 3.1 depicts overall research method to study PMFC in terms of biosystems principle. All the factors underlying can be interpreted into three main domains. The first one is biocontrol structure which comprises plants and light that initiates the formation of exudates/ substrates/ fuels for microbial world to generate electron. Secondly, the microbial world is termed as bioprocess structure and finally, all operating conditions internally or externally which are required for operation of PMFC were termed as favorable conditions. The result revealed that plant types, support medium, configuration, and physical parameters all are the drivers for system performances of the PMFC. Given that the multiple factors prevailing within the system and dynamic nature of biosystems, it is quite difficult to identify the boosting and declining factors. Therefore, this method of study provides foundation for conducting PMFC research in coming days. Overall methodology can be further divided into three major steps. First one is microcosm set up which includes choice of plants, substrates or support, design configuration. After microcosm set up, next step would be data collection deploying online and offline methods and finally data analysis for various subjects under studies.

Thus, in this study, different experiments were designed to assess various factors, changing at least each parameter of biosystems principle in one experiment.

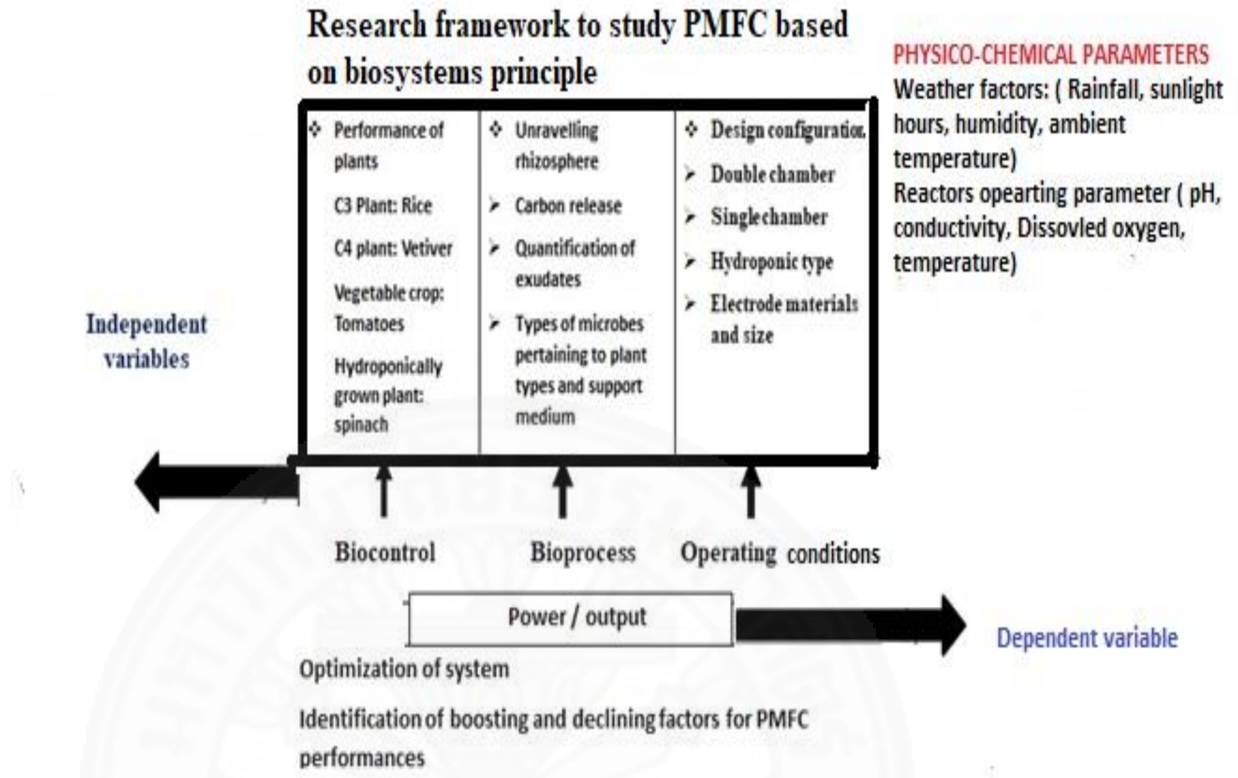


Figure 3.1 Research frame work

3.2 Choice of plants

Different plants were investigated based upon their morphology and physiology viz *Chrysopogon zizanioides (L.)* (vetiver), *Oryza sativa* (rice), *Solanum lycopersicum* (Tomato), and water spinach *(Ipomea aquatica)* (Figure 3.2). Vetiver has strong adaptive characteristics against harsh conditions along with rapid growth, fibrous root system and heavy metals removal ability. Moreover, it is widely spread all over Thailand. Rice is the mostly consumed food crops in Asia and has been advocated in PMFC for the green house mitigation and bioelectricity production. Tomato is popular economical and can be grown ubiquitously. Such type of plant has hardly been explored in the field of PMFC. It is hypothesized that bacterial colony devised by each root system of plant has role in the biofilm formation and contribution in the current generation. Most importantly, choice of these plants might clarify the better understanding about the relationship between photosynthesis with concurrent bioelectricity production. Vetiver is a C4 plant and other plants are C3 plant. In principle, C4 plants have the efficient Photosynthetic activity than C3 plant. To elucidate the effect of growth stage, it was an interesting to see the trends of voltage generation between Vetiver, rice and tomato. Since, the first one is devoid of growth stages and the latter two have life cycle with distinct growth phases (Vegetative and reproductive).

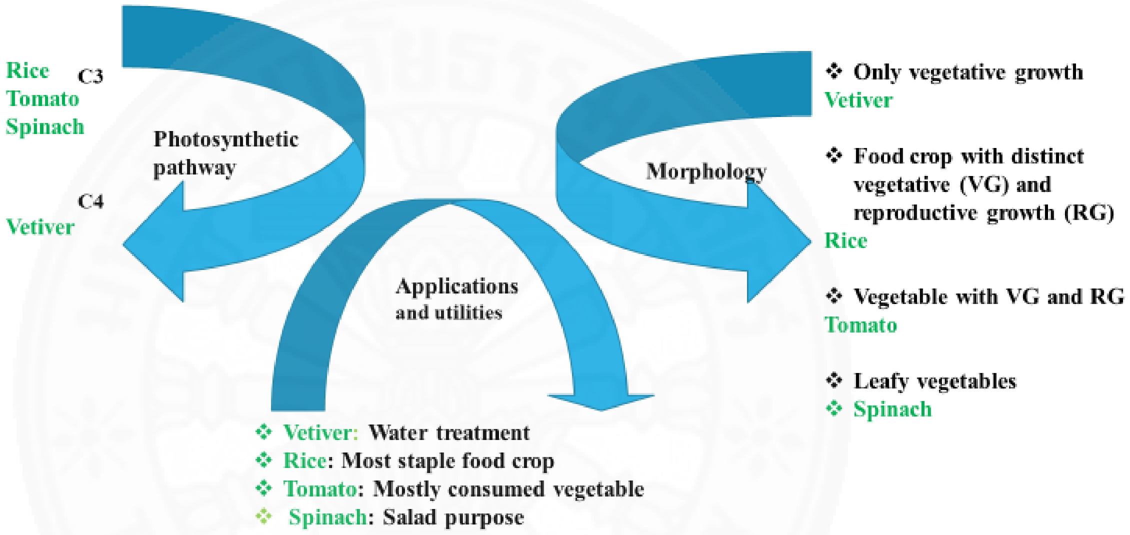
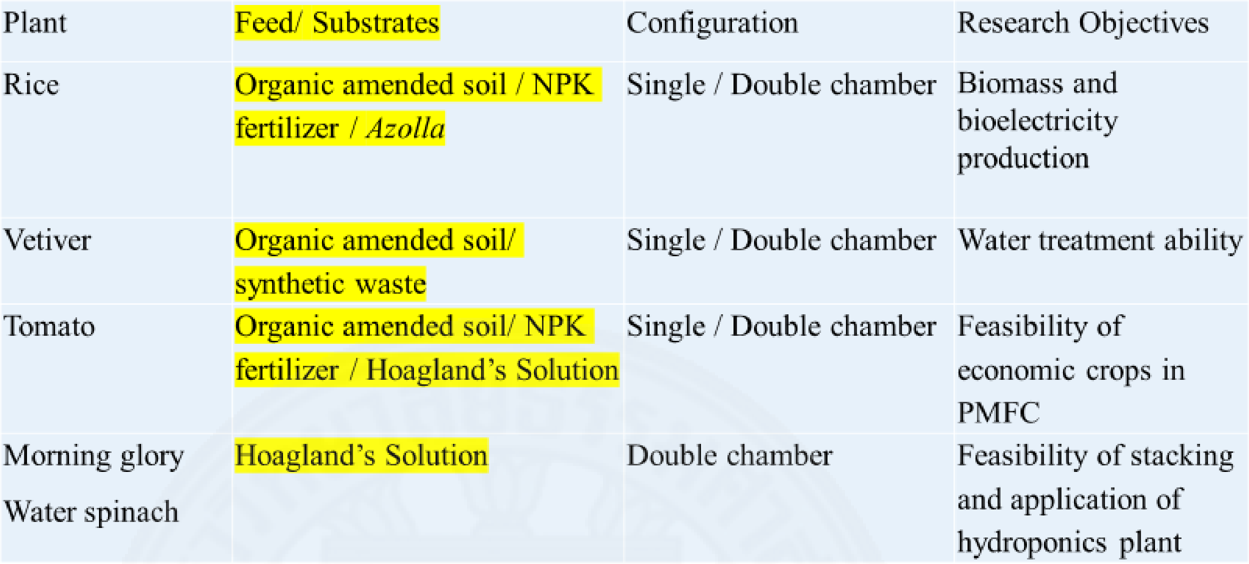


Figure 3.2 Determinants for choice of plants

3.3 Substrates/growth medium used

Commercial garden soil, rice field soil, synthetic wastewater, and Hoagland’s solution are main substrates/ support used in this study. Rice, vetiver, and tomatoes were grown strictly in soil medium for at least one complete cycle in an initial phase to mimic the natural microcosm. Furthermore, vetiver was investigated both in a synthetic wastewater and soil to see its water treatment ability. Spinach was grown on Hoagland’s solution hydroponically with soil as an inoculum. Characterization of substrates used in this study is given in Appendices.

Table 3.1 Summary of microcosm set up

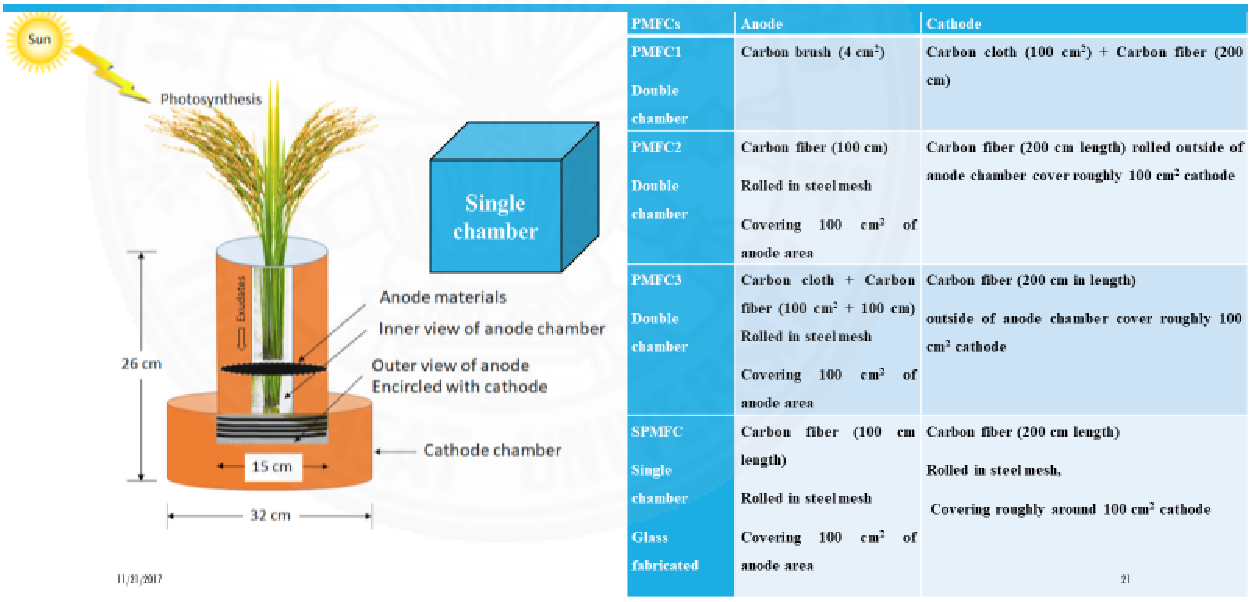


Plant	Feed/ Substrates	Configuration	Research Objectives
Rice	Organic amended soil / NPK fertilizer / *Azolla*	Single / Double chamber	Biomass and bioelectricity production
Vetiver	Organic amended soil/ synthetic waste	Single / Double chamber	Water treatment ability
Tomato	Organic amended soil/ NPK fertilizer / Hoagland's Solution	Single / Double chamber	Feasibility of economic crops in PMFC
Morning glory Water spinach	Hoagland's Solution	Double chamber	Feasibility of stacking and application of hydroponics plant

3.4 Design configuration

Different kind of reactors were designed in this study. Double chamber and sediment single chamber were two main configurations. Earthen material was used as material and membrane unit while operated as double chamber (Figure 3.3 and Figure 3.4). In addition, for sediment single chamber commercially available glasses were used. The detail about their construction and operation is described in the respective chapter. Construction were done mainly based on life cycle and morphological characteristics of plant. Smaller unit in term of volumetric capacity were used for hydroponic plant operated PMFC while almost double the working volume were used for the construction of unit for higher plants like rice, tomato and vetiver. However, the electrode size was used almost in the same range for all fabricated reactors on an average of 100 cm^2 unless mentioned anything else. The real units operated in this study is shown in Figure 3.5.

Figure 3.3 Fabrication of double chamber hydrophytes operated PMFC



PMFCs	Anode	Cathode
PMFC1 Double chamber	Carbon brush (4 cm^2)	Carbon cloth (100 cm^2) + Carbon fiber (200 cm)
PMFC2 Double chamber	Carbon fiber (100 cm) Rolled in steel mesh Covering 100 cm^2 of anode area	Carbon fiber (200 cm length) rolled outside of anode chamber cover roughly 100 cm^2 cathode
PMFC3 Double chamber	Carbon cloth + Carbon fiber (100 cm^2 + 100 cm) Rolled in steel mesh Covering 100 cm^2 of anode area	Carbon fiber (200 cm in length) outside of anode chamber cover roughly 100 cm^2 cathode
SPMFC Single chamber Glass fabricated	Carbon fiber (100 cm length) Rolled in steel mesh Covering 100 cm^2 of anode area	Carbon fiber (200 cm length) Rolled in steel mesh, Covering roughly around 100 cm^2 cathode

Figure 3.4 Schematic for earthen material double chamber PMFC

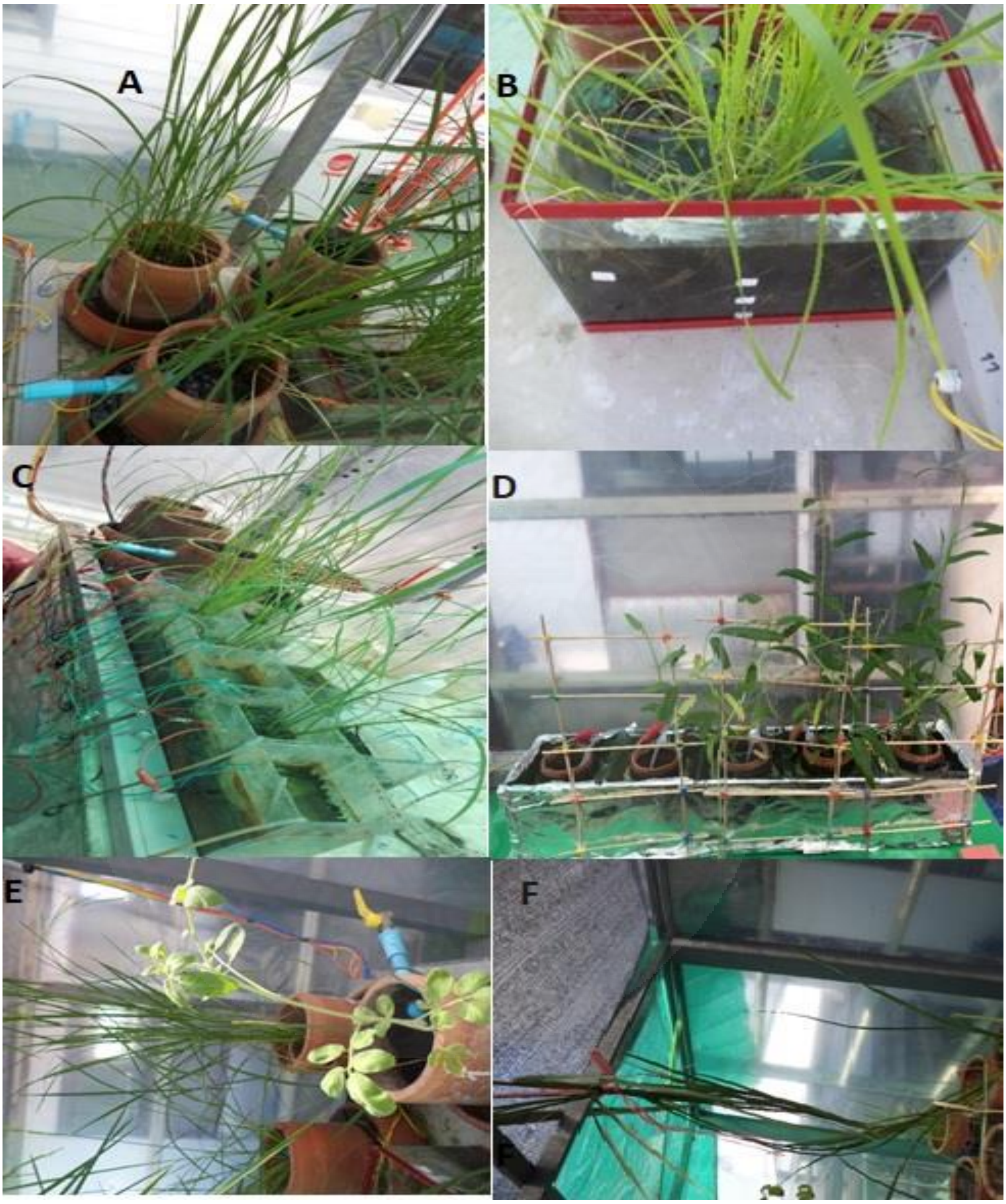


Figure 3.5 Different units used in this study

3.5 Analyses

3.5.1 Bioelectricity production

The voltage across the external resistance was measured online with Wisco Data acquisition system (Figure 3.6) and offline with multimeter (UNI-T 30B). The current was calculated by Ohms law (V =IR), where voltage (V) was recorded from data logger, R was the resistance (Ω), and A was the anode surface (m^2) and/or above plant growth area. Maximum power was determined by the Polarization curve by imposing variable resistors using resistor box (GAMMA CO 6DECADE). At maximum power point the external resistance on the system imposed is equal to the internal resistance. Internal resistance was determined by the linear regression of IV curve (Figure 3.7). Average current density and power was computed with $(\sum V)/ R$ and $(\sum V)^2/ R$ (100 Ohm), respectively. Analyses and collection of data was somewhat modified based upon the objective of each experiments.

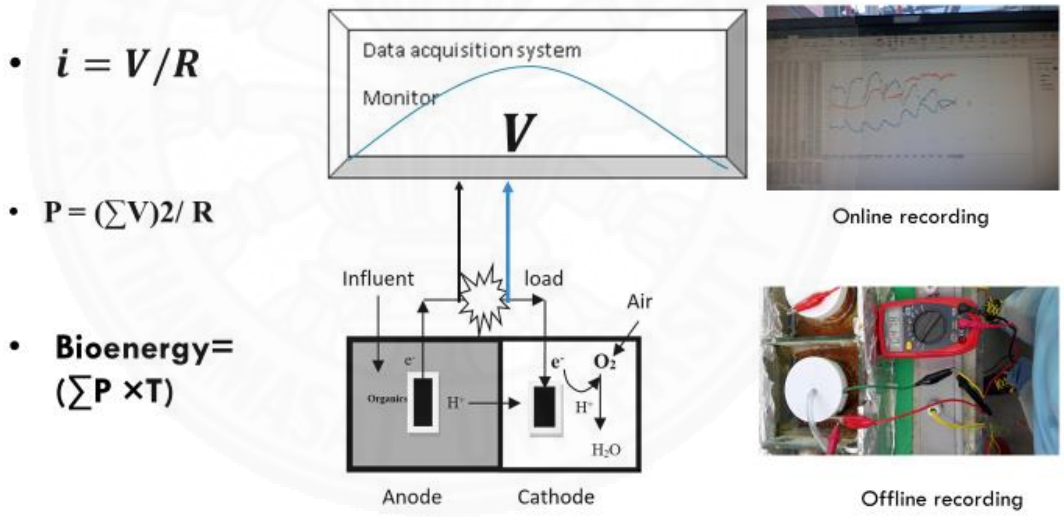
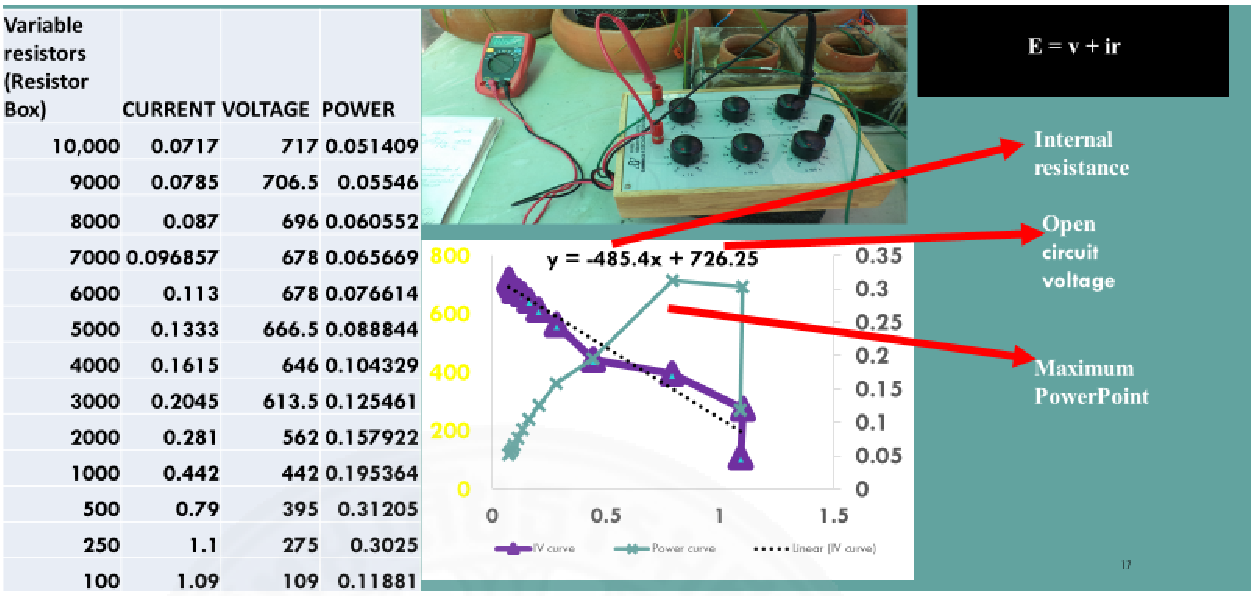


Figure 3.6 Methods for data collection and analysis for quantifying electrical performance



Variable resistors (Resistor Box)	CURRENT	VOLTAGE	POWER
10,000	0.0717	717	0.051409
9000	0.0785	706.5	0.05546
8000	0.087	696	0.060552
7000	0.096857	678	0.065669
6000	0.113	678	0.076614
5000	0.1333	666.5	0.088844
4000	0.1615	646	0.104329
3000	0.2045	613.5	0.125461
2000	0.281	562	0.157922
1000	0.442	442	0.195364
500	0.79	395	0.31205
250	1.1	275	0.3025
100	1.09	109	0.11881

E = v + ir

Internal resistance

Open circuit voltage

Maximum PowerPoint

Figure 3.7 Maximum power point and internal resistance calculation for fuel cell characterization

3.5.2 Biomass production

Growth of plants was monitored visibly and with the help of a digital camera. Any detrimental biotic and abiotic effects were analyzed. Record of plant height, growth nature, root and shoot biomass were analyzed comparing with the typical growth behavior of plants.

3.5.3 Physical parameters

Conductivity, pH and dissolved oxygen were monitored with the respective probes. COD test was carried either to know the organic added by plant or water treatment ability of system. Overall soil and earthen membrane properties was evaluated as mentioned elsewhere (Ghadge et al., 2014). Moreover, to see the effect of weather conditions like atmospheric temperature, humidity, sunlight hours, rainfall, etc., data were collected from meteorological office, Pathum Thani.

3.5.4 Surface morphology analysis

To gain clear picture about the microbial biomass attachment on the electrode surface, SEM images was employed (Bond & Lovley, 2003). Furthermore, to see the distortion on the layer of earthen membrane, EDS pattern was done for unused and used membrane (Ghadge et al., 2014).

3.5.5 Electrode materials and treatment

Carbon brush, carbon fiber, and carbon cloth were employed in various configurations. Varied size was studied to know the effect of electrode size on the system performances, mostly 100 cm^2. Electrodes were used either with treatment or without treatment. In case of treatment, they were alternately dipped into acid and base for 24 hours. The description of detail configuration is provided in respective microcosm preparation of results and discussion. Figure 3.8 shows some of materials used in this study.

Figure 3.8 Some materials used in this study

Chapter 4

Results and discussion

4.1 Assessment of electricity generation from paddy microbial fuel cell in single sediment and double chamber configuration throughout the growth stage

4.1.1 Microcosm set up and operation

Earthen material was used as structural and membrane materials. Earthen cylinder (26 cm height and 15 cm diameter) was used as the anode compartment and positioned into the widened cylinder (32 cm diameter and 16 cm height) that worked as the cathode chamber (Figure 4.1). Garden soil enriched with an organic matter and indigenous microbial community was packed in the reactor without any pretreatments to provide the natural condition for electricity generation. Commercially available fertilizer was amended once in tillering stage and another in panicle stage principle constituent of NPK along with various micronutrients. Water and soil surface was maintained at 12.5 cm from the depth of the anode chamber (working volume of 2.2 L). The cathode chamber was filled with tap water with a working volume of 2.5 L. Twenty-six days old KMDL 105 cultivar obtained from Thammasat rice research center, Thailand was transplanted in anode compartment. Prior to plant in PMFC the rice seeds were germinated in an ambient environment condition with the same soil that was later used in the PMFC system (3-4 seedlings). Either carbon brush, carbon fiber, and carbon cloth singly or in combination with carbon fiber were used as electrodes. Four different PMFC were set up and given the name PMFC1, PMFC2, PMFC3, and SPMFC. The prior three were operated as a double chamber however vary in electrodes used and size and the last one was operated as a traditional sediment set up. Carbon fiber and effective area in an anode was chosen based upon the previous study which provided the enhanced voltage generation (Moqsud et al., 2015). Detail configuration description is given in methodology section.

The day when the seedlings were transplanted in reactors was considered as a day 1. The life cycle of rice lasted for 93 days, 26 days as a germination until the seedlings and 67 days in a PMFC system. All the reactors were operated under OCV until 19 days to provide acclimatization of microbial community in electrodes thereafter 100 Ohms of an external resistor was connected. Comparison and analysis of data therefore was done from day 20 to day 67, the final day of operation, when the current became zero in almost all reactors. The week (3rd to 9th) when reactors under closed circuit (active energy harvesting) was further divided into different growth stages to know the effect of these stages in the bioenergy harvest. All the data were normalized to two broad growth stages viz vegetative and reproductive phases. When all the tillers developed into the panicle, the stage was supposed to be entered reproductive phases (Mosleh et al., 2015). No external organic substrates and the fact of varying exudates at different stages, the detailed about each stage helps to know the role of exudation in current generation. Tap water was periodically added to the reactors to nullify the water loss through evaporation only in case of cathode and loss via evaporation and uptake by plant in anode. After the maximum greenness and highest current generation in double chamber during tillering stage, the water in cathode chamber was ceased through day 31 to 41 that means no compensation of catholyte lost and its effect on voltage output was analyzed. All the reactors were placed under polyhouse to better control the ambient weather conditions.

Figure 4.1 Lab-scale paddy microbial fuel cell: Double chamber(A), Single chamber (B)

4.1.2 Electrical output behavior in PMFCs

The average OCV at 11 AM for 19 days of operation was amounted to be 409 ± 8 mV, 450 ± 13 mV, 704 ± 51mV, and 261 ± 51 mV for PMFC1, PMFC2, PMFC3, and

SPMFC, respectively. After day 20, all the reactors showed the quick current generation (active energy harvesting) indicating the acclimatization of bacteria in the system accompanied with the excessive vegetative growth except SPMFC**.** It took 2 days for SPMFC to generate electric current which might be due to low OCV recorded. Quick start up voltage generation revealed the presence of electrigen in soil inoculum which initially degraded the organic matter present in soil and root exudates to some extent. The ohmic resistances calculated by current interruption technique further revealed the best performing reactors in terms of bioenergy harvest, exhibited the lower internal resistance (Table 4.1). Polarization tests conducted characterized the electrical behavior at two different phases is summarized in Table 4.2 and 4.3. Interestingly, fluctuation between maximum and minimum voltage in SPMFC was higher than the DPMFCs (Figure 4.2). Such phenomenon of current generation is probably attributed to battery built system in PMFCs (Strik et al., 2008) . Moreover, at night bacteria can oxidize the dead roots, slough of cells and soil organic matter itself which is not dependent on the photosynthesis (Timmers et al., 2012). From day 63, current generation in PMFC2 was stopped, however, other reactors still produced small amount of electricity. Sudden devoid in current generation might be due to contamination of the cathode chamber which was evident with accumulation with organic matter, the scrap of outer anode material and algae growth which might decrease the cathode potential thereby decreasing the current. The separate chamber for catholyte therefore has effect in current generation at night since other conditions are identical in the system. The current generation showed the peak period in an excessive vegetative growth which comply with previously reported results (Deng et al., 2016; Moqsud et al., 2015). Figure 4.3 gives active voltage generated from PMFCs across 100 Ohms.

Table 4.1 Electrical performance of PMFC

PMFCs	OCV mV	Potential across 100 Ohms	Current	Ohmic resistance
PMFC1	419	123	1.23	241
PMFC2	738	270	2.70	173
PMFC3	765	416	4.16	84
SPMFC	189	45	0.45	320

Table 4.2 Polarization test for vegetative phase

PMFCs	OCV mV	Internal Resistance (r) Ohms	Power Anode area mW/m^2	Power Above plant growth area (PGA) mW/m^2	Power Anode volume (PAV) mW/m^3
PMFC1	374	1329	50	1	8
PMFC2	623	317	60	17	120
PMFC3	710	162	70	40	280
SPMFC	180	76	28	8	56

Table 4.3 Polarization test for reproductive phase

PMFCs	OCV mV	r	Power Anode Area mW/m^2	Power PGA mW/m^2	Power PAV mW/m^3
PMFC1	221	1178	35	0.80	5.6
PMFC2	96	345	1.4	0.40	2.8
PMFC3	248	293	7.4	4.24	30
SPMFC	387	2773	2.8	0.80	5.6

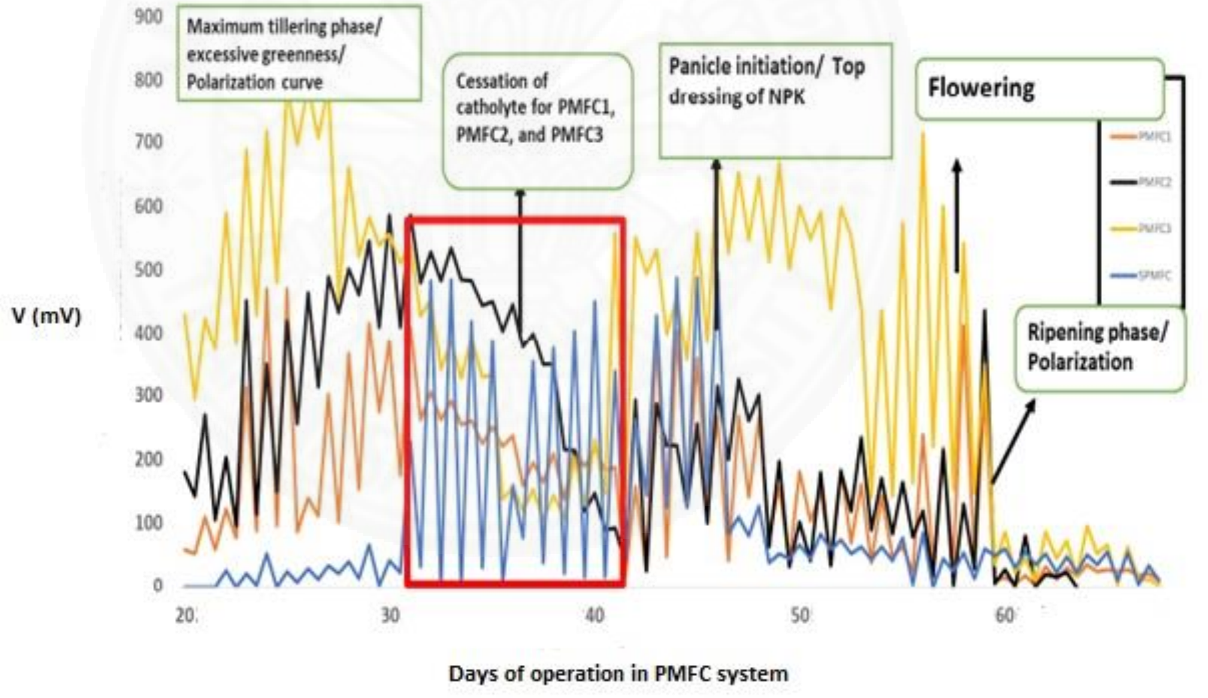


Figure 4.2 Maximum and minimum voltage generation under close circuit

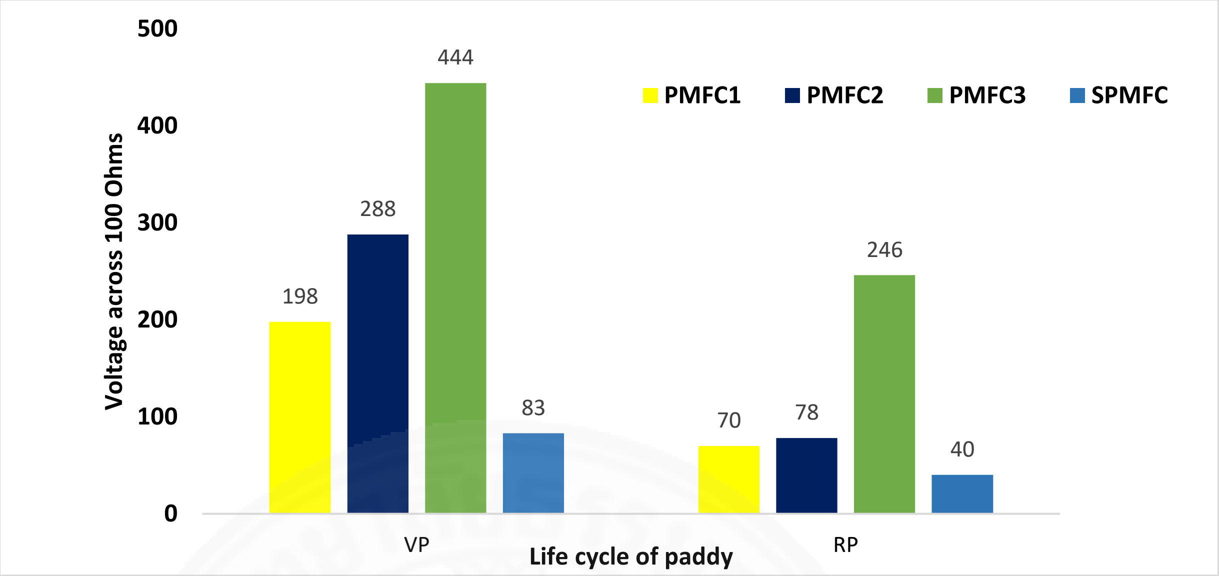


Figure 4. 3 Average voltage generation during vegetative and reproductive phase

4.1.3 Termination of catholyte addition decreases current generation in double chamber

Catholyte plays an important role in reduction reaction of oxygen to water in a cathode chamber. Previous study reported that catholyte cessation can affect the current generation (Gajda et al., 2015). After day 30 when all PMFCs achieved the highest voltage generation, catholyte addition has been ceased until day 40 to see whether decrease of catholyte compromised with decrease in current. All PMFCs after cessation of catholyte significantly showed a decreasing trend in current generation (Figure 4.4). The cumulative evaporation of catholyte and voltage generation trend showed linear relationship (R^2= 0.98) which signifies the role of catholyte that it is important in current generation in PMFCs. After addition of water on cathode chamber on day 41, all PMFCs showed an incline trend suggesting the root exudates/substrates were present in anodes. Therefore, given the enough substrates the cathode environment would be the limiting factor. The claim can be further supported by the trend of current generation in SPMFC, where no decline in current generation was observed since the condition was not changed since beginning. All the plants in the reactors were in the same growth stages (tillering phase), it can be consequently concluded that even with enough anodic substrates, the catholyte could play an important role in current generation. In addition, catholyte volume, cathode area has also significant impact for power generation. In this study, PMFC1 has been operated with smaller anode, however, cathode area is relatively larger than other PMFCs. Overall, lesser current generation of PMFC1 than PMFC2 and PMFC3 is attributed to less microbial niche for oxidation of organic matter due to smaller anode area when same amount of exudate is available for smaller and larger anode PMFCs. Nevertheless, while normalized to the anode area, the power generation value was higher than that of PMFC2. Normalized to PGA, PMFC 1 exhibited lesser power throughout the stages, which was further supported by polarization curve, where maximum power for PMFC1 was very less than that of PMFC2 and PMFC3. However, in comparison of PMFC1 and PMFC2, the decline rate of average voltage generation in PMFC1 was smaller which might be due to larger cathode area. At reproductive phase, exudates are not enough in all reactors, therefore microbial attachment and exudate oxidation is limited. Therefore, it can be predicted when anode conditions are same, catholyte and cathode area is an important for electricity generation in PMFC.

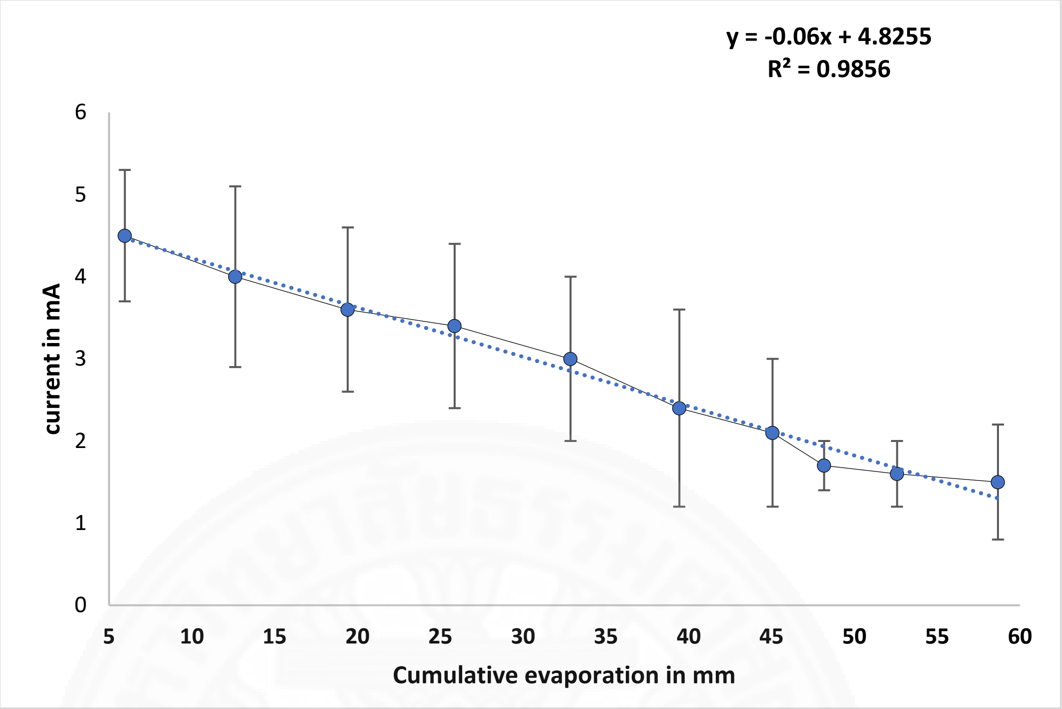


Figure 4.4 Effect of catholyte cessation in current generation

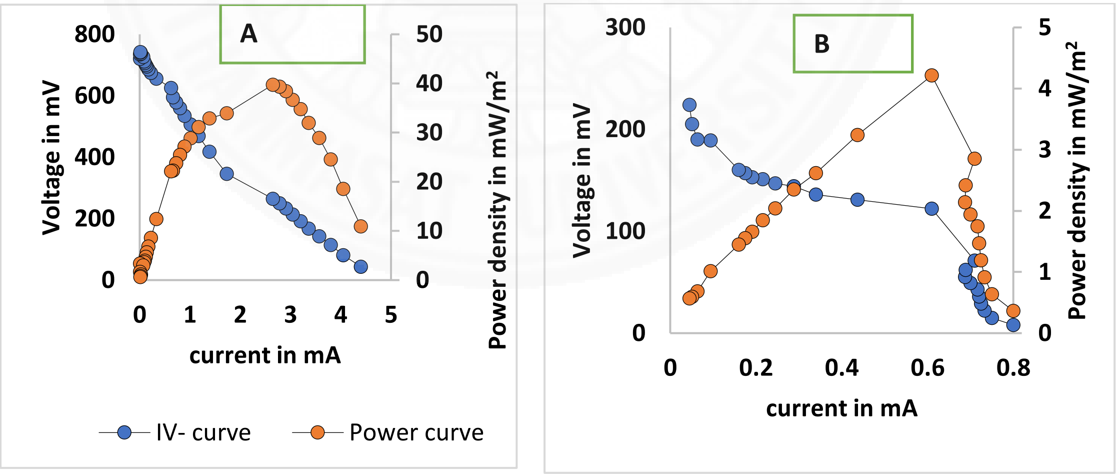


Figure 4.5 Polarization curves for PMFC3: Vegetative phase (A) and reproductive phase (B)

4.1.4 Growth stages affects the bioenergy harvest

Throughout the experiment, no external substrates have been amended to enforce the utilization of rhizodeposition and organic matter in soil for bioelectricity production. Trends of root exudates was reported to be vary according to the growth stages in rice (Bacilio et al., 2003). Highest current generation in vegetative phase was probably due to higher exudations. However, once plant started developing the panicles, glucose transfer to root might be lowered thereby limiting the substrates for bacteria (Deng et al., 2016; Moqsud et al., 2015). Polarization curve further confirmed the maximum power density in reproductive phase was decreased maximum by 10 - folds than in the vegetative phase. Figure 4.5 entails the maximum power harvested in vegetative phase and reproductive phase for best performing reactor PMFC3. Similar trends have been achieved in other PMFCs. The trend of polarization curve and maximum power density after 3 weeks of operation in single chamber PMFC normalized to anode area was in the line with previously reported results (Takanezawa et al., 2010) i.e. 14 mW/m^2 (anode area). It is to be noted that previous study used platinum as cathode catalyst. The internal resistance increment near the end of life cycle can be accompanied with the replenishment of exudates in the anode chamber thereby starving of microbial communities. Table 4.4 shows the amount of organic matter calculated by COD analysis. Therefore, electricity generation from paddy PMFC is life cycle dependent and maximum bioenergy harvest can be achieved in the vegetative phase. Furthermore, COD analysis revealed the higher value in vegetative phase than in reproductive phase meaning lesser organics available in the latter. The height of plants showed a sigmoid growth, the maximum of which was 110 cm, whereas the average panicle length was 19.8 ± 0.98 cm (n= 18) from all reactors and spikelet number per panicle was 31.75 ± 2.4. Figure 4.6 depicts the growth behavior of paddy in MFC at different stages.

Table 4.4 Quantification of organic matter at different stage of rice growth during operation

PMFCs	Transplanting	Max tillering	Flowering
PMFC1	90	112	40
PMFC2	80	104	20
PMFC3	120	96	40
SPMFC	90	112	44
Control	70	16	8
CODmg/l	95±8.6	106±3.8	36±5.4
CODmg/Av	237.5	265	90

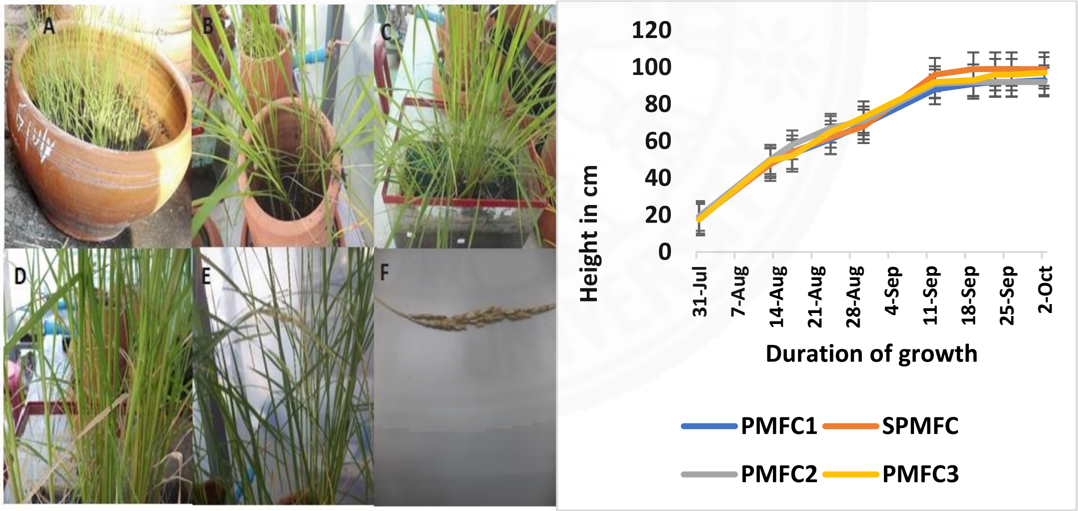


Figure 4.6 Different growth stages and biomass growth in Paddy PMFCs. Seedling raised for transplantation (31 July) A, After 2 weeks in PMFC system (14 Aug) B, Maximum greenness (28 August) C, Internodal elongation (4 Sep) D, Panicle formation (25 Sep), Panicle (2 Oct)

4.1.5 Enhanced performance with combined electrodes and double chamber

Figure 4.7 shows the power generated by PMFCs normalized to the weekly progression. Combination of carbon cloth and carbon fiber enhanced current generation significantly than carbon fiber alone although in both system the effective area on anode was kept same. This indicated that the higher the microbial surface attachment for bacteria in an anode region the, higher will be the current generation with an efficient formation of biofilm. In PMFCs for higher power output, root biomass need to be compatible with the electrode usage. Higher root biomass attachment in the carbon electrode might ensure the exploitation of hot spot (microbes and exudates) for electricity generation (Timmers et al., 2012). Figure 4.8 shows the firmly attachment of fine roots of rice in the carbon fiber. However, carbon brush electrode exhibited lesser power output since the effective surface area for microbial attachment was limited. However, carbon brush in double chamber performed better than carbon fiber single chamber suggesting the architecture of the reactors played the significant role. The higher current generation in double chamber might be due to its flat design and horizontal flow of the protons, whereas in single chamber the design mimics the tubular structure. Previous study reported that flat plate PMFC performed better than tubular PMFC (Helder et al., 2012). Therefore, configurations accompanied with the electrode materials largely affects the bioenergy.

This study reported the maximum voltage generation of 788 mV in Paddy PMFCs without use of any catalysts and additives so far in PMFC. Carbon cloth in combination of carbon fiber provided enough attachment of roots and microbial attachment in the electrodes hence higher current generation was achieved. Previous study reported maximum voltage of 700 mV while using carbon fiber electrode with 3% organic matter in paddy microbial fuel cell (Moqsud et al., 2015). Furthermore, leakage of sediment and organic matter to the cathode was prevented in case of double chamber thereby enhancing the current generation. Oxygen is, therefore, reduced efficiently in catholyte chamber. Another reason for the enhanced performance would be attributed to the catholyte volume which was higher than previous reported results in sediment single chamber. Moreover, support or growth medium also affects the current generation (Timmers, et al., 2012). Soil used in this study was enriched with high organic matters. Perhaps it is the reason the system enabled the enhanced performance in terms of electricity generation.

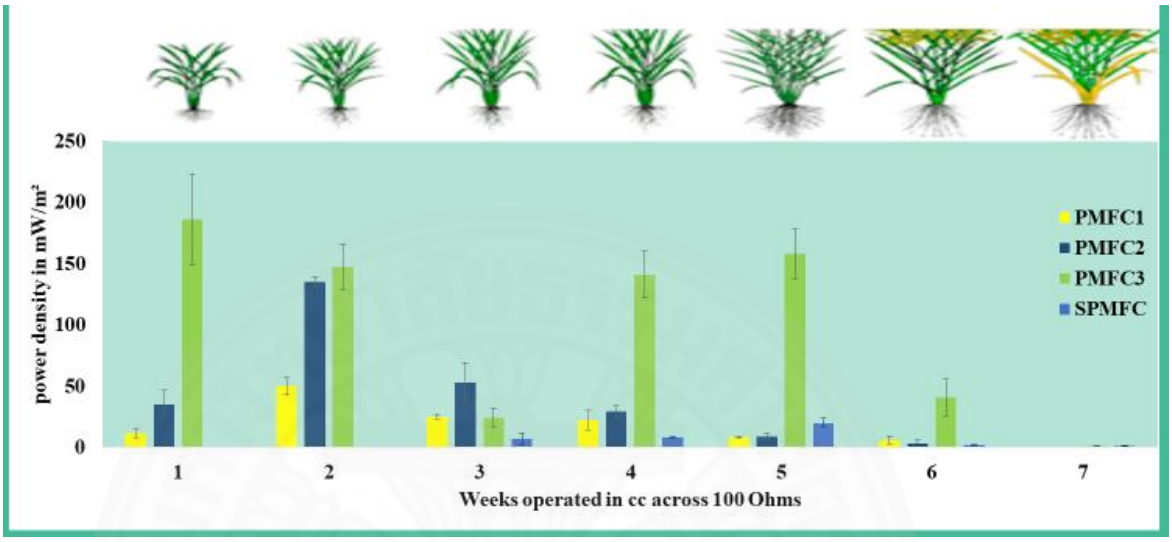


Figure 4.7 Average power density generated, error bar indicates standard deviation

Figure 4.8 Naked eye view and SEM image of carbon fiber

4.2 Paddy – *Azolla* biosystems for enhanced bioelectricity production in plant microbial fuel cell

4.2.1 Microcosm set up and operation

Sediment MFCs were constructed with soil collected from Thammasat rice field 14° 4' 12.576" N 100° 36' 24.9264" E from 0-10 cm. Soil suspension were filtered through sieve for removing any coarse debris. Glass container (20×15×10 cm) was used as a material unit for each reactor. Small pebbles were placed up to 2 cm for facilitating the anolyte collection and increasing the circulation of water in sediment. Untreated carbon cloth measuring 150 cm^2 was stuck with a silicon sealant just above pebbles. Cathode measuring the same size as anode was placed in soil-water interface. Plastic tube was purged vertically to collect sediment for analysis, and feeding of anode chamber has been facilitated through the same tube. Thin Titanium wire passed through the plastic tube was used as a current collector for both electrodes. Details of construction is shown in Figure 4.9. Table 4.5 provides summary for operational mode and objectives for this experiment.

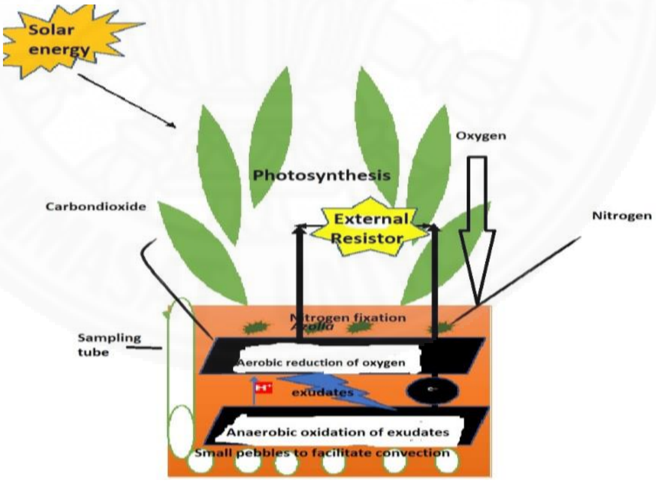


Figure 4.9 Schematic of paddy *Azolla* biosystems

Table 4.5 Different phase of experiment for paddy-*Azolla* biosystems

Days of operations	**Operating conditions**	**Objectives**
17	Only sediment: Open circuit Voltage	To acclimatize bacteria in an electrode
13	Close circuit	To investigate the electrical output behaviour from sediment
35	close circuit planted vs unplanted reactors	To inspect the efficacy of planted reactors over unplanted for power generation
25	close circuit *Azolla* amendment	To examine the role of Paddy-*Azolla* Biosystems for system performances
5	Stacking of reactors	To optimize the power output in stacked reactors for *in situ* operation

4.2.2 Start-up open circuit voltage (OCV) and close circuit from sediment

Figure 4.10 displays passive energy harvesting (OCV) (A and B) and Figure 4.11 active energy harvesting (C) from sediment MFC. OCV shows the increasing trend and reached maximum average around 630 mV, which indicated presence of electrigens in soil and efficient configuration. However, each reactor attains maximum OCV in different time which reflects dynamic nature of biosystems. For instance, for same soil consortium, the microbial shaping in different reactors might be different in terms of abundance and types. Nevertheless, there was similar trend exhibited by all reactors suggesting fuel cell behavior. There was no day and night fluctuation in voltage generations in OCV conditions however after connecting an external resistance, a clear trend of crest and trough was observed (Figure 4.11). In close circuit, electrons are continuously received in anode via bacterial metabolisms which later pass to load to complete circuit. Therefore, close circuit reflects the microbial activity and substrates nature in anode compartment while OCV was mostly physical in nature, and true bio electrochemical systems cannot be reflected, however OCV determines the fate of reactor performances and therefore it is important to investigate the maximum OCV develop for individual cells. In principle, OCV refers to the maximum attainable voltage for reactors, for example for same anode and cathode operating parameters, reactors having high OCV exhibited higher current generation. However, in PMFC due to dynamic nature of anode consortium, it is quite difficult to provide same conditions to all reactors.

Day and night fluctuations in current generations is attributed to the effect of light intensity in photosynthetic bacteria since there was no external supply of substrates. Another probable reason for high current generation during day time can be accounted to the variation on temperature. During day time, bacterial metabolisms might be better favored with optimum temperature leading towards peak in current generations. Previous studies reported both conditions of day and night cycles and devoid of it while operating sediment MFC. These results can be interpreted based upon types of microbial strains and its dependency on light intensity and temperature.

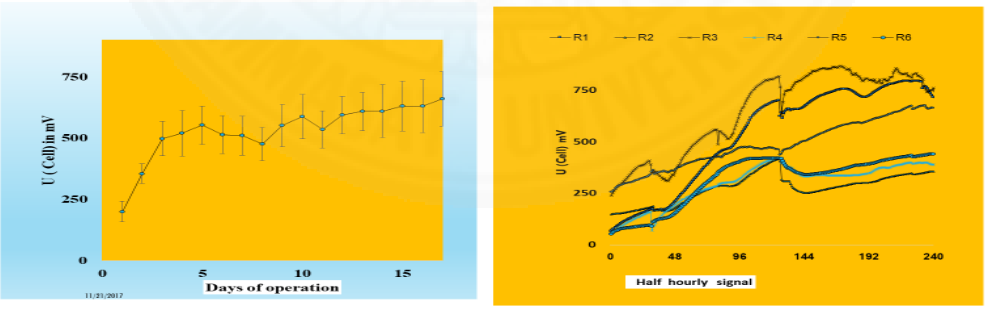


Figure 4.10 Average OCV for 17 days of operation and individual OCV for start-up period

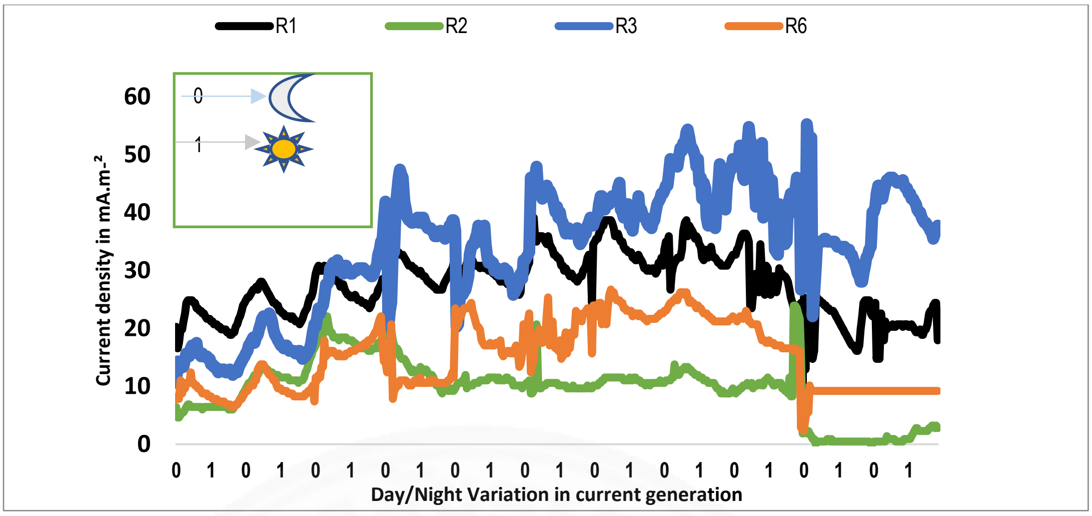


Figure 4.11 Circadian oscillation in current generation in close circuit from sediment

4.2.3 Enhancement of power generation by plants

Figure 4.12 shows the trend of current generations after transplanting of paddy and control sediment. Plants enhanced the current generations significantly. During close circuit in sediment, those reactors that shows high current generation was made control and low OCV was planted with paddy to better examine the effect of plants. Interestingly, after transplanting of rice seedlings, current generations were increased significantly in planted reactors however accompanied with enhanced fluctuation during day and night that indicated, rhizosphere of plant played a positive impact in enhancing current. However, for two control reactors, relatively R6 performed better than R1 which might be due to higher algal activity, after 28 days of operation, planted and unplanted reactors produced almost equal current which last for almost a week, algae bloom on the cathode therefore, enhanced the current generation due to efficient oxygen supply via photosynthesis. Since this study had no interest in knowing algal activity, the algae grown in R6 was removed before inoculation of *Azolla* in third phase. At the end of 35 days, after removing algae polarization curve was constructed to know the maximum power generation from planted and unplanted reactors. Figure 4.13 shows maximum power generation from planted reactors which was significantly higher than unplanted reactors.

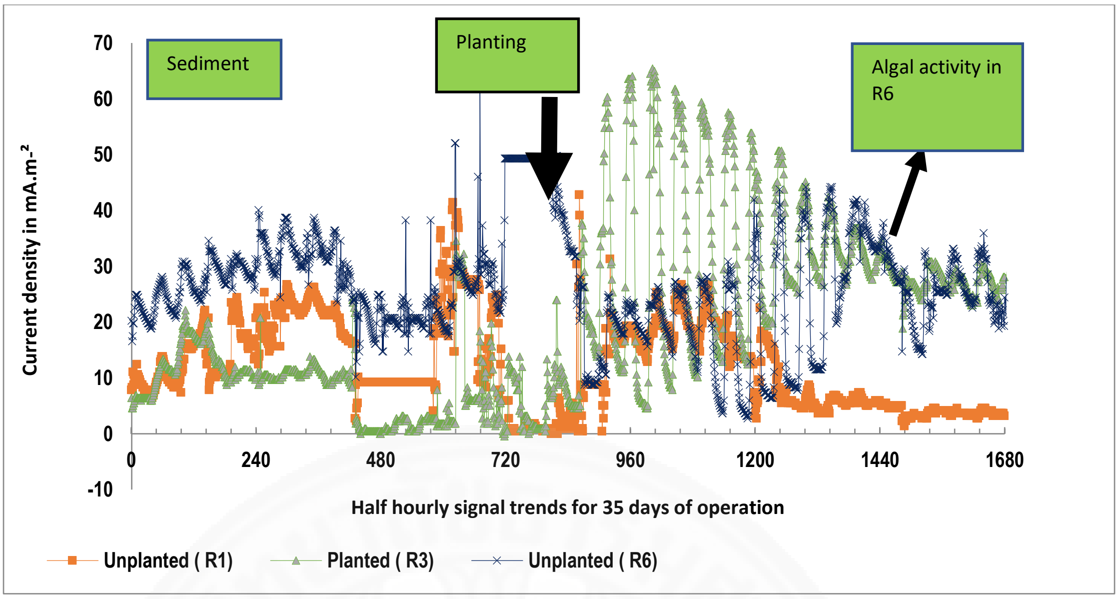


Figure 4.12 Representative current generation from planted and unplanted reactors

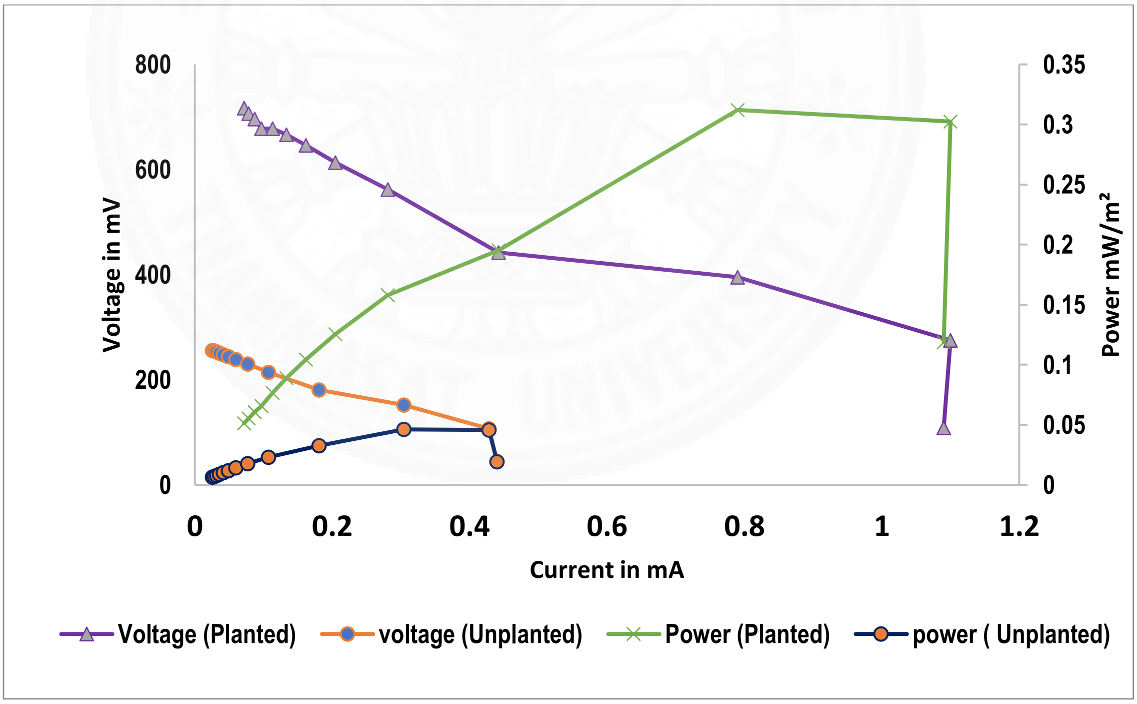


Figure 4.13 Polarization curve for planted and unplanted reactor

4.2.4 Enhance power generation with *Azolla* amendment

Figure 4.14 shows the average current generations after amendment. The result revealed that *Azolla* significantly enhanced the current generation. Improvement of system performance with Paddy- *Azolla* Biosystems, is accounted to enrichment of organic matters (Table 4.6). Moreover, as a cathode compartment *Azolla* can continuously supply nitrogen via nitrogen fixation to the plants thereby enhancing paddy growth which was evident from the nature of growth (Table 4.7) in amended and other reactors. Interestingly, overlying water in cathode compartment with *Azolla* was clear than that of treatment, because of absorption of nutrients and organic matters present in cathode compartment which can decrease the system performance otherwise. However, fluctuation in trend of current generation was higher in planted reactors than unplanted reactors due to circadian oscillation of plants.

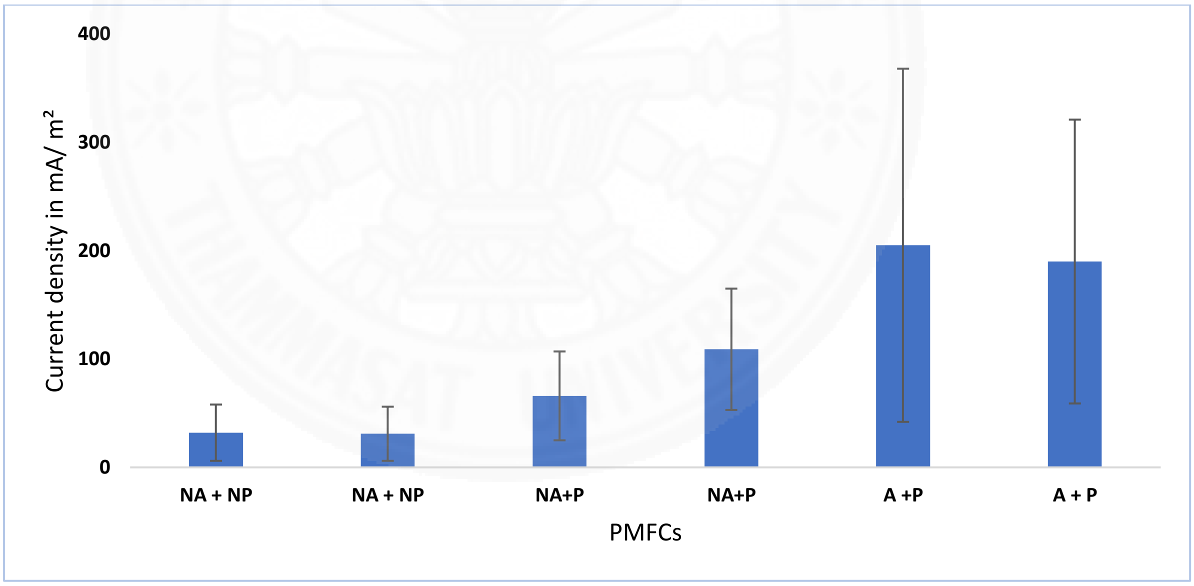


Figure 4.14 Comparison between *Azolla* amended and other reactors
NA + NP = No *Azolla* and No plant, NA + P = No *Azolla* and plant, A+P = *Azolla* + plant

4.2.5 Comparison of reactors performances in different phases

Table 4.6 summarizes the mean current density generated from three different phases. For control reactors, there was no significant difference in all phases meaning no external supply of substrates to trigger the current generation. The current generations were due to organic and inorganic decompositions of soil. Soil color changes further indicated the inorganic degradation of ferrous ions in anode chamber which was reported to be common biochemical reactions in sediment microbial fuel cell. In second phase, reactors were grouped into planted and unplanted whereas 4 reactors except R1 and R6 were planted. Later, third phases, R3 (yellowing of plant and mortality) and R6 were switch over as control to better know the effects of plants in current generation. During phase 2 after transplanting of plant, plant was still at a seedling phase, therefore initially, there was not much significant differences in current generation, which might be due to less rhizodeposition or acclimatization of the plants. After excessive vegetative growth and tillering phase, planted reactors performed significantly in current generation than unplanted reactors. The mean current density from four planted reactors before inoculation of *Azolla* was 36% higher than mean of two unplanted reactors. Moreover, during maximum tillering phase (third phase), current generation from planted reactors was almost twice than that of control unplanted which suggests the exudations was pronounced in anode chambers which was consequently consumed by electroactive bacteria (EAB) for electricity generations. *Azolla* amended reactors enhanced the current generation almost by 4 – folds.

Table 4.6 Summary of three phases of current generation (mA/m^2)

	Negative Control NA + NP		Positive Control NA + P		Treatments A+P		Remarks
Phase 1	**Phase 2**	**Phase 3**	**Phase 2**	**Phase 3**	**Phase 2**	**Phase 3**	
27 ± 6	26 ± 12	32 ± 26					
9 ± 6			27 ± 16			205 ± 163	
35 ± 10			34 ± 13			190 ± 131	
37 ± 10			24 ± 6				
30 ± 10			20 ± 5	66 ± 109			
16 ± 7	9 ± 2*	31 ± 25		41 ± 56			
17 Days	35 Days + 25 Days		35 Days + 25 Days		35 Days + 25 Days		

4.2.6 Stacking of PMFCs and voltage rehearsal assessment

Active energy harvested from individual unit for power use is unlikely given the micro electrical performance of individual units. However, it can be used as a biosensor for monitoring various parameters like toxic ions present in soil, flora health, organics present in soil, etc. Stacking and upscaling of this technology is a real challenge the scientific community need to sort out. Regarding paddy PMFC, converting paddy field into electric field or stacking individual units with minimum loss is an utmost. After 90 days operation, when plants were healthy and in vegetative phases, however few panicles arise from some reactors, all six reactors were connected in series to maximize the voltage generations. The detail about stacked voltage and individual unit performance is shown in Table 4.8.

Table 4.7 Plant growth behavior, height in cm

	NA+P	NA+P	NA+P (OCV)	A+P	A+P	A+P (OCV)	Remarks
During transplanting	18	22	17	19	22	17	
30 days after planting	50	70	40	60	70	60	
45 days after planting	85	110	75	107	115	98	Panicles arise from A4 and A5

Table 4.8 Individual and stacked voltage generation

Date	**PMFCs**	**Individual (V)**	**Stacked (V)**	**Percentage loss**
3 June 2017	NA + NP	0.64	2.6	32
90 days of operation	A+P	0.68		
	NA +NP	0.73		
	A+P	0.48		
	NA+P	0.55		
	NA+P	0.70		
5 June 2017	NA +NP	0.70	2.8	32
92 days of operation	A+P	0.75		
	NA +NP	0.75		
	A+P	0.60		
	NA + P	0.60		
	NA +P	0.70		
6 June 2017	NA + NP	0.70	2.5	37
93 days of operation	A+P	0.70		
	NA + NP	0.70		
	A+ P	0.60		
	NA +P	0.60		
	NA +P	0.70		

4.3 Hydrophytes (Morning Glory) operated plant microbial fuel cell for electricity generation

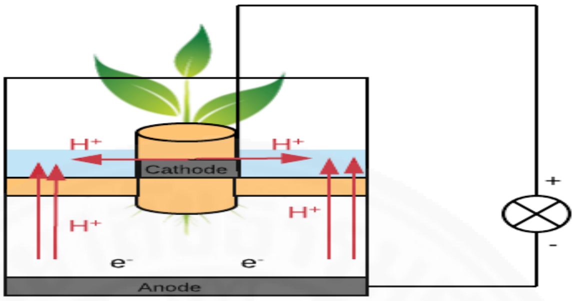


Figure 4.15 Schematic of hydrophytes operated PMFC

4.3.1 Microcosm set up and operation

Five individual PMFC were constructed using glass chamber (11.5×12 cm) by purging cylindrical earthen cup measuring 12 cm for anode consortium. At 7 cm from bottom of glass, earthen plate was inserted horizontally running throughout the glass chamber from left to right, a hole analogous to the diameter of the cylinder was made and earthen cylinder was inserted to support plant growth. Glass chamber was used to visually observe the rhizosphere region/ nature of root growth. Prior of inserting the cylinder, pretreated carbon cloth projected area 100 cm^2 was carefully glued at the bottom of the chamber with acetate free silicon glue. To perform the electrical measurement, copper wire was stuck in the carbon cloth. For cathode electrode, carbon fiber measuring 99 cm was encircled on the outer part exposed earthen cylinder. The reactor was wrapped with aluminum foil. Figure 4.15 gives schematic of PMFC set up. The system was operated either in open circuit or/and close circuit. In case of close circuit, 100 Ohms of an external resistor was connected. The substrates used for anode chamber was soil suspension and Hoagland solution. Previous one was used as inoculum and substrates, the latter one provided nutrition for the growth of plants. Each PMFC was operated as standalone unit and three different scenarios have been investigated; role of plant for electricity generation, ii. role of plant as anode (lower compound substrates) and cathode (Oxygen supply), and morning glory iii. extracts (higher compound substrates) and plant in cathode (Oxygen supply). Figure 4.16 shows two unplanted reactors and three planted reactors in triplicates.

Figure 4.16 Growth behavior of morning glory while electricity generation in PMFC

4.3.2 Start-up potential and current generation in PMFC

Figure 4.17 shows the average open circuit potential generated from unplanted (control) reactors and planted reactors. During first five days, OCV of control was higher than planted reactors which can be attributed to acclimatization of Biosystems in reactors phase. However, accompanied with plant growth, OCV increased significantly until 10 days. Planted reactors showed higher fluctuations than unplanted due to dynamic rhizosphere region, and pronounced effect of light intensity, and weather conditions in plant rather than control. After day 28, potential in planted reactors decreased. After day 35, additional plants have been transplanted to anode chamber thereafter, incline trend was observed. On day 37, an external resistor of 100 Ohms was connected to duplicates of reactors, while one reactor was operated in open circuit conditions.

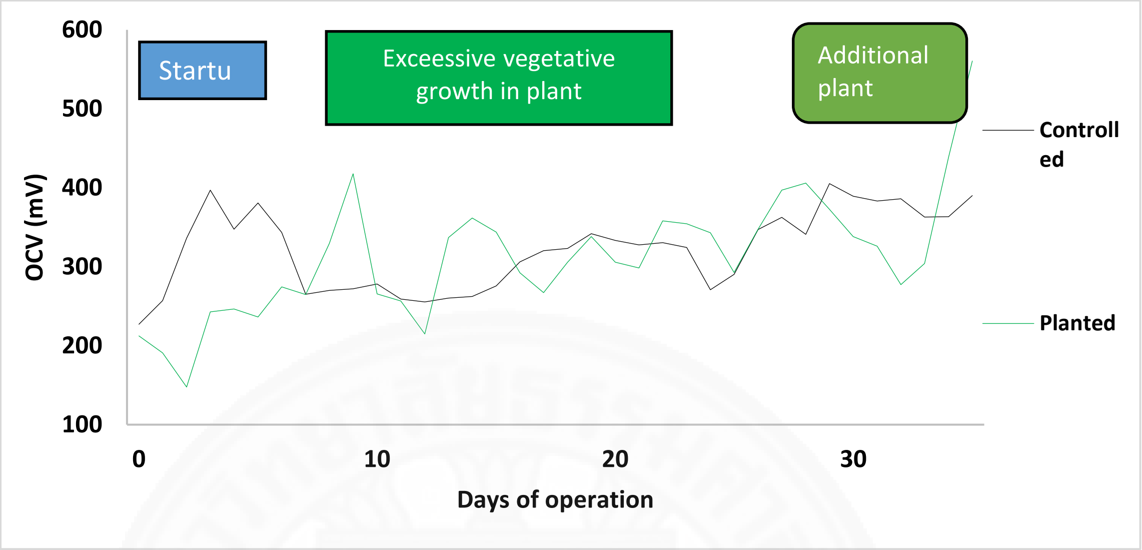


Figure 4.17 Start-up open circuit condition in hydrophytes morning glory PMFC

Figure 4.18 depicts the daily average current generation from planted and unplanted reactors which shows, plants increased active energy harvesting in PMFC than control. Average power density generated during this phase was 24 mW.m^{-2} which was 58 % higher than unplanted one. Thus, obtained power generation was however lower than reported from paddy, vetiver and tomato due to tenderness of plants, lower extent of exudates, and operated as hydroponic, where no organic rich soil was enriched. Moreover, reactor architecture was smaller in this case. However, enhancement of power from morning glory provides the further pathways for use of such classes of plants in electricity generation.

4.3.3 Power performance: Maximum Power and internal resistances

Figure 4.19 elucidates the maximum power and internal resistances for planted and unplanted reactors. The maximum power obtained for planted reactor was higher by 84 % than unplanted one. Highest current generation was attributed to lowest internal resistances of the system which was decreased by 10%. Therefore, substrates enriched via plant rhizodeposition and exudates decrease the ohmic loss due to higher transfer of electron. Moreover, in addition to the know whether planted cathode enhances the current generation by oxygen supply, two reactors were planted in cathode and anode was supplied with leaf extract which supplied different chains of organic compounds. Figure 4.20 elucidates the extract supplied anode and planted cathode provided higher current generation than planted anode only. This result suggested that root exudates from morning glory is not enough for higher current generation. Besides, oxygen leaked through the plant roots might have hydrolyzed the organic compounds limiting the current generation. To confirm whether the average current trend comply with fuel cell behavior, polarization test was conducted. Figure 4.21 shows that reactors with extract in anode exhibited higher current generation than other reactors. SEM images for anode and cathode electrodes shows that microbial attachment. Rod shaped bacteria are well attached in the carbon fiber cathode. Those bacterial proves that plant root form adapted biosystems in PMFC system.

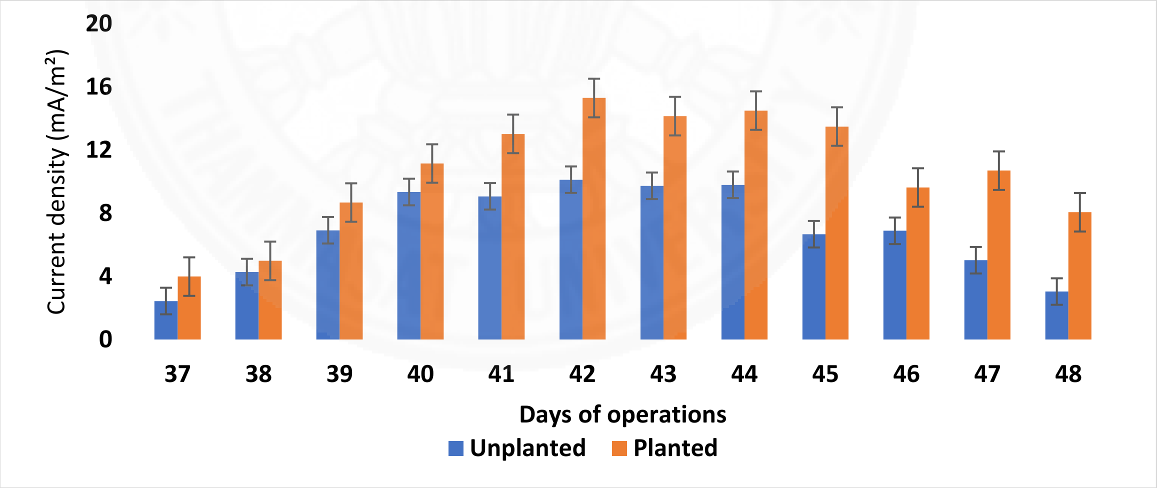


Figure 4.18 Comparison between planted and unplanted reactors

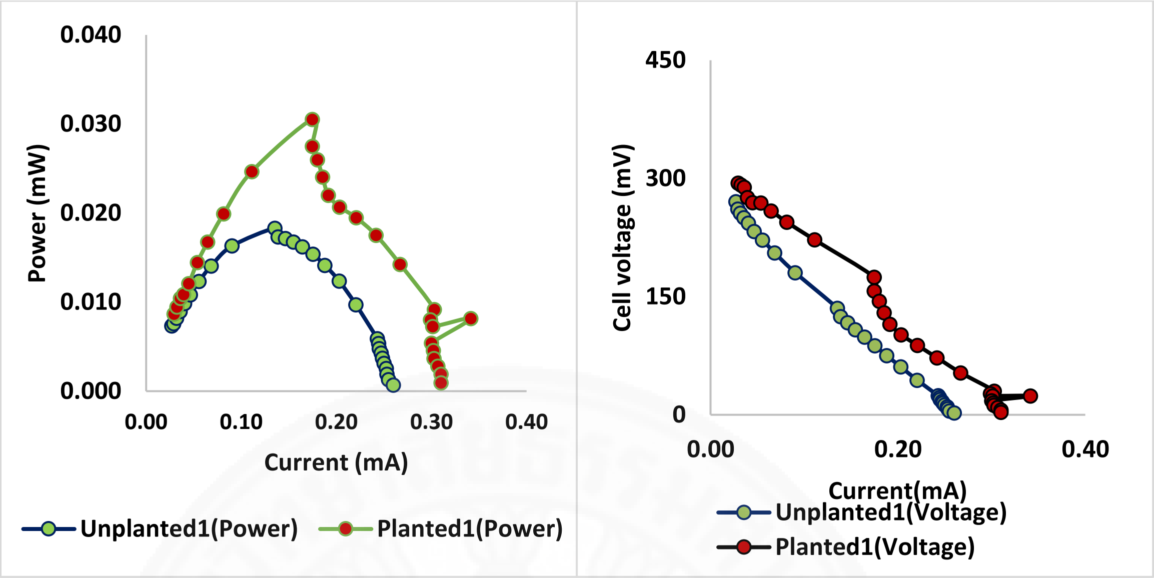


Figure 4.19 Polarization curve for planted and unplanted reactors

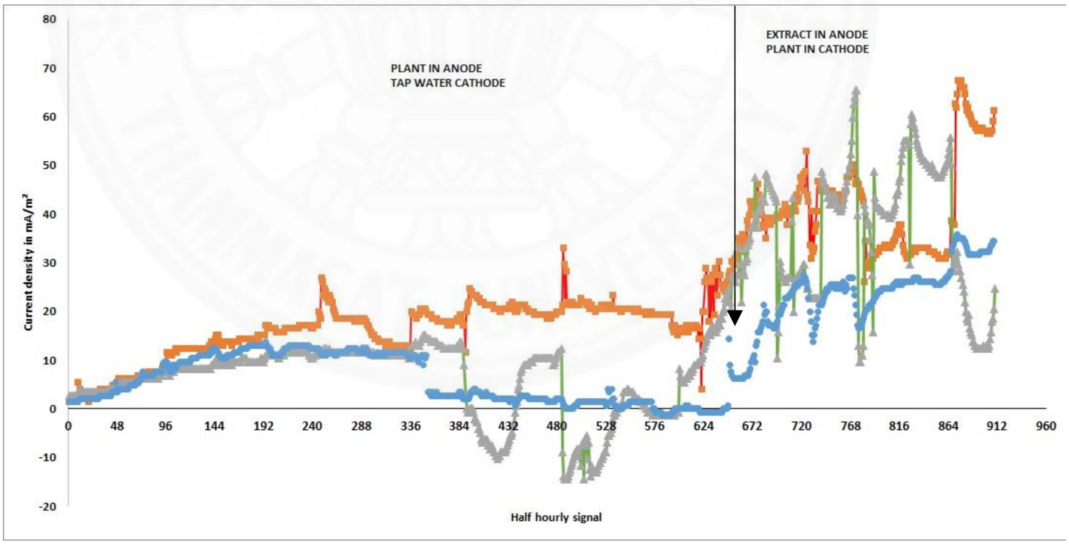


Figure 4.20 Comparison output voltage from panted anode vs planted cathode

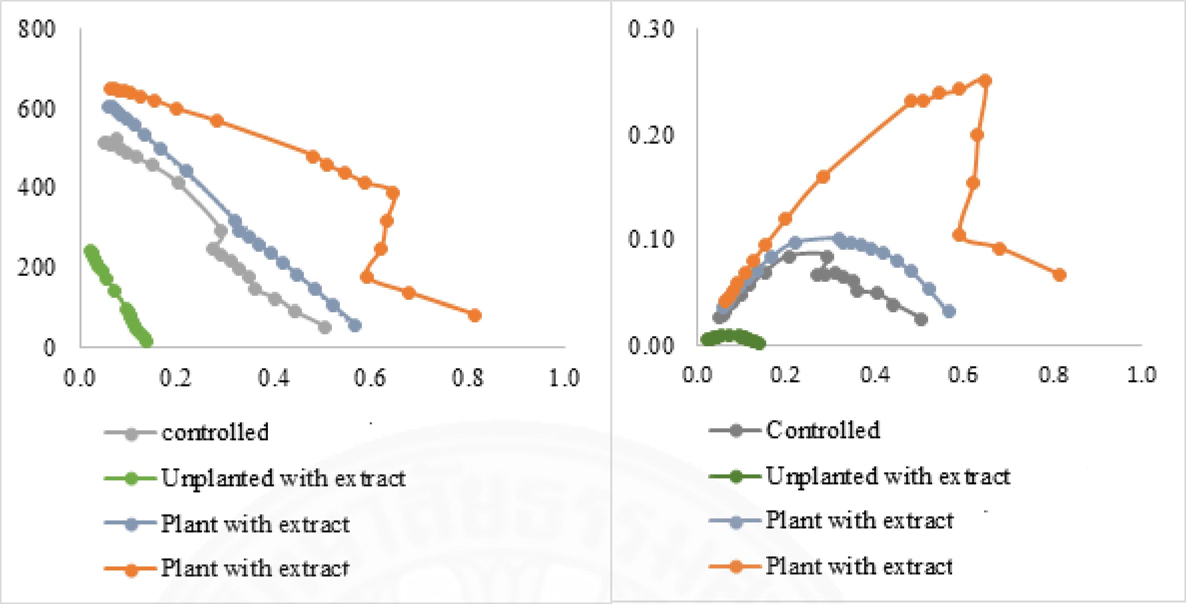


Figure 4.21 Polarization curve for controlled, unplanted cathode with extract at anode and duplicate planted cathode with extract at anode

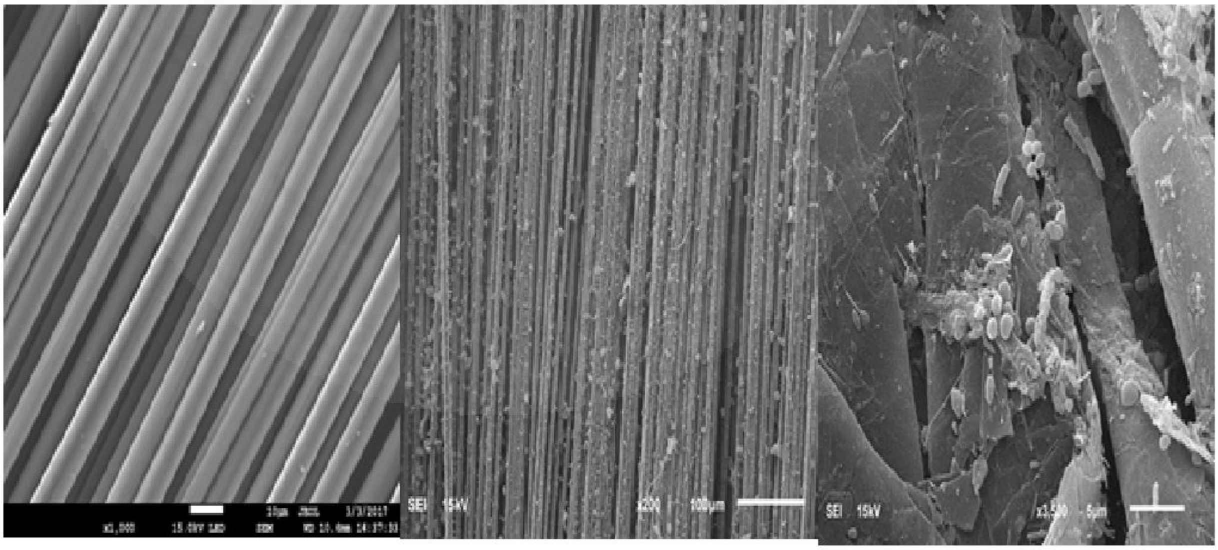


Figure 4.22 SEM images for unused electrode (A), anode electrode (B) and cathode electrode (B)

4.4 Long term electric signal of higher plants in plant microbial fuel cells

4.4.1 Effect of growth and vitality of plant in electricity generation

Role of plant via its morphology and physiology can affect the electric output in plant microbial fuel cell system. Root biomass, photosynthetic pathways, growth nature, and nature of rhizodeposition are the priming factors for the power output of the PMFC system (Nitisoravut & Regmi, 2017). Rhizosphere region of plant accompanied with its microbial community is dynamic in nature which incites difficulty in controlling the variables for system performance of PMFC (Timmers, et al., 2012). Previous chapters studied about two plant species paddy and morning glory. In this chapter, two plant species vetiver and tomatoes were fabricated in a similar way, and their electric signals have been recorded for almost for five months. Since these plants vary significantly in terms of their physiology, morphology, and utility which has been described in methodology chapter under choice of crops. Figure 4.23 depicts vitality of plants in PMFC system.

Figure 4.23 Plant growth behavior of higher plants, tomato (left) and vetiver (right) in PMFC system

4.4.2 Long term electric signal behaviors in PMFCs

Figure 4.24 depicts the representative voltage generation from tomato and vetiver operated PMFCs. The ambient weather conditions, configuration designs, and substrates used were similar for both types of PMFCs, alteration in output signal, therefore, can be attributed to the plants role. While start-up of reactors transplanted tomato seedlings (10 cm) were comparatively small than vetiver (50 cm) the latter one exhibited strong growth nature. Vetiver plants are robust in nature and develop vigorously in comparison with the tomato. Vetiver, therefore, provides quick current generation than that of tomato. The signal coming from tomato can be attributed to the organic and inorganic degradations of soil used rather than rhizodeposition. Moreover, vetiver can hold the water around its dense roots and tolerate saline condition which provides convention of anolyte for mass transfer of ions. Perhaps the reason vetiver performs better and quick start-up. However, after 3 months of operation, tomato generated significant current as comparative or more than vetiver. Previous studies reported around 1-3 months of period for significant current generation while using different types of plants (Timmers et al., 2010) (Strik et al., 2008) . After four months of operations, one of the PMFCs operated with tomato started getting wilted resulting in decline in current generations (Figure 4.25). In wilted plants, plant became yellowish and started dying meaning no photosynthesis and rhizodeposition, however, current can be generated due to slough of, dead cells, and soil used. Flora health can be therefore connected to the signal trends in PMFC system. Recent study shows the voltage output from plant operated PMFC as a function of floral health. This result therefore helps to understand the role of plants in PMFC to some extent. Moreover, economic plants like tomatoes can also be used for electricity generation in PMFC.

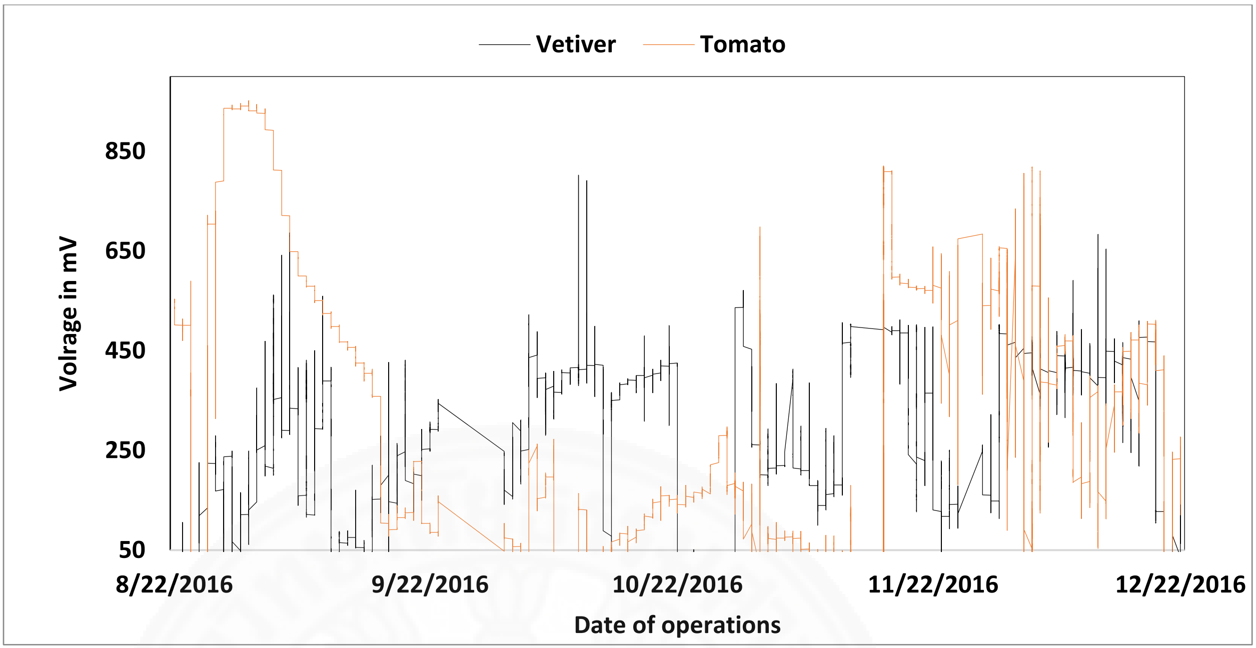
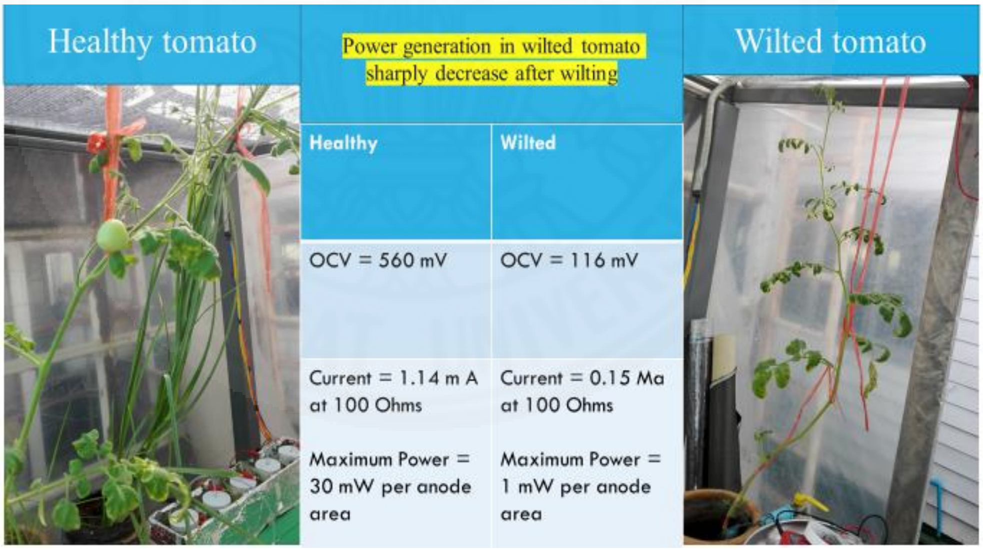


Figure 4.24 Representative long term electric signal from tomato and vetiver


Healthy tomato

Power generation in wilted tomato sharply decrease after wilting

Healthy	Wilted
OCV = 560 mV	OCV = 116 mV
Current = 1.14 m A at 100 Ohms Maximum Power = 30 mW per anode area	Current = 0.15 Ma at 100 Ohms Maximum Power = 1 mW per anode area

Wilted tomato

Figure 4.25 Effect of plant health on power generation

4.4.2 Power performances of vetiver and tomato

Table 4.9 shows fuel cell behavior after 5 months of operations which shows maximum power harvested from tomato plants. During this phase tomato performed better for average power density generation. Both vetiver PMFC exhibited similar trend across the varying external resistors. Interestingly, wilted tomatoes reactor performed worst in the system. Though the polarization curve depicts maximum 17 mW/m^2 (plant growth area) in tomato, long-term power was harvested from vetiver plant. Polarization curve data vary from average power density among reactors. Therefore, for *in situ* operation average active energy harvesting need to be taken into consideration. Two conclusions can be drawn from this study grasses species like vetiver are ideal for PMFC which are robust and can withstand harsh environmental conditions. In addition to this, economic crops like tomatoes can generate electricity but these plants mostly depend in growth stage, take long start up period for current generation. Floral health is detrimental for system performance. To exploit the root exudates, plants need to be healthy and perform photosynthesis subsequently, releasing the unused photosynthates to the rhizosphere.

Table 4.9 Electrical behavior of vetiver and tomato PMFCs normalized to anode area

	Healthy tomato (T1)		Wilted tomato (T2)		Vetiver (V1)		Vetiver (V2)	
Resistance	Voltage	Power density	Voltage	Power density	Voltage	Power density	Voltage	Power density
10,000	548	5.3153808	116	0.2381712	448	3.5524608	443	3.4736073
9,000	544	5.820074667	105	0.216825	442	3.842158667	442	3.842158667
8,000	540	6.45165	99	0.216847125	441	4.302892125	442	4.3224285
7,000	535	7.237403571	93	0.218696143	428	4.631938286	428	4.631938286
6,000	527	8.1930055	86	0.218182	416	5.105152	416	5.105152
5,000	513	9.3161826	80	0.22656	403	5.7492786	403	5.7492786
4,000	496	10.886208	72	0.229392	390	6.730425	390	6.730425
3000	479	13.537019	71	0.297419	384	8.699904	384	8.699904
2,000	433	16.5927765	70.5	0.439867125	380	12.7794	380	12.7794
1,000	340	20.4612	70	0.8673	310	17.0097	310	17.0097
900	330	21.417	68	0.909386667	291	16.65393	291	16.65393
800	325	23.36953125	66	0.963765	271	16.24882125	271	16.24882125
700	319	25.73099571	59	0.880195714	245	15.17775	245	15.17775
600	306	27.62262	54	0.86022	166	8.12902	166	8.12902
500	288	29.362176	45	0.71685	147	7.649586	147	7.649586
400	260	29.913	38	0.63897	125	6.9140625	125	6.9140625
300	226	30.13484	31	0.56699	102	6.13836	102	6.13836
200	180	28.674	24	0.50976	70	4.3365	70	4.3365
100	114	23.00292	15	0.39825	40	2.832	40	2.832
90	98	18.88786667	8	0.125866667	33	2.1417	33	2.1417
80	85	15.9853125	7	0.1084125	30	1.99125	29	1.8607125
70	74	13.84645714	6.5	0.106832143	27	1.843328571	26	1.709314286
60	65	12.46375	5	0.07375	19	1.06495	19	1.06495
50	55	10.7085	4	0.05664	16	0.90624	16	0.90624
40	44	8.5668	3	0.039825	13	0.747825	13	0.747825
30	34	6.8204	2.5	0.036875	9	0.4779	9	0.4779

20	23	4.68165	1.6	0.022656	7	0.43365	6	0.3186
10	12	2.5488	0.7	0.008673	5	0.4425	5	0.4425

Chapter 5

Conclusions and Recommendations

This study evaluated the bioelectricity production from plant microbial fuel cell and examined different factors underlying the system performance. Growth stages, vitality of plants, design configurations, electrode materials and size, and volume of catholytes are decisive for the maximum power generation. Plants that undergoes distinct growth stages viz vegetative and reproductive phases is affected by stages of growth. Smaller hydrophytic plants like morning glory are very tender in nature and root exudates limit the maximum power generation in those plants. The grass like vetiver has been identified as a suitable plant for its long-term operation and higher power generation, devoid of reproductive stage, adaptions to extreme environmental factors. Economic plants like tomatoes need careful cultivation techniques to be complied with PMFC performance. Low water around the rhizosphere region might cease power generation with no convention of anolyte causing negative voltage and higher water cause wilting conditions. Moreover, plant like tomatoes are easily attacked by pests and diseases which might have detrimental effect in output voltage which is evident in this study. The detail about maximum power generation subject to operational factors are shown in table 5.1.

* on table indicates most of findings are based upon the senior year project

Table 5.1 Summary of different plant performance in varied operating condition investigated in this study

Plant	Configuration	Substrates/ support	Maximum power (Anode area)	Duration (days)	Identified factors
Paddy	Single chamber	Commercial garden soil	14	93	Effect of design
	Single chamber	Thammasat rice soil + Azolla	32	93	Effect of substrate
	Double chamber	Commercial garden soil	70	93	Effect of electrode size and design Effect of catholyte
Vetiver	Double chamber	Synthetic waste	12	40	Volumetric effect
	Double chamber	Commercial garden soil	19	180	Long term performance
	Double chamber	Synthetic waste + Commercial garden soil	67	135	Effect of HRT and influent concentration *
Tomato	Double chamber		17	135	Effect of plant health
Morning glory	Double chamber	Thammasat rice soil+ Hoagland solution	3	48	Hydrophytes operated PMFC design

5.1 Future paradigms for PMFC applications: Trio for assessments

Given the low power generation from PMFC units, there is high time to do research in their other allied applications. Saying that PMFC might have its useful applications in tracking plant, soil, and microbe's health as discussed below.

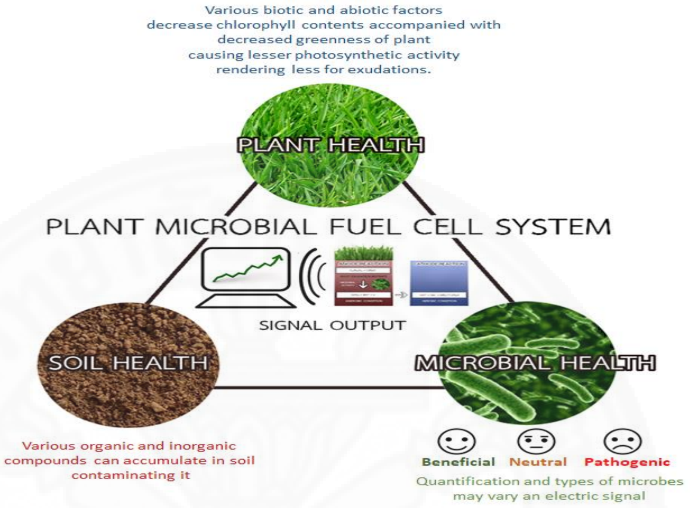


Figure 5.1 Future paradigm for application of PMFC

5.1.1 Plant health

With account of voltage generation by plants in PMFC as a function of root biomass, shoot biomass and high photosynthetic efficiency, healthy plants can perform well in the system (Deng et al., 2012; Helder et al., 2010; Timmers et al., 2012). It has been reported that liveliness of plant affects the voltage generation and replenishment of exudates at the latter part of life cycle results in decreased performances of the system (Moqsud et al., 2015). Monitoring the plant health is an utmost field for the scientists working in plant sciences. Modelling of PMFC varying in root biomass and number of healthy leaves in relations to the voltage generation given by the system will help to track what's going in the plant. A recent study reported the wireless monitor for plant health monitoring in PMFC (Brunelli et al., 2016). The most influential application of PMFC would be the manifestation of fate of plant pathogenic bacteria role for current generation. Does bioelectrical system at rhizosphere region helps to deviate the microbes? Recently, *in- situ* pathogen killing in earthen material MFC has been reported (Ieropoulos et al., 2017). Furthermore, white rot fungus commonly known as wood decay fungus was successfully used as a biocathode in MFCs. Isolation of pathogenic strains from PMFC and their role in plant disease and current generation would entirely add the new genera in the field of plant science with an application of this technology.

5.1.2 Soil health

Soil is considered as a living thing accompanied with its microbial health. Healthy microbes are achieved in a lump sum of healthy soil. A congruence in physical, chemical and biological properties in soil can be termed as soil health. One of the promising application of an MFC is its use as a biosensor in wastewater. MFCs are capable of spotting BOD content of wastewater (Di Lorenzo et al., 2009; Kim et al., 2003; Kumlanghan et al., 2007). A single terrestrial MFC was applied as a biosensor to detect the BOD content of synthetic rice washed wastewater (Logroño et al., 2016). Moreover, electric signal from an MFC is implied to detect copper stress in soil organisms (Deng et al., 2015). Furthermore, power-driven from microorganism in an MFC was reported to detect cadmium pollution in soil. Similarly, MFC was operated to monitor acidic toxicity (Shen et al., 2012). Those studies provide the examples of how PMFC can be further implied for monitoring soil health.

5.1.3 Microbes health

Microbes are core for MFC performances, since they are the drivers of electrons to the electrode. Investigation of quantity and quality of microbe’s strain in MFC performance is rapidly evolving research area. Growth of bacteria can be traced with the trends of voltage generation. Different models are proposed to establish the relationship between microbial growth and output voltage (Szöllősi et al., 2015). Traditionally, microbes present in soil is quantified by various methods such as serial dilution, UV spectrophotometer which are tedious. One of the promising applications of PMFC would be quantifying the microbes with a help of output signal.

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Appendices

Appendix A

Characterization of soil and earthen materials

Type of soil	**pH**	**size of particle**			**Soil texture**	**Organic matter**		**Phosphorus (mg/Kg)**	**Potassium (mg/Kg)**	**Calcium (mg/Kg)**	**Magnesiu (mg/Kg)**
		sand	silt	clay		%	level				
Commercial Garden soil	6.7	45	28	27	CL	3.33	middle	418	2100	5,545	1,525
Paddy field soil	5.2	17	24	59	C	3.46	middle	4	403	3,825	686

Element	**Unused earthen plate**		**Lower part of cylinder**		**side part of cylinder**	
	Weight %	**Atomic %**	**Weight %**	**Atomic %**	**Weight %**	**Atomic %**
o	**56.38**	**70.41**	**57.90**	**71.75**	**53.68**	**68.64**
Na	**0.37**	**0.32**	**0.31**	**0.27**	**0.51**	**0.46**
Mg	**0.71**	**0.58**	**0.60**	**0.49**	**0.73**	**0.62**
Al	**11.91**	**8.83**	**10.21**	**7.50**	**10.56**	**8.01**
Si	**23.97**	**17.05**	**24.51**	**17.30**	**25.17**	**18.33**
K	**2.11**	**1.08**	**1.83**	**0.93**	**2.19**	**1.15**
Ca	**0.56**	**0.28**	**0.65**	**0.32**	**0.93**	**0.47**
Ti	**0.40**	**0.17**	**0.38**	**0.16**	**0.79**	**0.34**
Fe	**3.59**	**1.29**	**3.59**	**1.27**	**5.43**	**1.99**

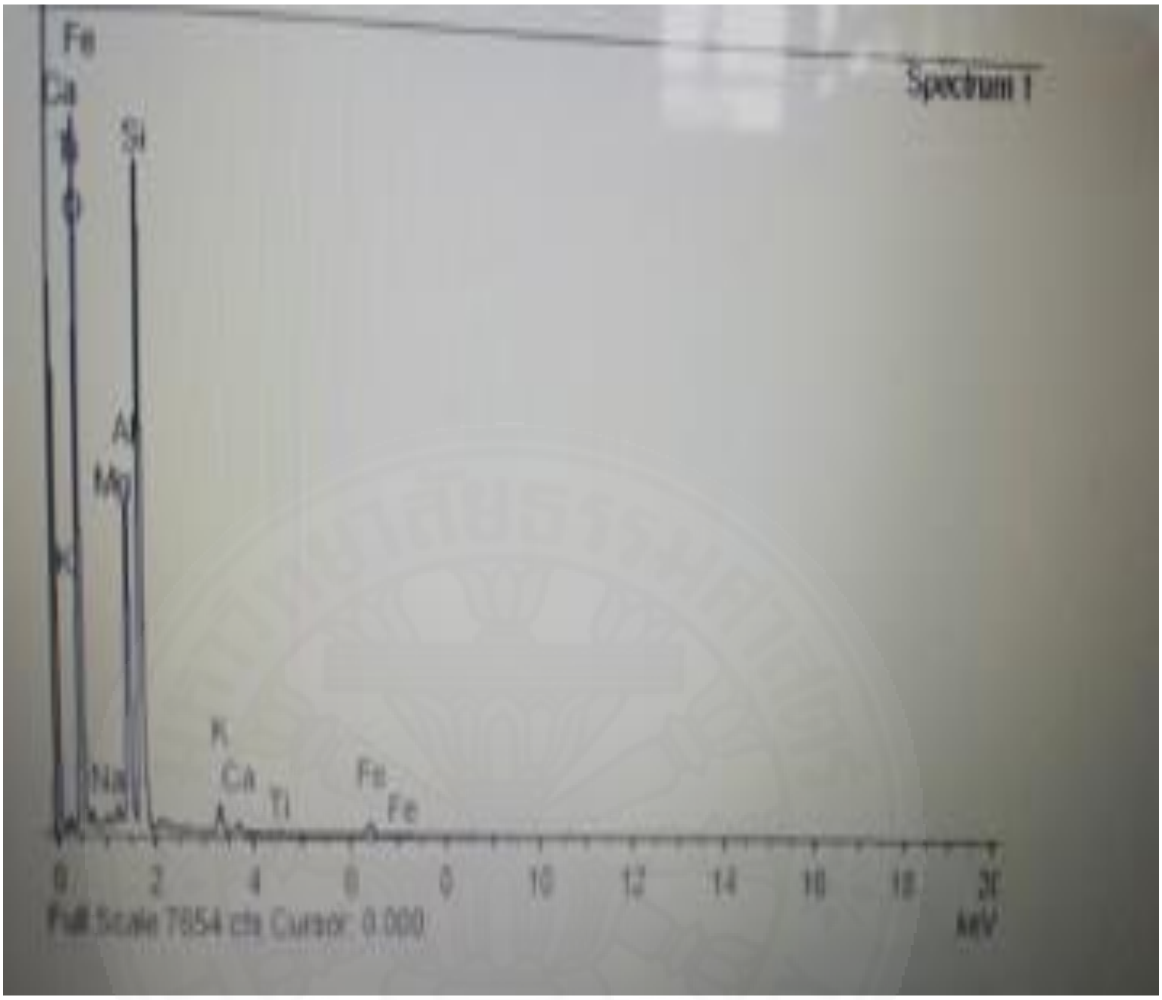

Figure 1A. EDS spectrum for earthen material

Appendix B

Physical and weather parameters recorded

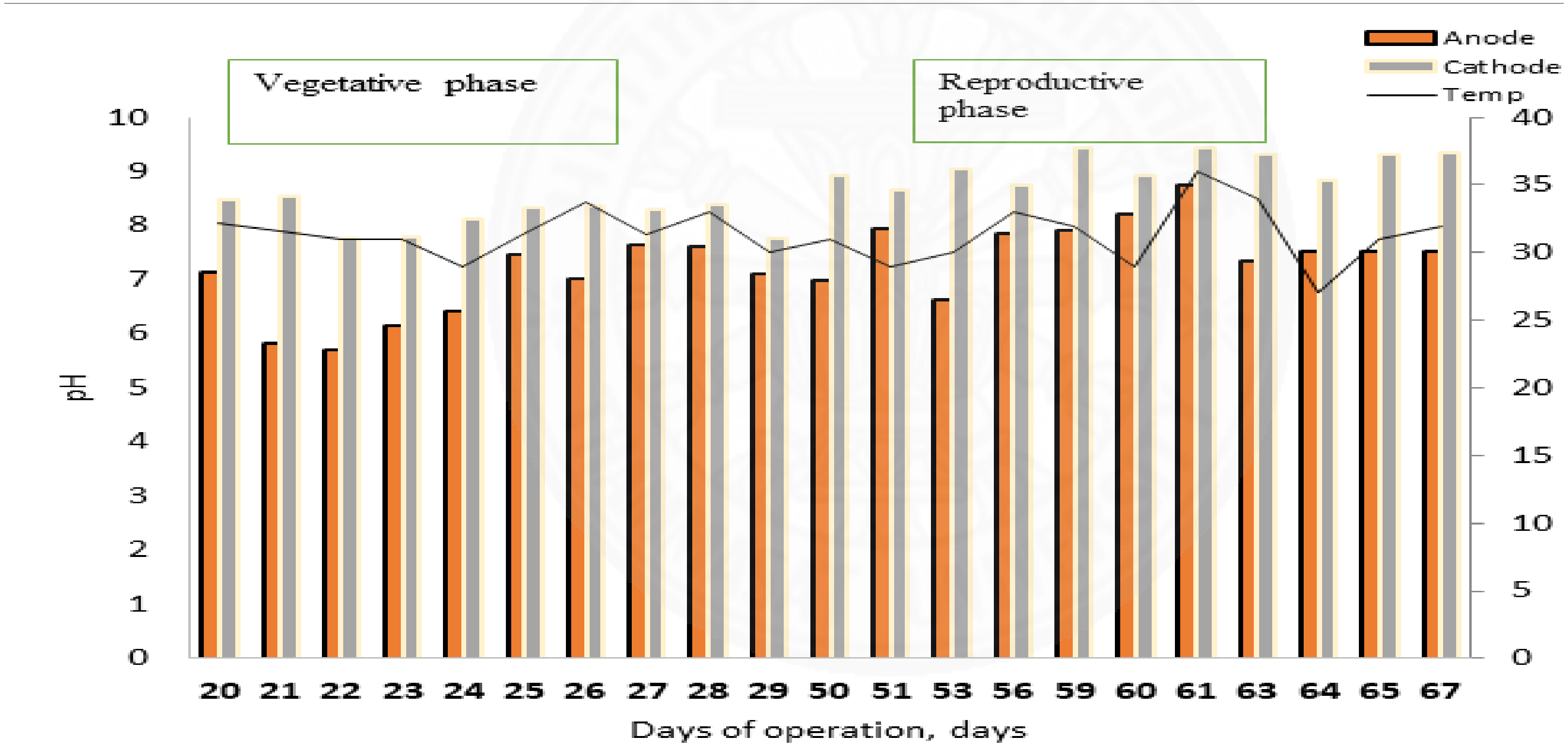

Figure 1B. pH, anode and cathode temperature fluctuation during paddy microbial fuel cell operation (chapter 4.1)

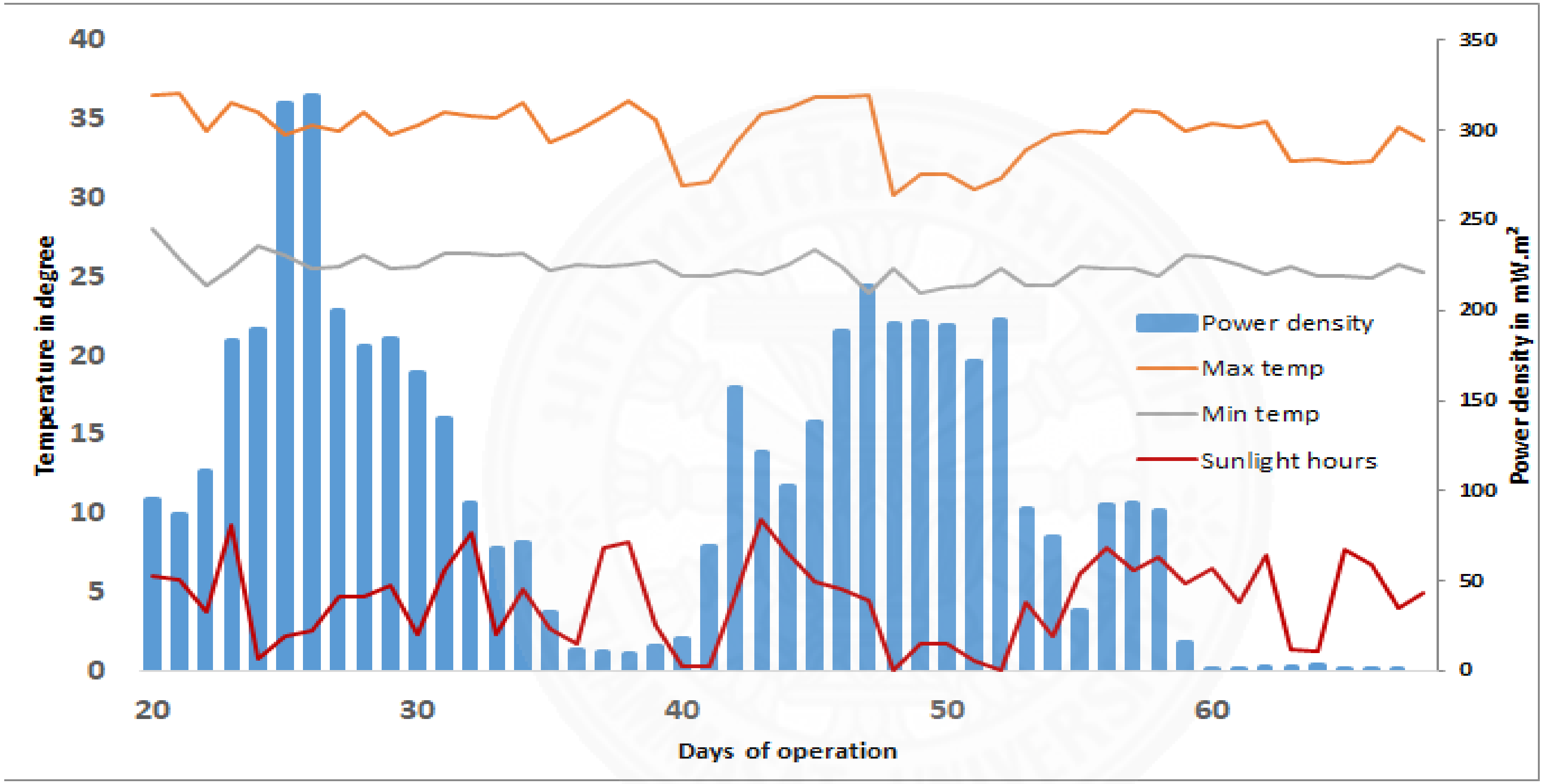

Figure 1C. Relation of weather parameters with High performing reactors (PMFC3) (Chapter 4.1)

Table 1A pH and conductivity measured for hydrophytes operated PMFC (Chapter 4.3)

Days	pH										Conductivity									
	Anode					Cathode					Anode					Cathode				
	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5
Influent	5.85	5.75	6.28	5.72	5.76						709	825	851	867	893					
0	7.01	6.46	6.68	6.53	7.07		8.51	8.49	7.96		891	1303	1338	1246	875		438	423	432	
1	6.79	6.54	6.65	6.64	7.03	7.78	8.08	8.23	8.17	8.18	857	1241	1322	1197	557	512	485	448	472	482
2	6.77	6.63	6.77	6.73	6.94	7.86	8.32	8.45	8.3	8.25	843	1210	1285	1149	764	781	528	476	547	540
3	6.8	6.78	6.76	6.77	7.19	8.03	8.74	8.73	8.66	8.7	810	1185	1299	1178	788	836	559	494	569	527
5	6.86	6.78	6.74	6.75	7.14	8.05	8.88	8.6	8.63	8.89	838	1205	1336	1192	795	816	629	534	578	618
6	6.9	6.77	6.69	6.68	7.15	8.08	8.76	8.63	8.81	8.77	825	1210	1360	1226	801	787	625	551	586	606
7	7.14	6.95	6.75	6.7	7.25	8.17	8.2	8.11	8.17	8.01	769	1116	1264	1122	733	545	485	477	478	582
8	7.29	7.2	6.92	6.73	6.95	7.78	8.22	8.26	8.32	8.2	762	1098	1247	1066	735	712	714	496	500	662
9	7.12	6.92	6.75	6.77	6.95	7.89	8.11	8.18	8.26	8.19	766	1110	1249	1034	747	751	612	500	524	677
10	7.09	6.93	6.71	6.74	6.98	7.75	8	8.08	8.16	8.17	767	1096	1246	1019	751	775	648	511	517	741
12	7.21	7.1	6.78	6.73	6.75	8.03	8.61	8.57	8.7	8.46	774	1082	1223	1006	757	1074	708	533	549	774
13	7.16	6.94	6.6	6.24	6.6	7.79	8.27	8.3	8.38	8.28	790	1097	1260	1008	774	1091	717	527	534	695
14	7.31	7.03	6.61	6.06	6.63	7.8	8.43	8.52	8.46	8.17	786	1050	1216	945	759	1014	725	522	533	659
15	7.07	6.61	6.22	5.61	6.6	7.92	8.17	8.41	8.44	8.27	715	1061	1242	976	765	1111	809	551	584	397
16	7.17	6.58	6.09	5.97	6.68	8.45	8.48	8.49	8.52	8.42	790	1065	1248	970	767	535	456	429	440	519
20	6.66	6.16	5.71	5.98	6.07	8.08	8.18	8.37	8.71	8.22	770	1056	1271	914	774	559	570	438	484	573
21						8.99	9.03	8.95	8.97	8.04						574	578	42.4	490	583
22						8.62	8.8	8.495	8.5	8.03						571	576	413	504	544
23						8.57	8.76	8.75	8.68	8.24						632	580	427	519	528
26						7.13	7.6	7.73	7.94							763	736	506	616	
27						8.04	7.95	7.89	7.85	7.47						419	405	388	408	429
28	4.45	2.7	5.67	6.04	6.13	7.88	8.05	7.97	8.23	7.97	850	1857	1356	819	776	435	427	412	423	472
29	5.57	5.34	6.03	6.18	5.62	8.15	8.22	82.85	8.25	8.07						449	446	427	431	483
30						8.29	8.23	8.06	8.1	8						444	469	434	459	494
31						8.24	8.04	8.05	8.26	8.04						444	499	435	467	482
33						7.4	7.58	6.95	7.85	7.74						463	550	447	523	494
34						7.43	7.55	7.61	7.74	7.76						477	569	451	533	480

Table 1B Dissolved oxygen for morning glory operated PMFC (Chapter 4.3)

Days	Dissolved Oxygen in cathode (mg/l)				
	R1	R2	R3	R4	R5
6	3.51	3.4	3.23	3.44	3.65
7	3.05	2.84	2.61	2.75	2.692
8	2.51	2.6	2.58	2.68	2.84
9	3.71	3.15	2.98	3.02	2.98
12	3.33	3	3.02	3.09	3.25
13	2.61	2.64	2.38	2.59	2.48
14	2.86	2.8	2.71	2.66	2.23
15	3.44	3.06	3.08	2.99	2.24
16	3.03	3.03	3.18	3.19	3.26
20	1.47	1.91	1.88	1.88	2.04
21	1.97	2.36	1.87	2.2	1.78
22	2.15	2.13	2.07	2.12	2.01
26	3.39	3.45	3.45	3.6	0
27	1.72	1.76	1.78	1.81	1.56
28	1.56	1.51	1.51	1.6	1.51
29	2.1	2.07	2.04	2.13	2.08
30	2.49	2.56	2.58	2.67	2.56
31	2.95	2.29	2.46	2.64	2.5
33	2.31	2.31	2.38	2.23	2.11
34	2.08	2.26	2.29	2.18	2.25
55	1.53	1.44	1.4	1.38	1.22
58	2.23	2.25	2.24	3	0
59	1.54	1.48	1.15	1.48	1.42
60	2.09	2.45	2.47	2.45	2.49
61	1.47	2.15	2.07	2.31	2.42
64	0.65	2.36	2.23	2.41	2.91
65	3.61	3.81	3.57	3.91	3.97
66	2.12	2.02	1.8	1.84	1.47
69	1.84	2.58	2.96	2.57	2.07
70	1.14	1.1	1.56	1.47	1.83
79	0.8	1.38	1.65	1.66	1.71
80	1.17	1.26	1.33	1.43	1.47
81	1.55	1.58	1.51	1.68	1.69
83	0.84	1.64	1.67	1.72	1.85
86	0.39	0.84	0.92	1.42	1.32

Appendix C

Polarization data for vegeative and reproductiv phase for PMFCs

	Maximum greenness (VP)												
SPMFC					PMFC3			PMFC2			PMFC1		
R	I	V	P	PD	I	V	PD	I	V	PD	I	V	0.077509
10	2.45	24.5	0.060025	3.398443	4.4	44	10.96108	2	20	2.264685	0.37	3.7	0.130899
20	1.65	33	0.05445	3.082803	4.05	81	18.57381	1.9	38	4.087757	0.34	6.8	0.186837
30	1.6	48	0.0768	4.348195	3.8	114	24.52654	1.87	56	5.944798	0.33	10	0.203822
40	1.5525	62.1	0.09641	5.458471	3.575	143	28.94409	1.65	66	6.227884	0.3	12	0.221939
50	1.44	72	0.10368	5.870064	3.36	168	31.95924	1.62	81	7.429299	0.28	14	0.265393
60	1.32	79.2	0.104544	5.918981	3.2	192	34.78556	1.57	94	8.492569	0.275	16.5	0.291522
70	1.235714	86.5	0.106889	6.051764	3.043	213	36.69526	1.562	109.4	9.681529	0.271	19	0.292144
80	1.155	92.4	0.106722	6.042293	2.9125	233	38.42038	1.56	125	11.04034	0.26	21	0.303921
90	1.12	100.8	0.112896	6.391847	2.78	250	39.3172	1.511	136	11.63482	0.244	22	0.299505
100	1.18	118	0.13924	7.883369	2.65	265	39.75938	1.48	148	12.45577	0.23	23	0.523425
200	0.7	140	0.098	5.548478	1.73	346	33.97028	1.19	238	15.8528	0.215	43	0.70109
300	0.503333	151	0.076003	4.30309	1.39	418	32.89455	1.01	303	17.55131	0.203	61	0.733758
400	0.3925	157	0.061623	3.488889	1.1725	469	31.13942	0.91	364	18.74027	0.18	72	0.74293
500	0.3216	160.8	0.051713	2.927857	1.014	507	28.87473	0.79	395	17.67021	0.162	81	0.783128
600	0.273333	164	0.044827	2.537957	0.892	535	27.17622	0.695	417	16.41897	0.152	91	0.906214
700	0.236714	165.7	0.039224	2.220725	0.8	560	25.47771	0.624	435	15.34324	0.151	106	1.019108

800	0.208625	166.9	0.03482	1.971381	0.724	579	23.77919	0.561	449	14.26752	0.15	120	1.114452
900	0.186333	167.7	0.031248	1.769178	0.66	595	22.25053	0.511	460	13.30502	0.148	133	1.109696
1000	0.1748	174.8	0.030555	1.729939	0.625	625	22.1373	0.47	470	12.51238	0.14	140	0.962491
2000	0.09025	180.5	0.01629	0.9223	0.33	656	12.45577	0.268	536	8.152866	0.092	184	0.758669
3000	0.0606	181.8	0.011017	0.623755	0.225	674	8.605803	0.19	563	6.227884	0.067	200	0.672611
4000	0.0456	182.4	0.008317	0.47091	0.172	686	6.794055	0.144	576	4.699222	0.0545	218	0.736023
5000	0.03656	182.8	0.006683	0.378382	0.14	695	5.661713	0.1167	584	3.855626	0.0512	256	0.849257
6000	0.0305	183	0.005582	0.316008	0.12	701	4.755839	0.098	588	3.283793	0.05	300	0.843595
7000	0.026157	183.1	0.004789	0.271161	0.101	707	3.963199	0.085	592	2.830856	0.046	325	0.79264
8000	0.0229	183.2	0.004195	0.237525	0.089	712	3.623496	0.074	595	2.491154	0.042	335	0.849257
9000	0.020367	183.3	0.003733	0.211364	0.008	720	3.397028	0.0664	598	2.2477	0.041	369	0.905874
10000	0.01833	183.3	0.00336	0.190227	0.073	727	3.000708	0.0605	605	2.094834	0.04	400	0.481246
	NEAR HARVEST		(RP)										
10	0.14	1.4	0.000196	0.011097	0.8	8	0.36235	0.27	2.7	0.041274	0.26	2.6	0.038273
20	0.145	2.9	0.000421	0.023808	0.75	15	0.636943	0.26	5.2	0.076546	0.21	4.2	0.049936
30	0.14	4.2	0.000588	0.033291	0.733333	22	0.913423	0.256667	7.7	0.111894	0.186667	5.6	0.059184
40	0.1425	5.7	0.000812	0.045987	0.725	29	1.190375	0.25	10	0.141543	0.16	6.4	0.057976
50	0.144	7.2	0.001037	0.058701	0.72	36	1.467516	0.246	12.3	0.171312	0.16	8	0.07247
60	0.143333	8.6	0.001233	0.06979	0.716667	43	1.744751	0.241667	14.5	0.198396	0.16	9.6	0.086964
70	0.142857	10	0.001429	0.080882	0.7	49	1.941967	0.234286	16.4	0.217539	0.158571	11.1	0.099654
80	0.1375	11	0.001513	0.085633	0.6875	55	2.140835	0.23	18.4	0.239604	0.1575	12.6	0.112357
90	0.144444	13	0.001878	0.106314	0.688889	62	2.41818	0.225556	20.3	0.259237	0.156667	14.1	0.125067
100	0.15	15	0.00225	0.127389	0.71	71	2.854069	0.222	22.2	0.279032	0.157	15.7	0.139556

200	0.13	26	0.00338	0.191366	0.61	122	4.213447	0.18	36	0.366879	0.145	29	0.238075
300	0.106667	32	0.003413	0.193253	0.436667	131	3.238688	0.15	45	0.382166	0.135667	40.7	0.312619
400	0.0975	39	0.003803	0.215287	0.34	136	2.617976	0.12875	51.5	0.375407	0.12625	50.5	0.36097
500	0.108	54	0.005832	0.330191	0.288	144	2.348025	0.113	56.5	0.361472	0.119	59.5	0.400878
600	0.108333	65	0.007042	0.398679	0.245	147	2.039066	0.100833	60.5	0.345388	0.113333	68	0.436329
700	0.101429	71	0.007201	0.407724	0.215714	151	1.844182	0.090714	63.5	0.326135	0.107571	75.3	0.458606
800	0.09875	79	0.007801	0.441684	0.19125	153	1.656688	0.0825	66	0.30828	0.102125	81.7	0.472391
900	0.098889	89	0.008801	0.498294	0.174444	157	1.550617	0.075556	68	0.290886	0.111556	100.4	0.634122
1000	0.1	100	0.01	0.566171	0.16	160	1.449398	0.07	70	0.277424	0.1108	110.8	0.695068
2000	0.085	170	0.01445	0.818117	0.0945	189	1.01121	0.04225	84.5	0.20213	0.085	170	0.818117
3000	0.068667	206	0.014145	0.800868	0.063333	190	0.681293	0.029433	88.3	0.147146	0.0606	181.8	0.623755
4000	0.05925	237	0.014042	0.795032	0.05125	205	0.594834	0.02255	90.2	0.11516	0.045575	182.3	0.470393
5000	0.051	255	0.013005	0.736306	0.0448	224	0.568164	0.01824	91.2	0.094182	0.03786	189.3	0.405769
6000	0.043667	262	0.011441	0.647738	0.0385	231	0.503524	0.01535	92.1	0.080042	0.03175	190.5	0.342442
7000	0.039571	277	0.010961	0.620597	0.034857	244	0.481537	0.013214	92.5	0.069204	0.027657	193.6	0.303152
8000	0.035925	287.4	0.010325	0.584563	0.032	256	0.463808	0.0116	92.8	0.060947	0.024975	199.8	0.28252
9000	0.033511	301.6	0.010107	0.572227	0.029444	265	0.441771	0.010333	93	0.054409	0.022222	200	0.251632
10000	0.0306	306	0.009364	0.53014	0.0275	275	0.428167	0.00933	93.3	0.049285	0.0201	201	0.228739

Appendix D

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