Electricity Production from Plant Microbial Fuel Cell (PMFC) and Isolation of Active Electrogenic Bacteria

By

Erum Shahzadi

Registration No. 00000327617

Supervisor

Prof. Dr. Imran Hashmi

Institute of Environmental Sciences & Engineering
School of Civil & Environmental Engineering
National University of Sciences and Technology
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A thesis submitted in partial fulfilment of the requirement for the degree of the Degree of Master of Science in Environmental Science

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Ms. Erum Shahzadi

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Supervisor: ....
Professor
Dr. Imran Hashmi
SCEE (IESE) NUST

GEC Member: …………………………………
Dr. Zeshan
Associate Professor
SCEE (IESE) NUST

GEC Member: ………………………………..
Dr. Aamir Alaud Din
Assistant Professor
SCEE (IESE) NUST

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327617.

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List of Abbreviations

PMFC	Plant Microbial Fuel Cell
IEA	International Energy Index
HDI	Human Development Index
GDP	Gross Domestic Product
CO2	Carbon Dioxide
SDG	Sustainable Development Goal
UN	United Nation
MFC	Microbial Fuel Cell
PBT	Payback Time
PAR	Photosynthetic Active Radiation
IPAR	Interception of Radiation
APAR	Absorbed Photosynthetic Active Radiation
GAC	Granular Activated Carbon
PD	Power Density
CE	Conversion Efficiency
LMW	Low Molecular Weight
C/N	Carbon/Nitrogen
PEM	Proton Exchange Membrane

Abstract

The Plant Microbial Fuel Cells (PMFCs) are environmental friendly fuel cells developed to produce renewable and sustainable bioelectricity from flooded agricultural fields. Active electrogenic bacteria serves as crucial catalytic machinery in PMFC, which perform redox reaction. The PMFC relies on rhizospheric zone to power exo-electrogens at anodic compartment with excretion of rhizodeposits with concomitant bioelectricity production. The PMFC may be capable of continuous output of bioelectricity without depending on biomass (biofuels) by utilizing waste streams of plant carbon sources, i.e., rhizodeposition, for their functioning. In this study, paddy based PMFCs were designed in horizontal configuration for both summer and winter experiments. Continuous daily readings were recorded for 320 days during both experiments. Both experiments were performed in different climatic condition with same setup, configuration and experimental site. The performance of both PMFCs was evaluated in terms of Voltage (*V*), Current (*I*) and Power (*P*). In addition Voltage (*V*) and Current (*I*) were recorded in each progressive paddy growth phase which includes Nursery Phase, Transplantation Phase, Tillering Phase, Panicle Phase & Ripening Phase. The existence of exo-electrogenic bacteria was also determined to conclude that electricity production is directly proportional to relative abundance of these active bacteria in mature biofilm formed at surface of anode. The results revealed that peak voltage produced in summer and winter experiment was 1436.6 mV and 1063.5 mV respectively. Similarly peak current generated in summer and winter experiment was 1376.6 mA and 1037.6 mA respectively. In addition power generated in Summer and Winter experiment was 1977.6mW and 1103.4mW respectively. The maximum energy was harvested in active Tillering phase while minimum energy was recorded in Ripening phase of paddy growth. A total of 13 species were isolated from anodic sample of PMFC. Among these 13 microbial species, 3 isolates were in abundance which were strongly attached to the surface of anode this evident that these bacteria possess a significant role in electricity production. Outcomes of research, Voltage (*V*), Current (*I*) and Power (*P*) produced in summer experiment was 40%, 30% and 44% greater than winter experiment respectively.

Keywords: PMFC, Rice Paddy Photosynthesis, Root Exudates, Active Electrogenic Bacteria, Rhizodeposition

Chapter 1

Introduction

1.1 Background

Modern human lifestyle is totally dependent on an efficient energy source such as electricity. Electricity can be used directly or indirectly very efficiently to perform energy-associated factors. According to International Energy Agency (IEA), the delivery of reliable and affordable electricity must be ensured to enhance the global economies (Jadhav *et al.*, 2020). Human development aspect directly associated to free access to green, clean and modern energy source. Global human development is interlinked with electricity consumption per capita (in kWh) in positive correlation with human development index (HDI) and gross domestic product (GDP) from 120 countries around the globe (Gebreslassie *et al.*, 2021). Currently fossil fuels such as oil, gas and coal are contributing the major part in electricity generation while renewable energy sources are still at ignorant stage except conventional energy sources. All natural sources that have ability to replenish naturally are known as renewable sources. Renewable energy sources comprised of all natural potential sources derived either from natural phenomenon (e.g. tidal energy from moon and geothermal energy from earth core) or directly (e.g. photo-electric, photo-chemical and thermal) and indirectly (e.g. hydropower, wind power and energy produced as a result of photosynthesis) from the sun (Zhang *et al.*, 2019). Fossil fuel sources (~70% of total generation) are still dominant in total global electricity generation mix. Modern world economies progress still lie on the availability of oil, coal and natural gas as conventional energy sources. However, increased consumption rate of fossil fuels causing exploitation of theses natural sources along with other negative environmental impacts such as elevated health risks and enhanced global climate change (Kumar *et al.*, 2021). As assessment on life cycle emissions has illustrated that emissions through power generation via conventional energy systems is higher compared to renewable power generation systems. Therefore, switching from energy sources with a high carbon content to renewable energy sources with a low carbon content must happen right away. (Gupta *et al.*, 2021). Today policies at global scale are showing firm political concern by taking on Sustainable Development Goals (SDGs) to achieve 2030 agenda. SDG 7 is about to have complete access to continuous, affordable, clean, green and renewable energy source (Aiyer *et al.*, 2021).

Socioeconomic and environmental instability is the foremost challenge in pursuit for green and clean alternative energy technologies especially in view of rising global climatic concerns to fulfill the future energy requirements along with challenge of development and adaptation to newly emerging technologies for the cleaner and greener production of bioenergy (Kumar *et al.,* 2020). Currently Microbial Fuel Cells (MFCs) are seeking attention for generation of electricity just because of its cleaner and greener production and problem solving nature of waste management. Electric energy produced in MFCs is an output of metabolic activities of electro-chemically active bacteria. MFC as an ingenious technology is applied to generate electric energy as a result of oxidation process carried out by active electrogenic bacteria to organic fraction of renewable biomass and environmental waste (Apollon *et al.,* 2021). Cheng *et al.* (2021) reported two major MFC associated problems still exist which needs to be addressed before commercialize MFC technology. The complete application of the available organic content by the microorganisms resulted in decreased or a cessation of power after a certain time period, which is the first issue that needs to be taken into consideration. The relatively low voltage generation magnitude by MFC and these two factors render this technology inappropriate for commercial scale application (Bose *et al.,* 2020) addressed the second issue that needs to be concentrated on. The organic portion of biomass act as substrate in MFC hence uninterrupted supply of organic matter must ensure for MFC performance. One of the greatest creative solutions was the introduction of plants at the anode location as a supplement substrate supply for bacteria (Yaqoob *et al.,* 2021).

The plants secretes rhizodeposits through root exudation as a results of photosynthetic activity and these exudates utilized by the microbial communities present at the rhizospheric zone of plant to produce electricity (Shaikh *et al.,* 2020). The electrons are produced as the breakdown of exudates by bacteria takes place in rhizospheric zone of plant. The released electrons then trapped and transferred from the anodic region by the electron collectors and passed through membrane and directed to opposite pole. Resultantly gradient formation between two electrodes cause the power to generate and electricity generated termed as Bioelectricity (Yan *et al.,* 2015). During whole process, exudates through plants act as a concurrent source of substrate for MFC. But still there are a lot of driving factors and aspects which needs to be addressed to upscale MFC technology and to convert it to saleable products. Some driving factors included as; optimal microbial activity, light intensity for photosynthetic process, amount of rhizodeposits, plant selection and appropriate design configuration in need of modification in occurring issues associated to electricity generation through PMFC (Zhao *et al.,* 2019). Moreover, certain limitations are also applied including high operational cost of MFC and discontinuous generation of power because of interrupted parameters such as meteorological conditions associated with present technology. In stark contrast, PMFC can provide electricity continuously and uninterruptible for 24 hours every day (Jadhav *et al.,* 2017). Yet much research required to conduct for the application of PMFC as power source along with phytoremediation and waste treatment technology. "Payback time" (PBT) is a further notion to evaluate the effectiveness of PMFC as an alternative energy source similar to wind and solar. It refers to the amount of time needed to produce power with a value equivalent to the amount of energy used for its production and the durability of the materials used to create the PMFC assembly. Calculating and lowering the PBT of PMFC in comparison to other renewable energy sources is necessary to make PMFC an ideal alternative energy source (Lu *et al.,* 2020). Other bioenergy sources such as hydrogen and biogas should also be considered comparatively with PMFC to check its feasibility and performance at large scale. To ensure the social acceptance and viability of PMFC, consideration of already existing technologies can be vital. The Netherlands-based firm "plant - e" created and commercialized an efficient energy harvesting device based on the PMFC operating concept. The commercialization of PMFC technology for products is being aggressively pursued by a large number of commercial organizations, however the process is still in its early stages and additional technological advancement is needed (Maddalwar *et al.,* 2021).

1.2 Microbial Fuel Cell (MFC)

The Microbial Fuel Cell (MFC) is a sufficiently developed technology that treats wastewater by using microorganisms to break down organic substances in the wastewater while also producing power. The fundamental workings of MFC are based on an oxidation-reduction reaction carried out by active electrogenic bacteria, which generate electrons and protons through the anodic respiration of organic materials. The actively involved bacteria in oxidation of organic matter and transfer of electrons in MFC are generally termed as electricigens, responsible for electricity production (Khan *et al.,* 2022). The reduction reaction takes place at cathode to generate electricity after the transfer of electrons from anode to cathode. MFC can treat wastewater from different sources such as tannery, piggery, slaughter house, dye industry, households and pharmaceutical industry, etc. (Nagda *et al.,* 2022). Environmentally abundant microbes are responsible for the electricity production in all kind of MFCs. The organic matter present in the anodic chamber oxidized by the microbes act as biocatalyst in it. The electrons produced as a result of oxidation of organic matter reduce the anode and transferred to the cathode via external circuit to generate electricity. The electricity produced by this process is much cheaper as compared to other means as microbes are abundantly present in the nature with almost zero carbon output (Ananthi *et al.*, 2021). CO2 produced as a result of oxidation of organic matter and act ac energy reservoirs. The microbial growth in MFC is depends upon the available substrate and anolyte characteristics. Generally, electricity production through mix culture is higher than the pure culture of microbes in MFC; however, exceptions are always there, e.g., Rhodopseudomonas palustris DX-1 is responsible for higher power generation in MFC in comparison to mix culture. Additionally, electricity production increased with an increase in light intensity, although R. palustris ATCC 17001 produced no electricity (Xing *et al.,* 2008). With the aid of Shewanella oneidensis, Escherichia coli (acclimated, non-mediated), and Escherichia coli K12, the best electrical power of 3000 mW/m^2, 600 mW/m^2, and 760 mW/m^2 was generated via MFC, respectively (Wetser *et al.,* 2015). When using MFC, different substrates and microorganisms are used, however the substrates have a big impact on how much power is produced. Therefore, if microbe specificity is investigated, it will not only stop the production of superfluous products but will also allow for the selective differentiation of the microorganisms that produce the highest power density for an MFC. A large number of researchers from all over the world came up to show how effectively MFCs work as numerous value-added synthetic product devices with minor alterations at their individual electrodes (Bird *et al.,* 2022). Recent research have demonstrated an effort to raise the power generation rate in order to remove it from the list of criteria that limit the scaling-up of MFC. Plant microbial fuel cell (PMFC) technology has been developed to support this endeavor (Ahirwar *et al.,* 2023)

1.3 Plant Microbial Fuel Cell (PMFC)

Plant Microbial Fuel Cell (PMFC) is an eco-friendly cell and an emerging technology as an efficient bioelectricity generation source from plant photosynthetic process. In PMFC, CO2 and sunlight utilized by the plant to produce food by the well-known process called photosynthesis (De *et al.*, 2019). In this process, food neither use by the plant nor extracted to the soil. Microorganisms present near the roots of the plant, degrade the available low density compound cells and as result produce electron, proton and carbon dioxide (Di *et al.,* 2020; Regmi *et al.,* 2020). Bioelectricity can be harnessed by only placing two electrodes. For PMFC operation, different classes of plants were considered out of which three plant classes including macropgytes (hydrophytes), wetland grasses and artificial plants are used. Plant selection criteria include bacterial diversity in rhizospheric zone of plant, growth rate, root system perfection, density, location availability, collection and resistance, adaptation ability and amount of rhizodeposits (Regmi *et al.*, 2017). Usually anodes are inserted into the rhizospheric zone of plant, which is close to the roots and root surface. Microbial diversity supports by the release of large number of rhizodeposits by the roots of the plant. These depositions help in proper bacterial functions and provides place for bacterial attachment. Since roots vary within and between plant species, and microbial consortia vary in matrix or inoculation support and functional conditions, rhizosphere bacterial communities differ in PMFCs. During PMFC activity, a variety of bacterial species such as Natronocella acetinitrilica, Rhizobiales, Beijerinckiaceae, and Rhodobacter gluconicum can be found in the rhizosphere (Srivastava et al., 2019). The PMFC have the ability to offer potential in future as the ssafe, green, sustainable and renewable energy source at much lower cost as compared to other bioenergy sources. PMFC also have the significant potential to support agricultural production without disturbing the food. It referred as beneficial innovation for crop cultivation and energy production at the same time in the same fields. Furthermore, Chu *et al.*, 2021 reported the potential application of PMFC for wastewater treatment in terms of elimination of radioactive isotopes and carcinogenic metals. The application of PMFC expanded into plains and wetlands to make them useful by converting them into power generation sources. Due to rapid expansion and development urban areas suffer from green coverage which ends in poor air quality (Sonu *et al.,* 2022). Hence, PMFCs can be stagnant source of energy in urban areas with indoor and rooftop vegetation that generate electricity from living plants and conserve our environment (Mishra *et al.,* 2020). Currently, bioelectricity production from PMFC technology is very small dua to infancy stage; however, it has the potential to become a renewable source of bioenergy in the future. Hence, many researchers around the globe are putting efforts to make advancements in the development of PMFC through modifications in the design of nonchemical catalysts, electrode or long-term energy production capacity as bio-energy generating power source (Chakraborty *et al.,* 2020).

Description of the reaction that occurs in PMFC process:

a. Photosynthesis

$CO_2 + 6H_2O > C_6H_{12}O_6 + 6O_2$

b. Transport of organic compounds in the anode chamber

c. Oxidation of organic compounds by microorganisms in the anode chamber

$C_6H_{12}O_6 + 6H_2O > 6HCO_3 + 30H^+ + 24e^-$

d. Reduction in cathode chamber

$6O_2 + 24H^+ + 24e^- > 12H_2O$

1.4 Driving factors of PMFC Performance

Many researchers around the world reported and highlighted different factors which can potentially influence the performance of PMFC such as operational parameters, properties and The features of the wastewater, the type of microbial inoculum that is available, the chosen plant species employed in the PMFC, the chosen and adhered-to design configuration, and the design conformations (Khan *et al.,* 2021; Jain *et al.*, 2020). Before advancing to the optimal performance and design configuration selection criteria for PMFC, a number of additional minor aspects must be carefully addressed and studied. Here, we make a real effort to think about and address those areas that have been left out of PMFC research (Chu *et al.,* 2021; Lin *et al.*, 2021).

1.4.1 Intensity of Light

The most significant and highlighted driving factor in PMFC performance that needs to be considered is the photosynthesis using sunlight. Two dependent factors on which photosynthesis depends on are photosynthetic active radiation (PAR) light intensity and concentration of carbon dioxide for potential PMFC (Aftab *et al.,* 2021).In equatorial regions the PAR reported 10 times higher than the PAR in western Europe regions which is estimated up to 150 m/W^2. The wave band between the 400 – 700 in electromagnetic radiations are reported as PAR (Dubrawski *et al.*, 2020). The sunlight can reach to vegetative canopy of plant by adopting two possible pathways. Either through direct flux, indirect diffuse fluxes, or both. When the photons coming from sun travels through atmosphere in unscattered pattern than it termed as direct flux formation. When the photon beam scattered into the atmosphere due the presence of various barriers such as clouds, air molecules or aerosols than the formed flux known as diffuse flux. When this flux reached to the vegetative plant canopy, some fraction of it hits the other elements of plant such as leaves, from where the automatic removal of incident light takes place. According to Kakarla, (2020), the portion of the flux that affects the other plant components is known as the intercepted PAR flux or IPAR. The fraction of intercepted radiation (IPAR) is the only efficient and potential source of energy to carry out the photosynthetic process efficiently. Some fraction of intercepted radiations (IPAR) contributes for transmission of reflection by elements of interception. Therefore, the photosynthetic process unable to utilize it. So, In the vegetative canopy region, the absorbed photon of light is known as the absorbed PAR (APAR) flux, which is typically sufficient to carry out the photosynthetic process (Kumar *et al.,* 2020). Naturally, the APAR value is typically lower than the IPAR number. Therefore, the constant coefficient required for the calculations is 0.85. IPAR is equal to 0.85 APR. Before making any computations, it is important to understand the general relationship between APAR and IPAR. Therefore, it is advised to locate and evaluate the PAR data of the chosen region for the launch of PMFC. It would be helpful in the development of understanding and optimization of photosynthetic activity of the plants in the selected region but also useful for the estimation of power generation from PMFC (Ieropoulos *et al.,* 2016).

1.4.2 Selection of Plant

In earlier research, several broad standards were followed and advised for choosing appropriate plant species for PMFC composition. Which explicitly stated that in order to maintain a steady redox potential gradient by avoiding the oxygen interruption in anode chamber, preference should be given to plants with strong adaptation abilities to survive and thrive in waterlogged conditions (Shaikh *et al.,* 2021). For the formulation of PMFCs, plants with the highest photosynthetic rate and the capacity to produce the most biomass are reported (Apollon *et al.,* 2021). Due to their capacity for adaptation in wet conditions, high biomass production, and salt tolerance, marshy grasses are a potential source of bioelectricity generation for the construction of PMFCs (Shaikh *et al.,* 2021). Plants with the C4 or C3 route are recommended for PMFC applications because they produce biomass at a rate that is higher than plants with the C3 pathway and significantly lower than those with the CAM pathway (C4 > C3 > CAM). Depending on the kind of carbon fixation pathway they use during photosynthesis, plants are classified as C3 or C4 plants. Prior to beginning the Calvin cycle, C4 plants first produce the chemicals malate and asparate, whereas C3 plants first produce the three-carbon compound 3-phosphoglycerate during the Calvin cycle.

(Takanezawa *et al.,* 2010). According to Husk *et al.* (2017), C4 plants (grass plants and monocots) have demonstrated high photosynthetic efficiency, which increases rhizodeposition as a result of root exudation and acts as a substrate for PMFC. Numerous plant species have been investigated across the globe as sources of power generation in PMFC, and earlier papers have also examined their respective power generation efficiency. Although there is little discussion of the specifics of plant performance and naming in different design configurations. The maximum power reported with S. anglica was 222 mW/m^2, more than twice as much power as was previously achieved using the same plant. (Neethu *et al.,* 2017). There are several elements that affect the power generated by each plant in various settings. As a result, it's crucial to take into account significant factors such as plant type, plant health, light intensity, and the amount of carbon dioxide in the atmosphere at a given level. Also important for PMFC effectiveness are the microbial communities found in plant rhizospheres and their extended interactions with plant roots (Uzair *et al.,* 2020).

1.4.3 Microbial Communities in Soil

Rhizosphere refers to the area immediately around a plant's roots and the soil there (Fuke *et al.,* 2021). In the rhizosphere, which also acts as a place for the attachment of microorganisms, microbial activities, and the residence of distinct microbial communities, plant roots exude organic chemicals known as root exudates (Guan *et al.,* 2021). In terms of composition, inter- and intra-species concentration, the rate of root exudation varies with plant species. As a result, the richness of the microbial population in the rhizosphere varies depending on the inoculum, nutritional needs, and supportive media (Popat *et al.,* 2012). When potting soil was used as the inoculum in the rhizosphere PMFC system with rice plant, high abundance of colonies on the anode surface were reported for the Geobacter sp. and Desulfobulbus sp. families (Azri *et al.,* 2018). A few organisms have also been discovered to have a prominent position on the anode in rice paddy field PMFC, including Rhizobiales, Beijerinckiaceae, Natronocella acetinitrilica, and Rhodobacter gluconicum. Different plant varieties' rhizospheres have been observed to produce varying amounts of voltage. Voltage fluctuations were mostly caused by microbial diversity in various plants and the composition of root exudates. According to reports, the species Geobacter sp., Ruminococcaceae sp., Desulfobulbus sp., Bacillus, Geothrix, Pseudomonas, Shewanella, and Acidoba are the most prominent ones that can be used to generate electricity in a PMFC system (Apollon *et al.,* 2021). It is advised to use these microorganisms to increase the voltage in PMFC applications. The formation of microbial biofilm at the anode surface, which could impair output generation and overall PMFC performance, may be influenced by a number of factors, including the plant growing medium, supporting matrix for plant growth, system temperature, selected plant species, pH, diffused oxygen, humidity, and substrate composition (El Gamal *et al.,* 2018). The effective operation of each component creates a long chain of subsequent events that can be used to determine the output of power generation over time. The entire effectiveness of the PMFC for the production of energy depends on all parameters. Although the species that perform the PMFCs most efficiently have been identified, there are still a few unresolved issues with these, such as maintaining an environment that is conducive to the growth of the microbial species that are present on the anode surface. Optimal pH, optimum temperature, maintaining the redox gradient, optimal rhizodeposition through the plants in a quantitative and qualitative manner to allow the exchange of ions through the membrane, etc. are some of the aspects of unsolved issues (Bahru *et al.,* 2021).

1.5 Electrode Material and Surface Area

According to Abdelhady *et al.* (2018), the anode and cathode electrode materials used in PMFC formulation should have certain properties, including conductivity, chemical stability, biocompatibility, and affordability for the application. It is typically advised to utilize non-corrosive stainless steel metal electrodes instead of copper electrodes because of the latter's poisonous effects on germs. For use in PMFCs, a variety of carbon-based electrodes, such as carbon cloth fiber material, compact graphite plates, rods or granules of various sizes, paper felt, and foam-shaped objects are recommended (Abbasi *et al.,* 2021). Larger surface area electrodes are preferable because they can offer more surface area for microbial adhesion and biofilm formation. Its porous nature is one essential component that must be preserved in order to enable protons to pass through it successfully to complete the circuit and to maintain the redox gradient in the PMFC system (Bijjanki *et al.,* 2021). The material resistance properties can be used to determine the electrode material to be used in PMFC. For PMFC formulation, high resistance materials are typically preferred. However, due to their high economic cost, PMFC cannot use electrodes with the maximum efficiency, such as platinum coated electrodes or pure platinum electrodes. The anode resistance, which might impair the performance of the PMFC, is connected to the overall PMFC resistance (Chiranjeevi *et al.,* 2018). The kind of electrodes used in a PMFC determines both its overall cost and its performance. The chosen design configuration presents the main obstacle to lowering the cost of PMFC technology and enhancing its scalability (Caiz *et al.*, 2020). A rough surface, great biological compatibility, and effective electron transport between the bacterial community and the electrode surface are all required for electrodes in PMFCs (Flexer *et al.*, 2020). Giving in to some materials, such as activated carbon, that have the capacity to function even in the absence of catalysts during the operation of PMFCs is one way to reduce the cost of the cathode (Arun *et al.*, 2020). The possessed configuration of electrodes presents another obstacle to the viability and scalability of PMFC. According to configuration, the electrodes used in PMFC can be divided into plane electrodes and three-dimensional electrodes (Gadkari *et al.*, 2020). The availability of a broad surface area for microbial community adhesion is the first requirement for an optimum configuration of an electrode for a PMFC, and efficiency in current collection is the second. For an electrode-based chemical catalyst to perform an oxygen reduction process in three steps for water, catalyst, and air, an effective arrangement is necessary (Gomathy *et al.*, 2021). Carbon-based electrodes are typically employed as anodes, and they can be divided based on configuration into brush-like structures, structures with a fully packed arrangement, and structures with a plane shape (Govender *et al.*, 2020).

1.6 Effect of Additives on PMFC Performance

Due to its poor power generation, erratic performance, lack of viability, and high cost of PMFC technology was formerly thought of as a secondary technological feature used in waste-water treatment (Yokomatsu *et al.*, 2020). However, the practical demonstration of the potential for increased magnitude with the addition of additives in PMFC opened up new research opportunities to examine the performance of PMFC with the addition of various additives (Gulamhussain *et al.*, 2020). Researchers from all around the world have worked hard to determine how different additions affect boosting power outputs. Due to the use of varied design configurations, different electrode materials, variable substrate types and sources, as well as other environmental characteristics, it is difficult to compare and evaluate PMFC performance accurately (Nagda *et al.*, 2022). The main goal of adding additives to PMFC is to improve performance and produce power production at a sustained pace.

1.7 Growth Phases of Paddy Crop

An annual grass known as paddy plant has hollow, round, connected culms, flat, narrow sessile leaf blades attached to the leaf sheaths by collars, well-defined, sickle-shaped, hairy auricles, short, acute, two-cleft ligules, and panicles. Pakistan's rice varieties' life cycles ranged within 140-160 days from germination to maturity, depending on the variety and the environment. Usually paddy growth period consist of three to five phases, depending on followed nomenclature with total duration of 140-160 days that vary from plant species to species. The paddy plant growth can be categorized into five main agronomic phases of development which include nursery phase, transplantation phase, tillering phase, panicle phase and ripening phase. These growth stages influence the three PMFC components. First, the number of panicle per unit land area. Second, the average number of roots exudates with plant growth. Third, the microbial communities abundance actively involved in power generation, present the surface of anode. Some of the highlighted characteristics in each phase are illustrated in figure 1.

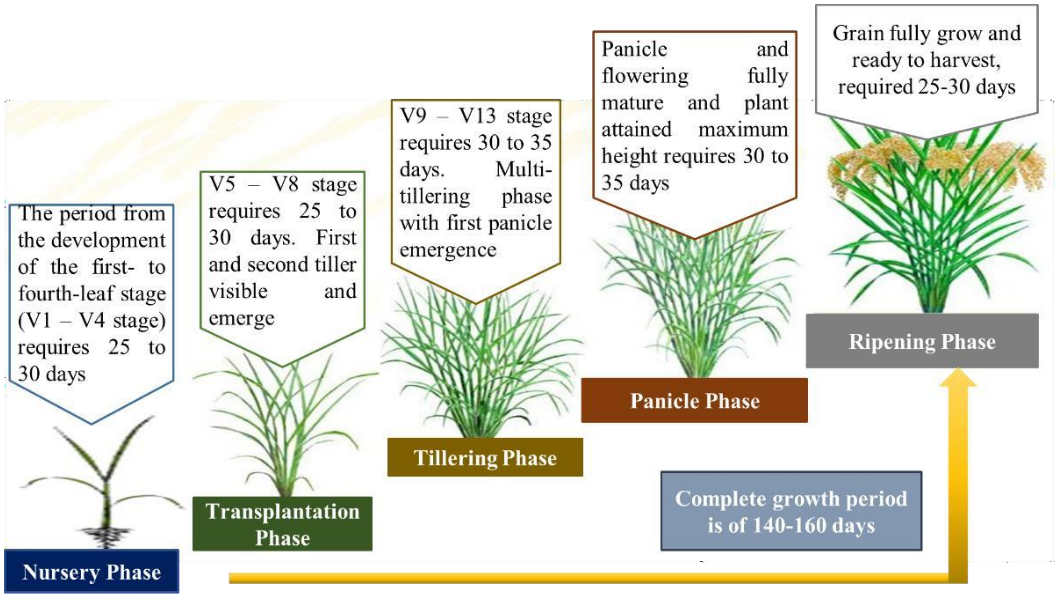

Figure 1: Paddy growth phases

1.8 Objectives of the Study

Present study was conducted with two research objectives;

1. To experimentally design and operate PMFC at different growth stages of rice plant.
2. To isolate the active electrogenic bacteria from PMFC and study microbial morphology and abundance.

Chapter 2

Literature Review

Strike et al. created the first PMFCs in 2008, paving the way for the production of bioenergy from rhizo-deposits. A sustainable form of energy, bioenergy from PMFC generates energy without endangering the environment. In a recent study, Helder et al. selected marsh species, which may flourish in standing water. PMFC systems do not compete for land with conventional crops and can be mixed with agricultural fields. It can also be used into locations not ideal for growing food, including wetlands or even rooftops. The PMFC could be used in these ways to stop deforestation (Timmers *et al.,* 2013). In a region known as the rhizosphere, plant roots exude many chemicals into the surrounding soil. The rhizosphere is more populated by microbes. The relationship between roots and soil bacteria is started and regulated by root exudates. One of the main mechanisms by which plant roots release soil organic carbon is called root exudation. According to Neethu and Ghangrekar (2017), plant species, plant age, and environmental conditions including biotic and abiotic stress all affect the properties of root exudates. In order to mineralize newly obtained nutrients and to mediate interactions between plants and microbes, organic chemicals released from roots are crucial (Popat *et al.*, 2012). The root exudates contain a variety of types of primary and secondary components, such as vitamins, amino acids, organic acids, phenolic acids, flavonoids, enzymes, fatty acids, nucleotides, tannins, steroids, terpenoids, alkaloids, and polyacetylenes. As a result, it may be anticipated that regulating growth and root branching in areas of nutrient-rich patches will coincide with enhanced root exudation, which may have an impact on the dynamics of nutrients. A larger concentration of root exudates would imply greater metabolic activity and hence greater bio-energy production because there would be more substrate available for the root microbes to metabolize. According to Tapia *et al.* (2017), they mediate both positive and negative interactions, such as symbiotic associations with beneficial microbes like mycorrhizae, rhizobia, and rhizobacteria that promote plant growth. They also mediate associations with parasitic plants, pathogenic microbes, and invertebrate herbivores. The main mechanism for mineralizing ingested nutrients and facilitating conversations between plants and microbes is the release of organic molecules from roots. Higher exudate concentrations may be caused by a variety of complicated factors, including microbial population near roots, pace of exudation, soil nutrient content, and more (Neethu and Ghangrekar *et al.*, 2017).

Porter first raised the possibility of bacteria generating electricity in 1910. It did not initially get much interest. The development of this technique was later praised by the scientific community because it turns waste into energy without leaving a trace on the environment. The MFC technology saw numerous improvements and alterations over time. A plant was intended to be included in the PMFC as a supply of substrates for bacteria in the anode region. Multidisciplinary subjects like the study of microbes, plants, electrochemistry, and many engineering specialties are all included in this technology. Therefore, investigating these fields in PMFCs appears to be crucial to comprehending the connections between them (Seth *et al.*, 2021). Viewing a PMFC system as a biosystem that includes biotic and abiotic components for the production of biomass and bioenergy is the most effective way to gain a comprehensive understanding of the interrelationships that exist among these aspects. Biosystems are made up of interrelated living and non-living parts that work together to provide food, protect the environment, grow the economy, and improve technology. One of the most well-known examples of a biosystem is the photosynthetic process, which converts solar radiation into sugars and then biomass. Few works have been reported in the field of PMFCs due to the expansion of research in MFCs. Review studies on a variety of MFC technologies, including technique, configuration, substrates, microbes, applications, cellulosic MFC, built wetland, domestic wastewater treatment, and phototropic organism in an MFC, have been presented in recent years. However, Strike *et al.* (2008) noted that there were just a few review studies for PMFCs.

2.1 Input signal

2.1.1 Light

The three components of light—intensity, quality, and photoperiodism—are the input signals that can influence both plant growth and PMFC system performance. Additionally, light is crucial for photosynthesis in PMFCs, which results in the simultaneous generation of biomass and bioelectricity. Research in a PMFC should focus on the effect of light in maximizing the root exudates with high photosynthetic activity in addition to the ideal circumstances for the heterogeneous microbial population in the rhizosphere. Several earlier research have established the role of light in PMFC performance. For instance, increasing the light intensity increased the voltage obtained (Taskan *et al.*, 2022). Similar to this, Kaku et al. discovered that shadowing of plants can reduce the electric production in PMFCs because photosynthesis is inhibited along with a drop in rhizodeposition. The physiology of the plant, including the synthesis of organic compounds, transportation of compounds to the root, release of exudates and bacterial absorption of the exudates, as well as the release of electrons, was accounted for in differences in time for attaining the maximum power (Zhang *et al.*, 2019). Therefore, plant physiology also impacts total performances, making light not the only element that limits power production. Therefore, plants with the physiology to convert photosynthetic materials into root exudates while also allowing for simultaneous microbe absorption are ideally suited for PMFCs since increased bioenergy harvest is possible. However, the aspects that need to be thoroughly explored inside PMFCs include the determination of the ideal light intensity for an effective photosynthesis, optimal microbial activity, and increased rhizodeposition (Nath *et al.*, 2021).

2.1.2 Photosynthetic pathways

A appropriate plant selection aids in maximizing a PMFC's power output. The photosynthetic pathway and rhizodeposition are likely the two most significant effects of plants in PMFCs. Plants are divided into the C3, C4, and CAM classes based on their photosynthetic activity. Each type of plants has a unique set of photosynthetic processes. C4 plants are more effective at photosynthesis than other plant types (Varanasi *et al.*, 2020). This indicates that C4 plants have a better rate of solar energy conversion into bioelectricity when used in a PMFC. Additionally, C4 plants outnumber C3 plants in extremely hot and dry conditions when it comes to fixing the available carbon for carbohydrate production, as they never experience light saturation (Khan *et al.*, 2021). Additionally, O. sativa is the preferred food crop under study, and its maximum production is close to or less than 20 mW/m^2. This could be a result of the growth stage, which is less resilient than grasses and necessitates careful cultivation methods (Vinayak *et al.*, 2019).

2.2 Biocontrol structure/plants

For a PMFC, mostly salt-tolerant marsh plants were studied. For instance, Glyceria maxima underwent its first test for the production of electricity. Later, significant work was completed in an MFC of the paddy variety (Oryza sativa). In order to feed the growing population of customers, rice must be grown (Mohan *et al.*, 2014). Because of how well they adapt to the system, how much biomass they produce, and how well they tolerate salt, marshy grasses appear to be ideal plants for testing in a PMFC. Pennisetum setaceum, Cyprus involucratus, Lolium perennee, Echinorriea crassipes, Acorus calamus, Ipomoea aquatica, Typha latifolia, Echinochloa glabrescens, and Canna indica are just a few of the grass species that have been studied for various purposes, primarily bioelectricity production and wastewater treatment within PMFCs (Wang *et al.,* 2018). MFC installation in commercial crops (vegetable and fruit) hasn't, however, been documented. This might be due to the short lifespan of commercial crops, which raises the cost of installation; the possibility of plant degeneration, which lowers fruit production; and their susceptibility to biotic and abiotic influences. Plants are highly suited to greenhouse settings and may resist pests and disease under carefully monitored operation conditions. Consequently, it is feasible to attain the dual benefits of harvesting the economic and bioelectrical components, as well as potentially many additional benefits that are not yet recognized. As underutilized carbon sources, root exudates can be utilized by MFCs to generate bioelectricity (Maddalwar *et al.,* 2021).

2.3 Bioprocess Structure

2.3.1 Microbial community

Microbes use the substrates in the rhizosphere zone to contribute electrons to the anode through either direct electron transfer or mediated electron transfer. The microorganisms and the root zone of the plants have a special association. The availability of food for the bacteria in the rhizosphere and the involvement of the microorganisms in improving plant nutrition intake are the classic manifestations of this interaction (Fuke *et al.,* 2021). The likelihood of increased system performance increases with the degree of the microbial community's system adaptation. However, the phylogeny and species of the plant have an impact on the microbial community. In PMFCs, efforts have been made to clarify the microbial community there (Mourya *et al.,* 2022). Most studies discovered members of the Geobacteracea family in the highest performing groups. The obligate anaerobes responsible for producing electricity in the sediment PMFCs are geobacteracea. In contrast to prior studies, Lu *et al.* (2019) investigated the possibility of the present production employing the C. indica plant in oligotrophic circumstances without the use of external substrates. According to the study, there is a connection between bacteria that digest food and those that are electrochemically active.

2.3.2 Characteristics of Soil

The soil-root consortium is a place where bacteria can live and keeps the connection between microbes and plants going. An effective PMFC would be difficult to create without an understanding of the function of soil (Lin *et al.,* 2021). The electrogenic activities of the soil were reported in the same study, which also examined the performance of three distinct types of soil in a soil MFC. It's interesting to note that 60% of the isolated microbial communities from the anode contained strains that could produce electricity and shared a few basic community characteristics. Different strains produced power 17 times greater than that of MFCs based on forest soil when agricultural soil was used as the inoculum. Power performance in PMFCs is impacted by the physicochemical and biological characteristics of the soil (Sonu *et al.,* 2022). The factors that influence the bacterial community are soil pH, nitrogen availability, soil texture, and soil structure. Inorganic matter in the soil, in addition to organic matter decomposing, can influence the redox potential (Lin *et al.,* 2021).

2.4 Different Design Configurations

The system's affordability, durability, use, and environmental friendliness are all crucial to its sustainability and usefulness. An PMFC's practical application is hampered by construction costs. For instance, the reactor alone accounts for 68.5% of the system cost, followed by the anode and cathode 8.2%, membrane 11%, mediator 1.4%, and collector 2.7%. Although a relatively expensive proton exchange material (PEM) is required, a PMFC can be operated similarly to an MFC by putting the anode and cathode materials in-situ (Kakarla *et al.,* 2019). The uniqueness of a PMFC is that it generates in-situ bioelectricity from the living plants' rhizodeposition. In order to scale up this technology, a number of bottlenecks must be resolved, including an increase in internal resistance, an over-potential during activation, concentration and Ohmic losses, a lack of electrical contact between the bacteria and the anode, etc. (Arun *et al.,* 2020). To increase efficiency, various factors should be taken into account. These include inoculate, the substrate (fuel), the PEM type (and its absence), the cell's internal and external resistance, the solution's ionic strength, the materials used for the electrodes, and the spacing between the electrodes. To identify the constraints on maximizing energy extraction, internal resistances in an MFC and a PMFC were quantified and characterized (Timmers et al., 2012).

2.5 Paddy based PMFCs

When modelling a PMFC, the depth of the anode areas, the size of the electrodes, and the relationship between the anode and the cathode are taken into account. The cathode in a paddy PMFC is often left on the water's surface, and the anode is typically buried 2 to 5 cm beneath the surface. While offering less space between the two electrodes, the soil cathode in a paddy PMFC was examined to absorb the oxygen generated by roots (Chen *et al.,* 2012). It has been observed that the power output from a 5 cm dipped anode was almost three times that of a 2 cm depth anode while examining the elements impacting the electric output from paddy PMFCs. Similar to this, a soil microbial fuel cell showed improved performance when the anode was buried 5 cm in the ground. From these findings, it can be inferred that finding a sufficient anodic zone is crucial for creating anoxic conditions and enabling microorganisms in PMFCs to utilize the released carbon (Takanezawa *et al.,* 2010).

Materials and Methods

The methodology of this experiments was categorized into four steps as listed in the below flowchart diagram.

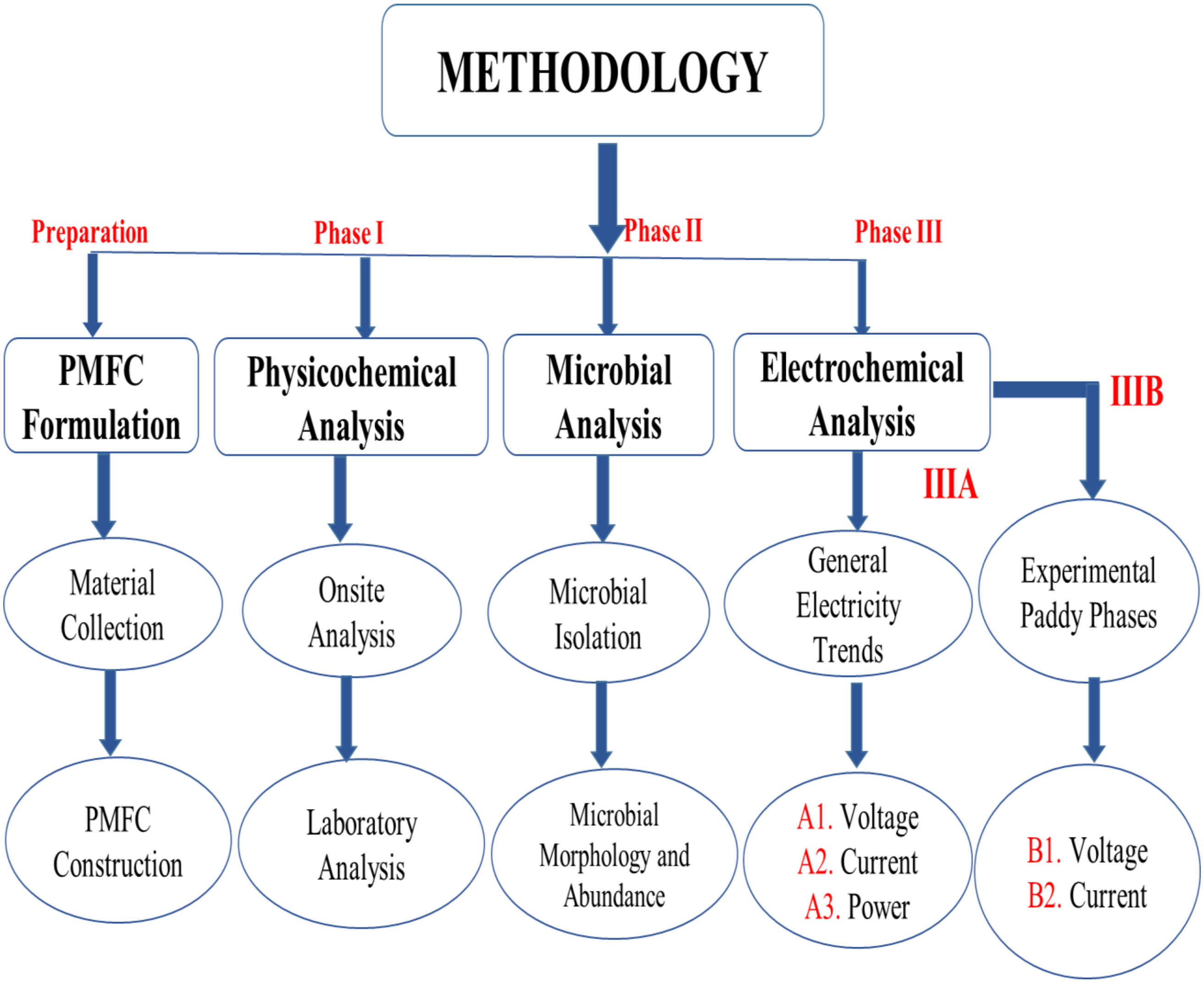

Figure 2: Methodology flowchart

The above mentioned experimental design was employed to achieve study objectives.

3.1 Materials Collection

Soil and seed were used as materials in PMFC formulation. National Agricultural Research Centre (NARC), Islamabad was selected as source to obtain these materials. A comprehensive picture of the sampled area was established after the onsite survey of site besides it aided in determining the sampling locations. Following standard book of soil sampling, soil was sampled in May 2022 from rice fields in rice department of NARC. Sampling was carried out from the upper layer of soil ranging from 0-20 cm depth. 24 sub-samples were collected from uniform agricultural fields of size 3 acre, after wheat harvesting. These sub-samples were combined to make one composite sample. After sampling pre-screening of soil was performed by Coning and Quartering method to obtain laboratory sample. Collected laboratory sample was than air dried and sieved up to 2 mm particle size.

In addition four rice seed (as plant source) varieties were selected on the basis of socio-economic value, water requirement, crop photosynthetic cycle, rhizodeposition and static water-logged conditions. After the pre-screening by seed germination method Super Gold was selected as experimental plant variety for PMFC formulation.

3.2 Sampling Area Map and Sampling points

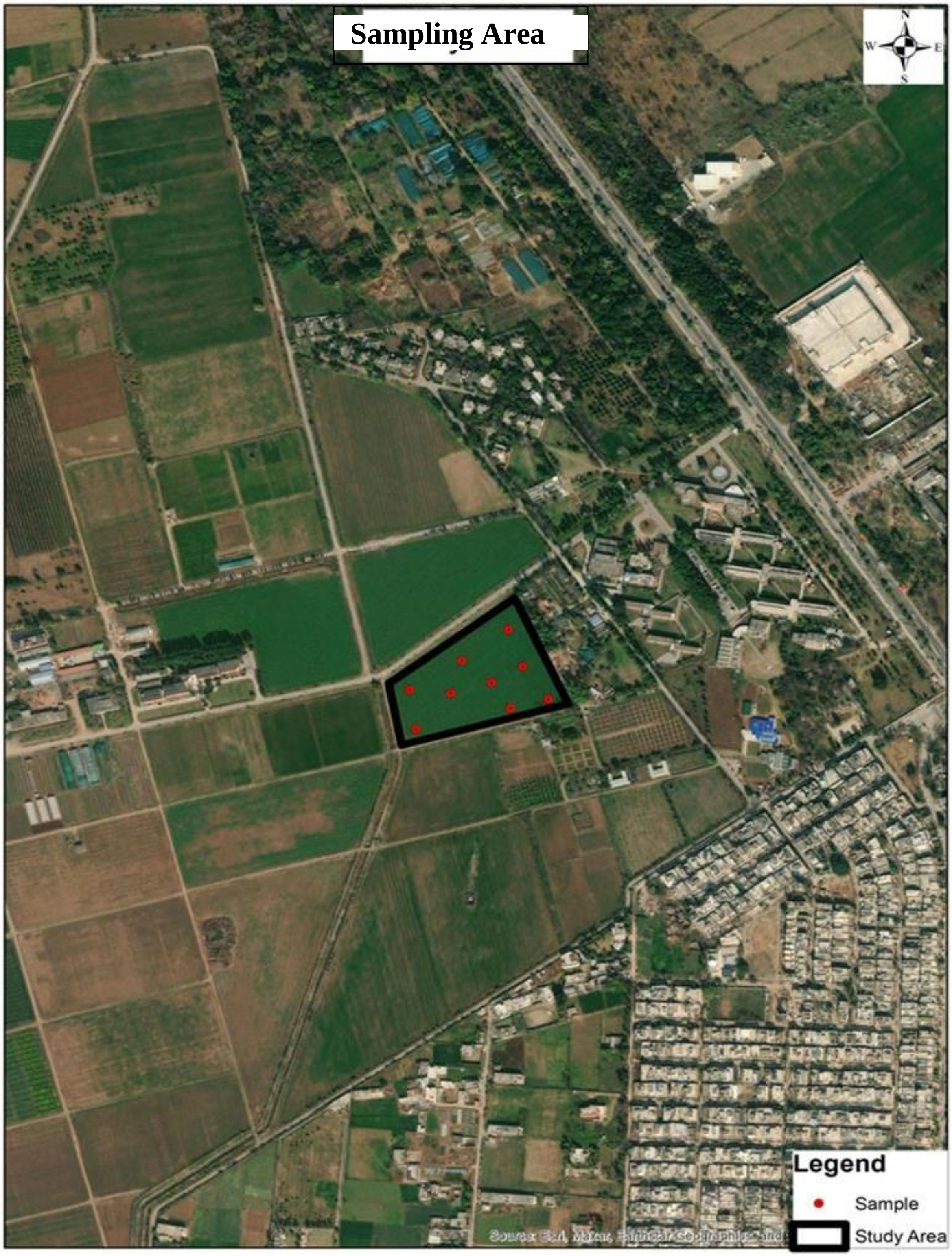

Figure 3: Sampling area map

3.3 Microcosm Setup

Two microcosms were setup in summer and winter seasons. Soil of paddy experimental fields of NARC was used to construct unplanted soil MFCs. Upper soil layer ranging from 0-20 cm was considered as an ideal depth for suitable performing PMFC. Soil coarse debris was removed through sieve the soil. Acrylic containers (25×20×10 cm^3) were used to construct basic model unit for each experiment. Basic functional unit of PMFC was assembled by proper method mentioned in (Regmi et al., 2020). At the bottom small pebbles were placed up to 2 cm to enhance the water circulation through the soil and to support the anolyte collection. Filled with soil up to 12 cm above the pebble layer to provide growth medium for plants. On top of all static water blanket were allowed to stand up to 4 cm. As horizontal design configuration was adopted in the light of previous literature, therefore electrodes (activated carbon graphite sheets of 2 mm thickness) were placed horizontally and a Nafion Proton Exchange Membrane (PEM) NC700 was sandwiched between the two electrodes.

3.4 Construction and Operation of PMFC

Single unit PMFC was constructed for each summer and winter experiments. Basic functional unit was constructed in acrylic container with 5 liter capacity. An activated carbon graphite anode, with length of 150 mm, width of 150 mm and thickness of 2 mm, was embedded horizontally in strictly anoxic conditions and cathode, with length of 150 mm, width of 150 mm and thickness of 2 mm, was submerged in upright position in strictly aerobic conditions. A Proton Exchange Membrane (PEM) of size 170×100 mm was used as partitioner between anode and cathode. Water surface of 4 cm was added above the soil surface. The anode and cathode of each PMFC were connected to electron collectors (wires) leading towards the assembly plate and ultimately attached to digital multimeter (MASTECH 360).

Both PMFCs were operated for 320 days in summer and winter seasons. During summer PMFC experiment was conducted in greenhouse and run for 160 days to evaluate the performance of PMFC. Created electrical potential and daily readings were manually recorded by multimeter during the experimental period. Direct current (DC) was measured in terms of electrical voltage generated, while current, power, power density and electrical density were calculated. Same experiment was conducted in winter in greenhouse. The only difference was the observed impact of meteorological condition in both seasons and overall performance rate of PMFC in each season to satisfy the research objectives.

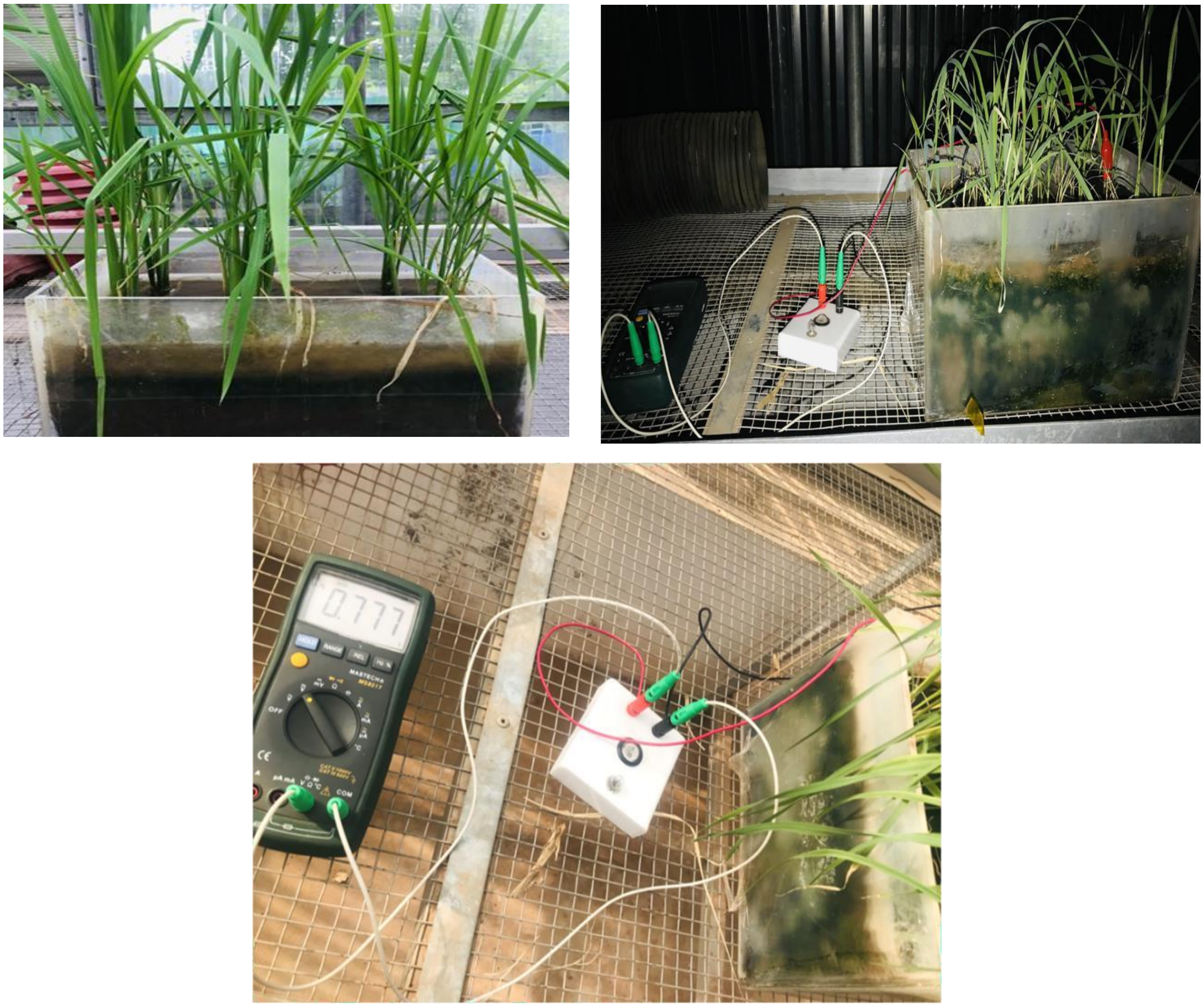

Figure 4: PMFC Construction and Operation

12-18 plant of Oryza Sativa (rice plant) were used per PMFC, transplanted after nursery preparation. Plant growth medium in the form of soil amendments was provided throughout the experiments. Plant-growth amendments (DAP and Urea) were added before the transplantation and gradually increased as the plants' water and nutrients demand increased.

Xing, D. (2008). Electricity generation by rhodopseudomonas palustris DX-1. Environ. Sci. Technol. 42 (11), 4146–4151.

3.5 Measurements and Analysis

Overall PMFC performance efficiency was analyzed in term of voltage, current, power, current density and power density. All five defining terms were monitored against the each experimental growth phase of rice plant. With prominent characteristics of growth phases of rice (Nursery phase, Transplantation phase, Tillering phase, Panicle phase, Ripening phase) fluctuation in these phases was recorded. Analysis of PMFC was categorized into three group of analysis such as, physicochemical, microbial and electrochemical analysis.

3.5.1 Physiochemical Analysis

1 kg of laboratory collected sample of soil was air dried for physiochemical analysis according to the Wu *et al.* (2013). Soil pH and conductivity of soil were analyzed by suspension of soil and water with the potentiometer and conductometer respectively. Humidity in air was calculated by humidity formula while moisture in soil was calculated by oven dry technique (Wu *et al.*, (2013).Total sodium potassium, total nitrogen and organic matter were determined by flame photometry. Mineral estimation was determined by acid digestion of soil samples. Nitrogen, potassium and phosphorus are known as growth enhancers in plants therefore, these factors were critically assessed to find about soil health for plant growth. Physicochemical characteristics of soil sample are listed in Table 1.

Table 1: Physicochemical Characteristics of Paddy Soil

Parameters	Units	Values	Interpretation
pH	-	7.57	Normal
EC	µs/cm	15.00	Less
TS	g/ha	78.78	High
Moisture	%	5.84	Normal
Temperature	°C	25.20	Normal
Nitrogen	Ppm	23.00	Sufficient
Phosphorus	ppm	21.00	Sufficient
Potassium	ppm	18.50	Deficient

3.5.2 Microbial Analysis

Microbial analysis was carried out after the 95 days of operation of PMFC. A part of soil of PMFC was selected and sacrificed for isolation of active electrogenic bacteria. A piece of anode was exposed after opening the soil and separated carefully from the anode. Without disturbing the biofilm at the surface of soil, anode was washed with sterile water several times. Phosphate-buffered saline (PBS) (0.13 M NaCl, 7 Mm Na_2HPO_4, 3 Mm NaH_2PO_4, pH 7.2) (Babu Arulmani *et al.,* 2016). Incubate the washed piece of anode in PBS solution for 16 h. Glass tubes were filled with PBS up to 9 ml and 1 ml suspension was added to these test tubes after 16 h incubation and then standard serial dilution technique was performed. Already prepared and incubated nutrient agar plates were used to spread the dilutions up to one hundred microliters. After streaking, these media plates were incubated again for 24-48 h. Colony counter was used to count the microbial colonies and their morphology was noted down.

3.5.3 Electrochemical Analysis

Precision MASTECH multimeter (MAS 345) was used to monitor the PMFCs. Continuous daily readings were recorded during experimental periods in summer and winter. Both experiments were performed in different climatic condition with same setup, configuration and experimental site. The voltage (*V)* was directly manually measured by digital multimeter. Current (*I*) was calculated by using Ohm's law (*V*=*IR*), where R was supposed to be constant as no external source was provided during the experiments. Power was calculated by the power law (*P=IV*). Power density and current density were determined by dividing the power and current values by the total anode geometric area (Jayaraj *et al.*, 2012).

Chapter 4

Results and Discussions

The results of both summer and winter PMFCs experiments are in coherence with the objectives of the present study. A suitable performing PMFC unit was successfully developed and operated at lab scale in compliance with conventional rice crop cultivation practices. Study demonstrated the possibility of energy generation from rice crop in off season under controlled experimental conditions with slight deviation from rice cropping season experiment. Active electrogenic bacteria-associated to anode were isolated to study their relative abundance and role in power generation rate. Direct current (DC) obtaining possibility was analyzed both experimentally and statistically in accordance with different rice growth phases. Bacterial biofilm at anode provided the relative abundance of microbial community genera considered as potent exo-electrogenic bacteria.

4.1 Site inspection and walkthrough survey

Site inspection and walkthrough survey was important to familiarize with the sampling area and for identification of sampling points according to different types of fields. As per standard book of soil sampling, sampling method, sampling tool and sampling depth may vary with the type of fields. Total sampling agricultural rice field area covered 3 acre facilitating around 110 experimental rice varieties. Below are glimpses of experimental sampling area captured during onsite survey.

Figure: 4.1 NARC Experimental Paddy Fields

4.2 Physicochemical Analysis of Paddy Soil

The physicochemical characteristics of paddy field soil samples collected from NARC experimental fields were analyzed and summarized in Table 1. Analytical parameters including pH, electrical conductivity, total solids, temperature of soil, moisture content of soil, nitrogen, phosphorus and potassium were analyzed to interpret the health of soil for plant growth. The pH, temperature and moisture content was found to be in normal range while total solids were in ample quantity. Contrary to this electrical conductivity of soil was less in comparison to normal rice field EC but normal in terms of EC of soil itself. Among the growth enhancers nitrogen and phosphorus was sufficiently present in soil to support the plants whereas potassium content was in deficient amount to support the growth of plant. According to these results additives amendments were made to ensure the availability of healthy growth medium before the transplantation of nursery.

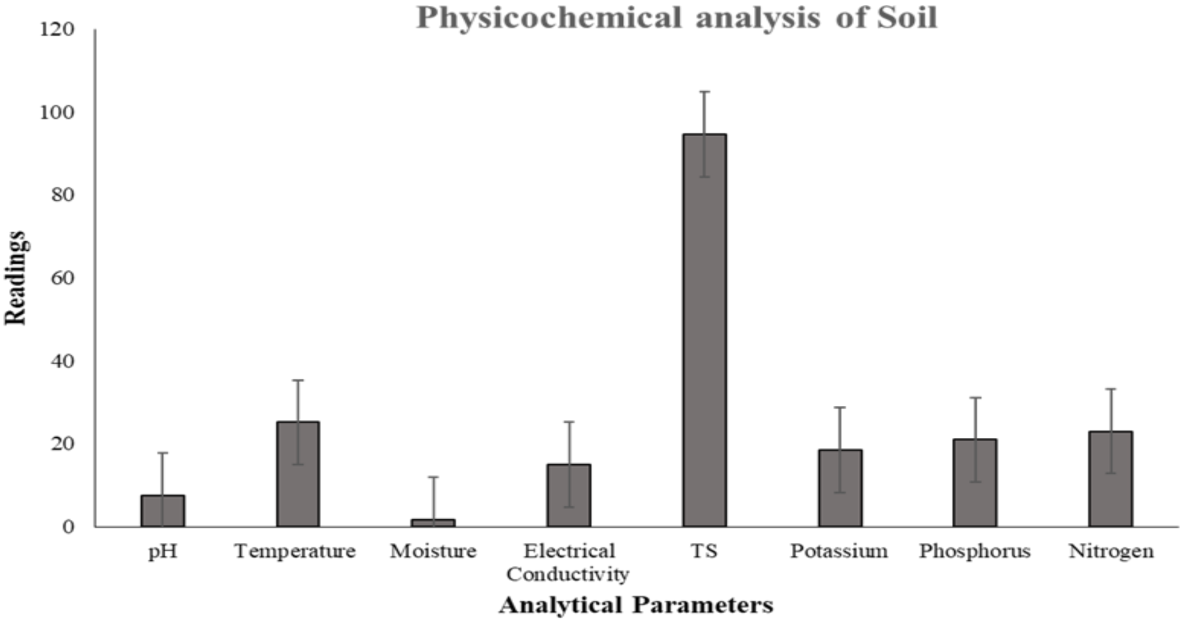

Figure: 4.2 Graphical Representation of Physicochemical Parameters of paddy soil

4.3 Microbiological Analysis of PMFC

After the 95 operational days of PMFC to assess microbial profile, microbial analysis was performed. A total of 13 different bacterial communities strains were isolated from anode sample. Biological morphology and relative abundance was determined by using colony counter. Among these 13 isolates, 3 were present in abundance at the surface of the anode therefore, these microbial colonies were considered as dominant species. Their relative abundance at anode surface as biofilm indicates that these exo-electrogenic bacteria-associated genera are an active group of bacteria actively involved in electricity generation in PMFC. More number of dominant bacterial species closely linked to the rate of electricity generation in PMFC. Complete morphology and abundance is assimilated in Table 2.

Table 2 Microbial Communities Isolated from PMFC

Species	**Shape**	**Elevation**	**Color**	**Margin**	**Texture**	**Size**	**Opacity**	**Number**
Specie A	**Circular**	**Raised**	**Transparent**	**Regular**	**Slimy**	**Medium**	**Transparent**	**78**
Specie B	Irregular	Raised	Milky White	irregular	Slimy	Large	Transparent	50
Specie C	Circular	Raised	Yellow	Regular	Slimy	Small	Opaque	50
Specie D	Circular	Raised	Transparent	Regular	Slimy	Large	Transparent	25
Specie E	Circular	Raised	White	Regular	Slimy	Medium	Transparent	29
Specie F	Irregular	Raised	Yellow	Irregular	Slimy	Small	Opaque	17
Specie G	Circular	Raised	Transparent	Regular	Slimy	Medium	Transparent	39
Specie H	Irregular	Raised	Milky White	irregular	Slimy	Large	Transparent	17
Specie I	**Circular**	**Raised**	**Yellow**	**Circular**	**Slimy**	**Medium**	**Opaque**	**69**
Specie J	Circular	Flat	Orange	Circular	Dry	Small	Opaque	14
Specie K	**Circular**	**Flat**	**Transparent**	**Regular**	**Slimy**	**Medium**	**Transparent**	**52**
Specie L	Irregular	Raised	Milky White	Irregular	Slimy	Large	Transparent	13
Specie M	Circular	Raised	Yellow	Circular	Slimy	Medium	Opaque	10

4.4 Electrochemical Analysis of PMFC

The Summer experiment was carried out in Greenhouse, during rice cropping season from May to August and winter experiment was carried out in Greenhouse, from October to January. The plants in both summer and winter PMFCs kept growing during the experiments period, allowing to acquire data for 320 days based on the performance of PMFC cell throughout rice growth phases. The performance of both PMFCs were evaluated in voltage (*V*), current (*I*), power (*P*), current density and power density. Similar electricity generation from PMFC in rice cropping season was reported in literature (Kumar *et al.*, 2020).

4.4.1 Voltage Produced in PMFCs

At the start of each experiment very less voltage was produced due to less potential difference created between the cathode and anode. The voltage gradually increased as the plant grow in size and number. It shows the close link between the rate of photosynthetic activity carried out by the pre-mature to mature plants, number of roots exudates associated with plant growth, bacterial rhizodeposition in rhizosphere zone of plants.

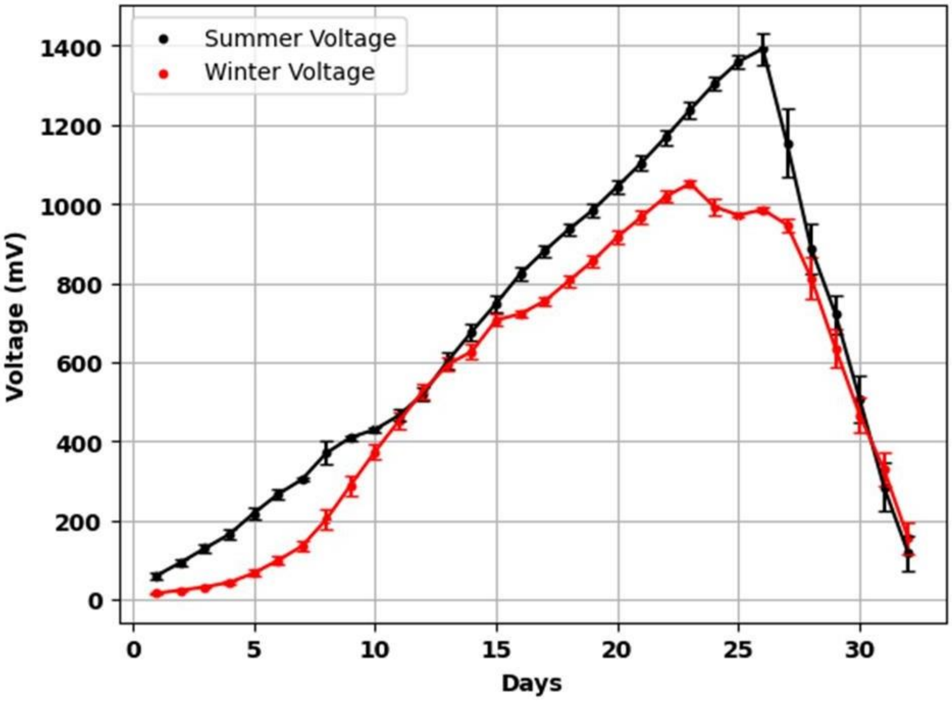

Figure 4.3 Voltage Curve of PMFCs generated in Summer and WinterExperiments

The peak voltage generated in summer and winter PMFCs was 1436.6 mV and 1063.5 mV respectively. The voltage produced in summer was 40% more than the voltage produced in winter. The root exudation was least at the beginning of the experiment, a gradual increase with plant growth till 135 days was observed followed by the subsequent decrease till the end of experiment.

4.4.2 Current Generated in PMFCs

Current is the flow of negatively charged ions (electron) therefore, current generation rate is depend on the rate of electron released as a result of oxidation reaction in PMFC. Current generation trend is almost similar to voltage trend because electron gradient initiated with potential gradient and electron stream began to flow PMFC. Factors affecting the voltage will almost have the similar impact on the current.

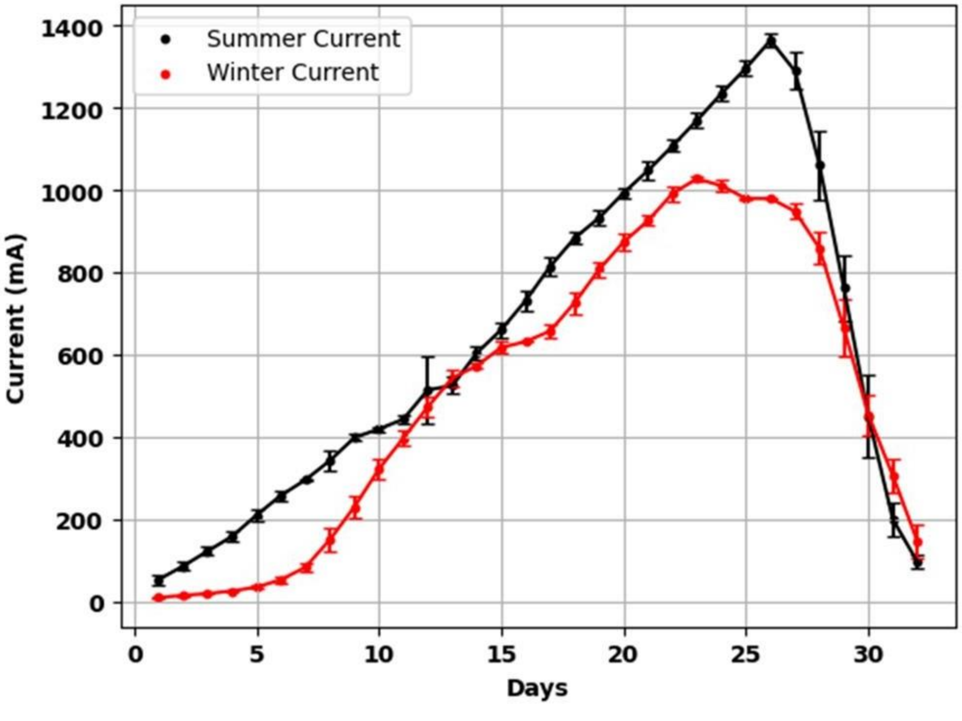

Figure 4.4 Current Curve of PMFCs produced in Summer and Winter Experiments

The maximum current generated in summer and winter PMFC was 1376.6 mA and 1037.6 mA, respectively. The current generated in summer was 30% higher than the current generated in winter. Minimum current at the beginning, maximum after the midseason and nearly equals to the initial rate at the end of the experiments was recorded.

4.4.3 Power Generation in PMFCs

Power curves were drawn against the product of voltage and current as per power law. As power is the amount of energy transferred or converted per unit time. In PMFCs power is the amount of electrons released transferred to cathode via electron collectors in unit time. There is clear depiction that power generation rate is also dependent on the amount of electrical potential created and flowed.

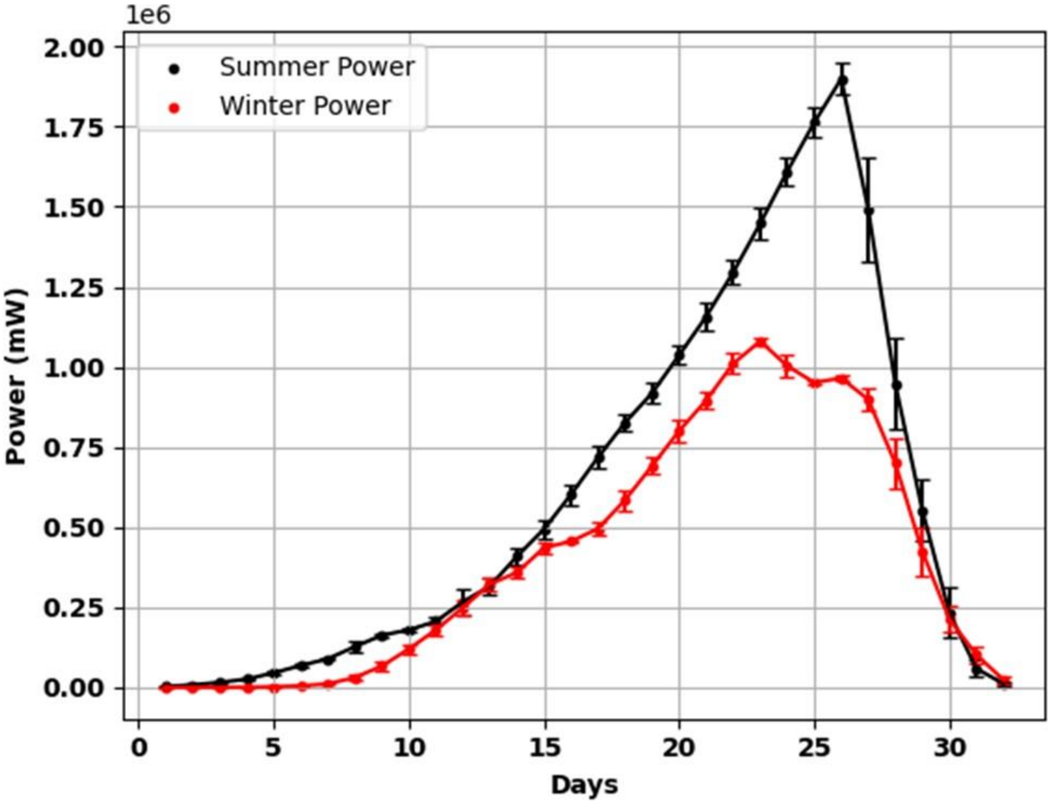

Figure 4.5 Power Curve of PMFCs produced in Summer and Winter Experiments

Summer and Winter PMFC was 1977.6 mW and 1103.4 mW, respectively. The power generated in summer was almost 44% greater than power generated in winter. Regular increase in power generation continued after mid-season from where drop in power till the end of the experiment was observed.

4.5 Experimental Rice Phases

4.5.1 Voltage Produced in Summer and Winter Nursery Phase

Nursery phase is the first stage of rice growth. Results of Summer and Winter experiments in nursery phase recorded separately. The voltage generated at the beginning is regarded as paddy soil voltage due to existing microbial activities. With seed germination at optimum temperature it increased gradually. In first 15 days of Summer experiment after the sowing electric potential generated at slow pace and right after it a delayed rise in voltage curve was observed due to emergence of first leaf (a folded leaf structure). The electric gradient rise as the plant grow from V1 to V4 (one leaf to four leaves) and roots developed up to one main root trunk with sub out roots. Seed germinated and very less roots developed at this stage. Voltage showed increasing trend over month. The maximum voltage generated in nursery phase during Summer experiment was 280.1 mV.

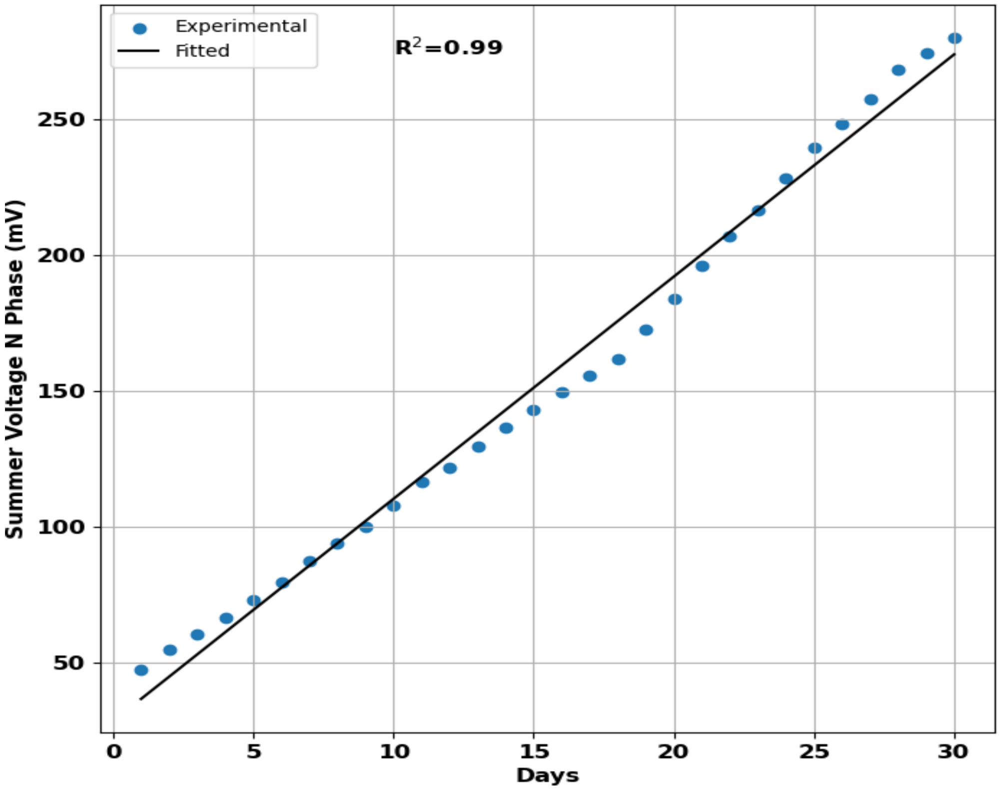

Figure 4.6 Voltage produced in Nursery phase of Summer Experiment

In Winter experiment the climatic conditions were different as compared to Summer experiment. Overall temperature was relatively low therefore, delay in germination of seed was observed. Voltage generation rate in Winter was comparatively low. In the absence of optimum temperature decrease in microbial activities was recorded which directly linked to the formation of potential gradient. Late Seed germination and roots developed at this stage. Voltage showed the delayed rise over the month. The maximum voltage generated in nursery phase during Winter experiment was 111.3 mV which is 60% less than the Summer voltage.

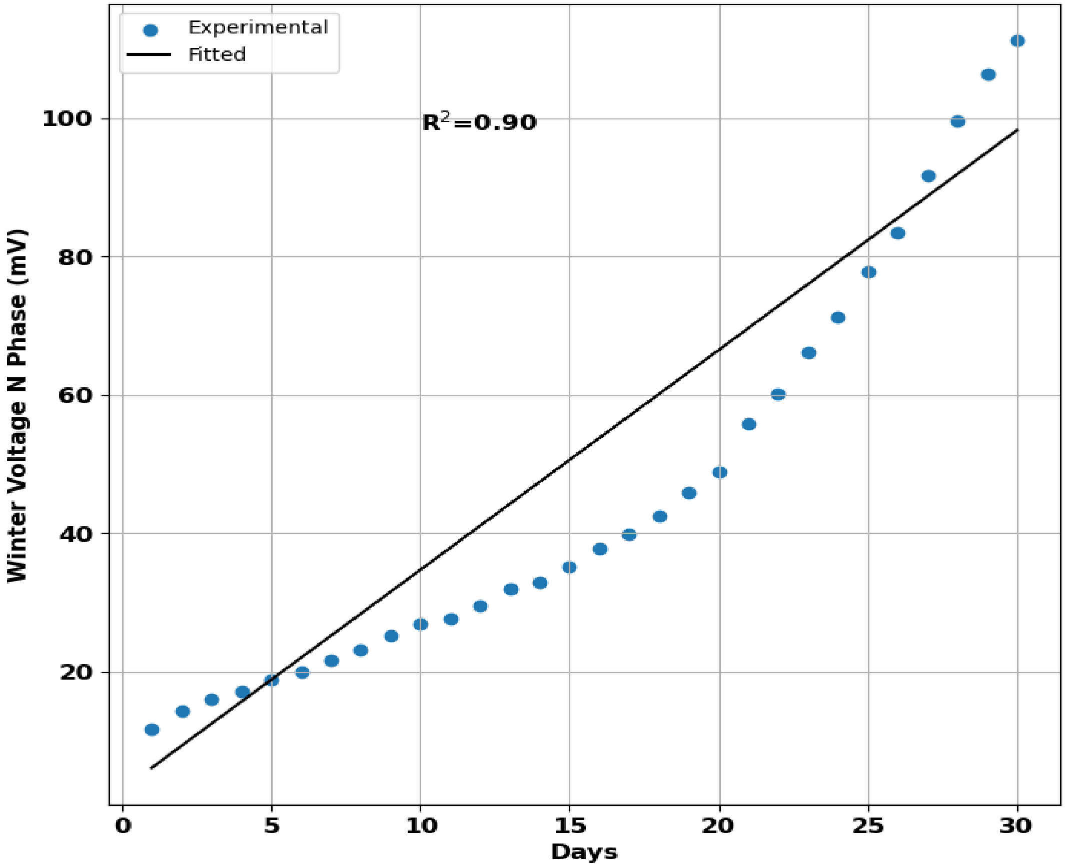

Figure 4.7 Voltage produced in Nursery phase of Winter Experiment

4.5.2 Voltage Produced in Transplantation Phase

The results of each Summer and Winter experiment in transplantation phase was recorded independently. Transplantation is the second phase of rice crop life cycle. Its V5 to V8 stage which means leaves continue to multiply till eight which directly associated to plant growth, root development, photosynthetic and microbial activity. In Summer experiment Voltage generation interrupted for one week after transplantation of nursery. This period refers to plant adjustment to new growth medium in which microbial activity sustained for few days. After the dormant period discontinuous voltage generation was observed. Voltage showed the irregular rise over the phase. The maximum voltage generated in transplantation phase during Summer experiment was 547.1 mV.

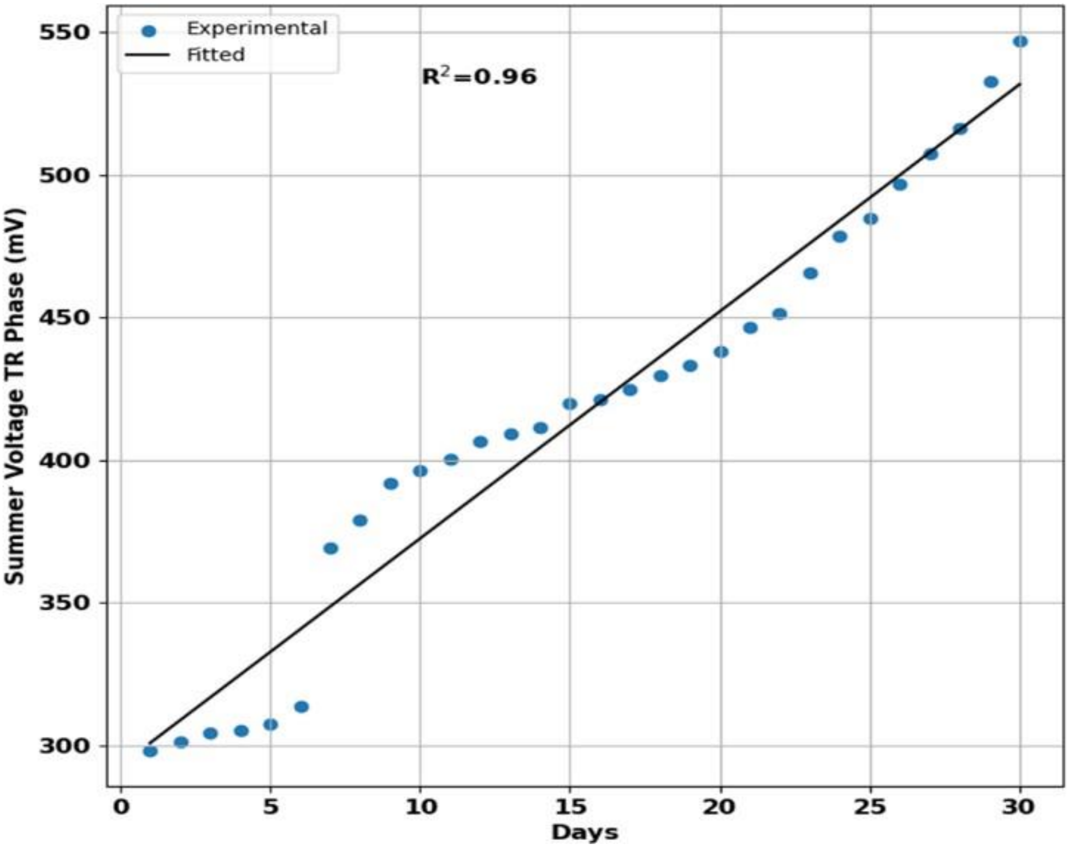

Figure 4.7 Voltage produced in Transplantation phase of Summer Experiment

In Winter experiment transplantation from one medium to another growth medium was avoided to maintain consistency in controlled conditions of greenhouse. The continuous rise in voltage was observed with continuous increase in root exudation. High photosynthetic activity resulted in high potential gradient. Voltage showed increasing trend over the month. The maximum voltage generated in transplantation phase during Winter experiment was 538.7 mV which is nearly equals to Summer voltage.

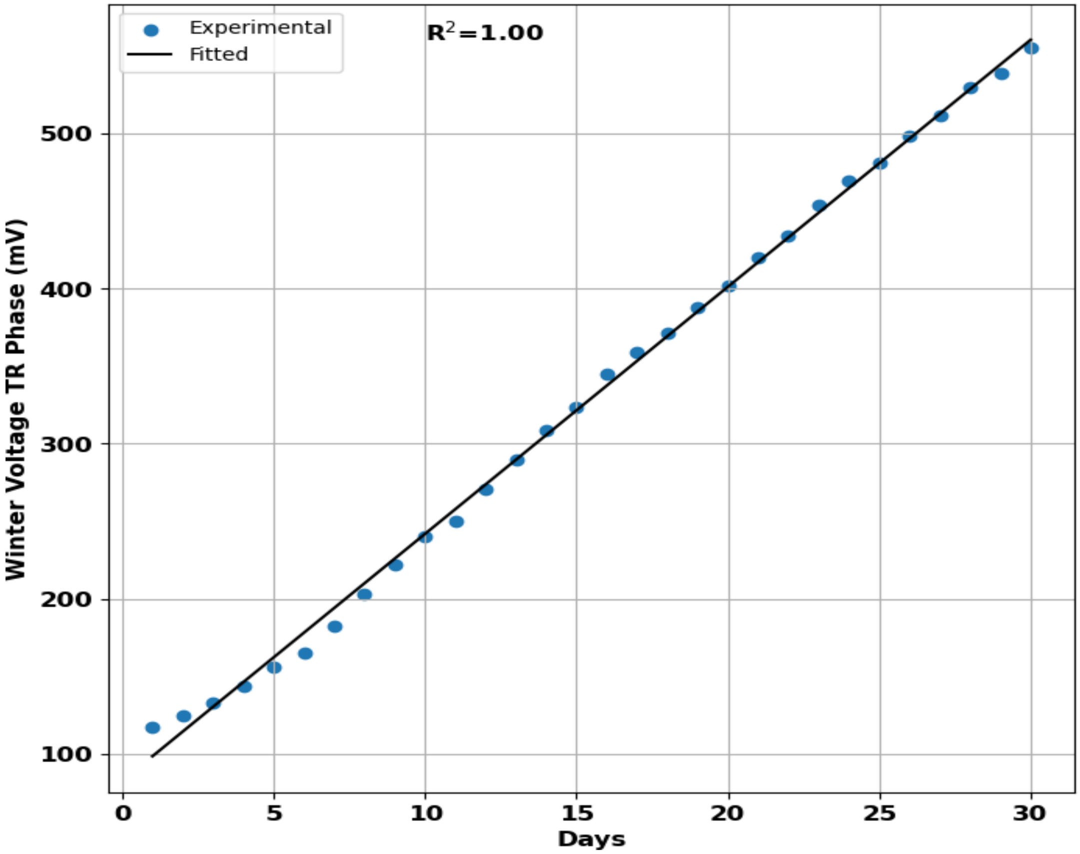

Figure 4.8 Voltage produced in Transplantation phase of Winter Experiment

4.5.3 Voltage Produced in Tillering Phase

Independent reading were recorded for each Summer and Winter experiment respectively in this phase. Tillering phase is the third phase of paddy crop cycle. It is reported as most active and important phase of cycle because major developments occurred in it. First tiller appeared at the beginning of phase along with transformation of V8 to V13 stage. High rhizodeposits in root zone of plants with maximum root development. In Summer experiment this is the most crucial phase in terms of energy generation due to high microbial activity, high root exudation. Voltage showed continuous sharp rise over this phase. The maximum voltage generated in tillering phase during Summer experiment was 984.6 mV.

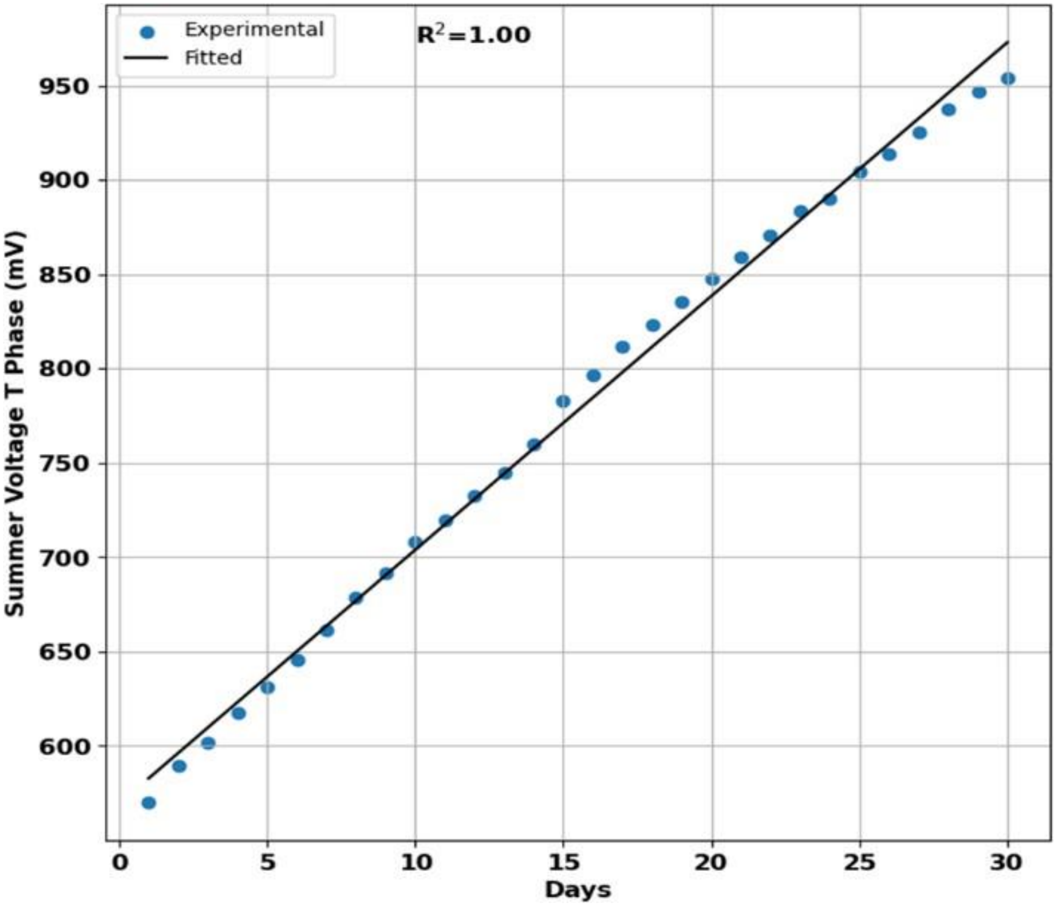

Figure 4.9 Voltage produced in Tillering phase of Summer Experiment

In Winter experiment during first 15 days voltage generation was uneven and irregular due to fluctuation in climatic conditions. Heavy rainfalls was experienced during these days leading to temperature drop from optimum level and suspension of remaining activities. For the first 15 days irregular pattern of voltage while the last 15 days almost smooth uprise trend was observed. The maximum voltage generated in tillering phase during Winter experiment was 856.3 mV which is 14% less than Summer voltage.

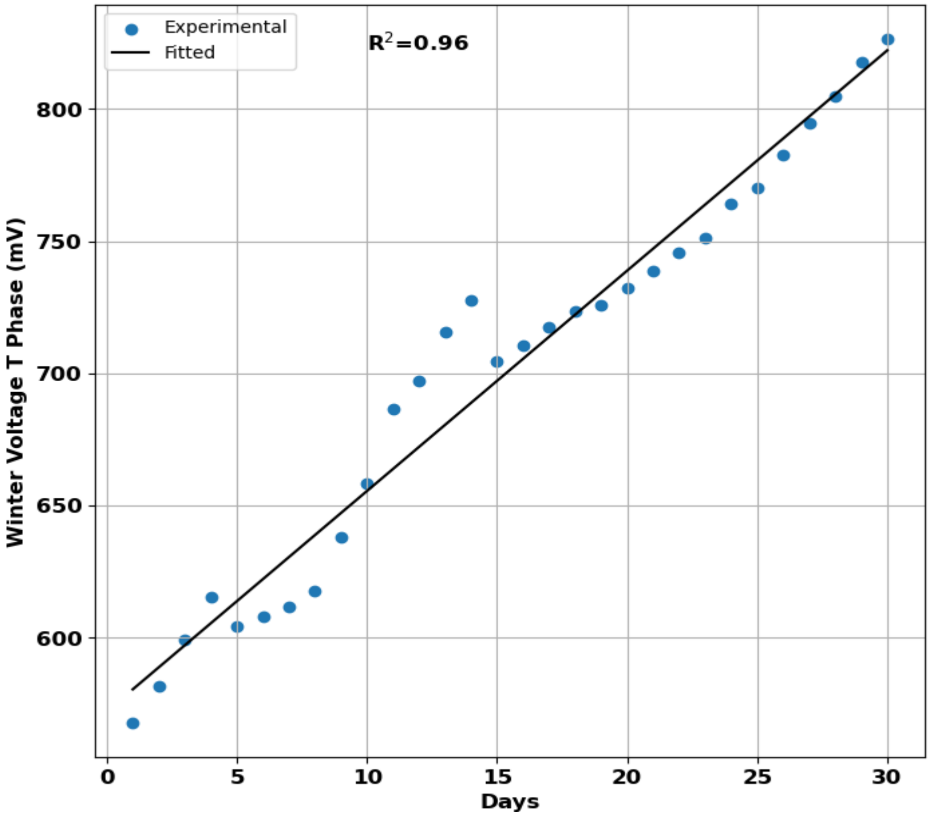

Figure 4.10 Voltage produced in Tillering phase of Winter Experiment

4.5.4 Voltage Produced in Panicle Phase

The results for Summer and Winter experiment were recorded separately for each phase. Panicle phase is the fourth phase of crop cycle. Plant attained maximum tillers and panicle showed appearance and visibility at later. Flowering matured and grain formation reached at mid. Phase of highest energy harness, peak voltage, maximum root exudation, top microbial activities and maximum photosynthetic process. In Summer experiment it attained as the most active phase in terms of energy generation in relation to peak in all driving factors. The maximum voltage attained during this phase. Voltage showed the continuous sharp lift during this phase. The maximum voltage generated in panicle phase during Summer experiment was 1436.6 mV.

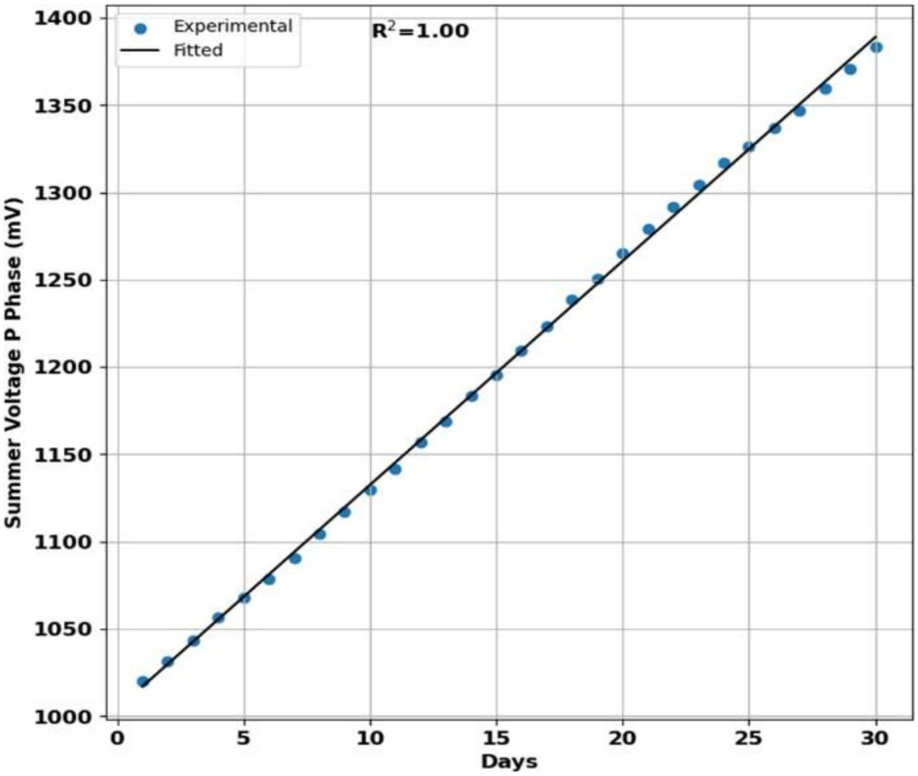

Figure 4.11 Voltage produced in Panicle phase of Summer Experiment

The measurements of Winter experiment was in the same order with summer PMFC with slight variations. Voltage generation achieved in smooth curve in first 18 days of the phase due to attained optimum condition. Heavy rainfall shorts were experienced due to abrupt change in climatic conditions resulting in sudden drop in temperature that ultimately slowed the ongoing power activities and cell faced an average decline in voltage lasting 25 days. Slight increase at 26 day was observed. For the first 18 days up right trend of voltage while the last 12 days declining trend with fluctuation was observed. The maximum voltage generated in panicle phase during winter experiment was 1063.5 mV which is 26% less than the summer voltage.

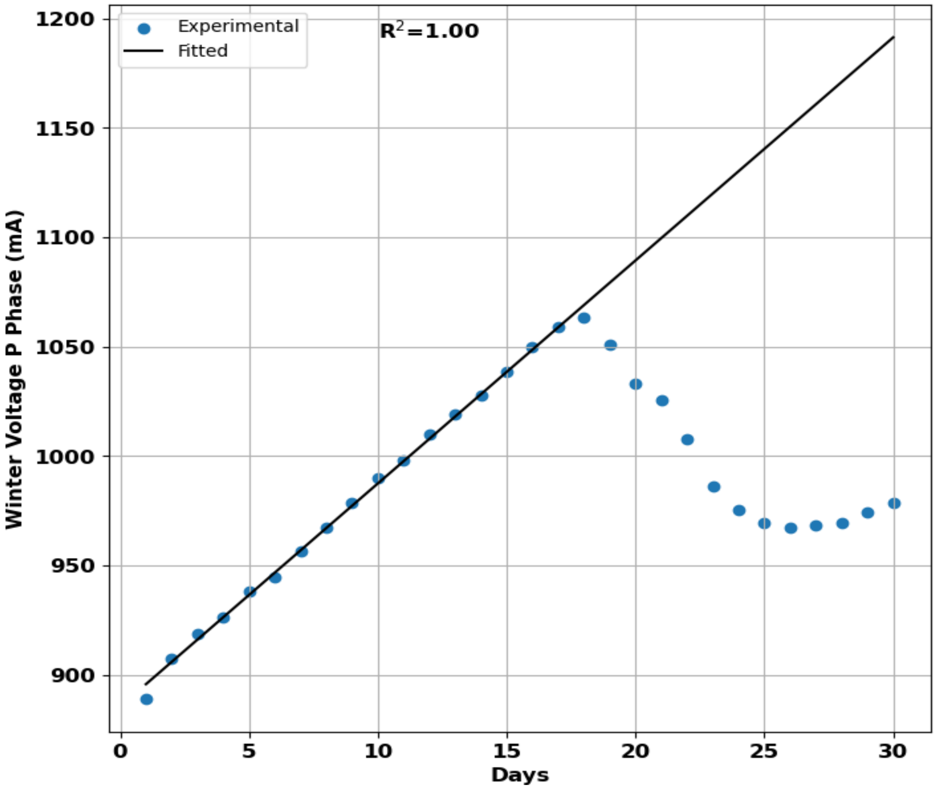

Figure 4.12 Voltage produced in Panicle phase of Winter Experiment

4.5.5 Voltage Produced in Ripening Phase

In the fifth and last phase of crop cycle daily independent readings were measured. Subsequent decline in all power generating factors was noticed. Reduction in rate at the beginning of phase and nearly stoppage at the end of phase was experienced. Grain fully matured and ready to harvest at this stage which indicate no further requirement of photosynthetic process. In summer experiment decrease in voltage started at the onset of last phase and continue till the end of phase. Voltage showed sharp declining trend till the end of phase. The minimum voltage attained at the end of ripening phase during summer experiment was 54.2 mV.

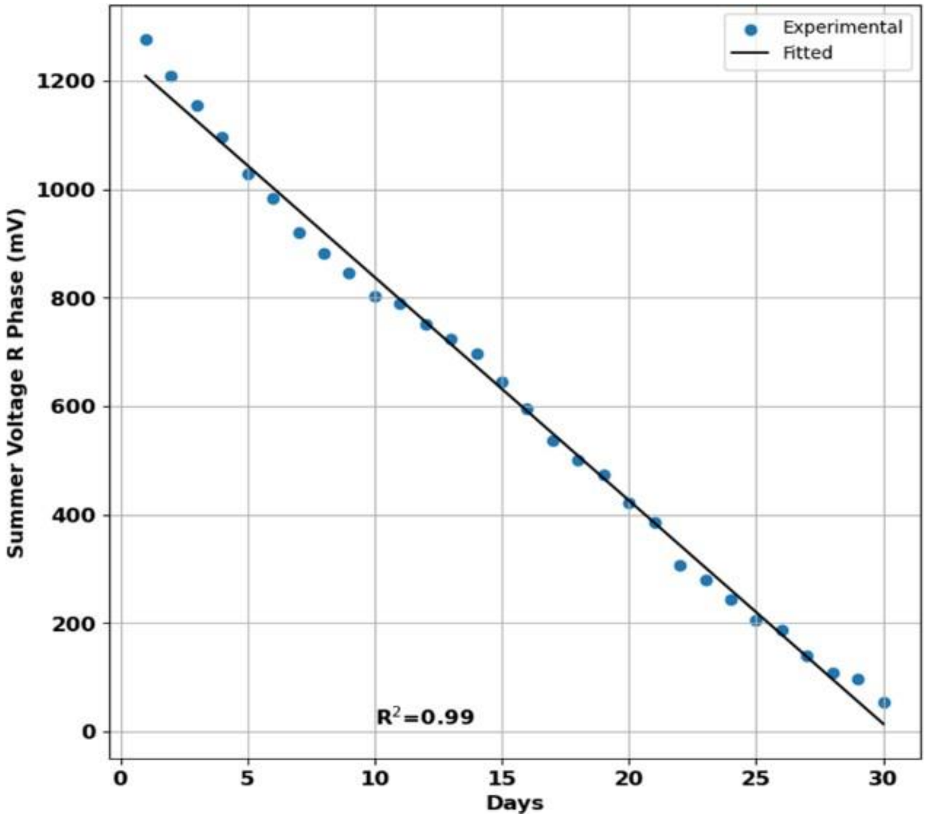

Figure 4.13 Voltage produced in Ripening phase of Summer Experiment

Similar to Summer experiment all essential activities were at minimum to produce a handsome amount of voltage. Ripening phase in Winter experiment showed nearly similar voltage trend to summer voltage trend due to more similar prevailing climatic conditions, provided in greenhouse. But it only differ in total voltage produced during winter is quite less than the Summer voltage. Decline in voltage started with the onset of last phase and reached to minimum level till the end. The minimum voltage attained towards reaching the end of ripening phase during Winter experiment was 100.3 mV which was 46% greater than the Summer voltage.

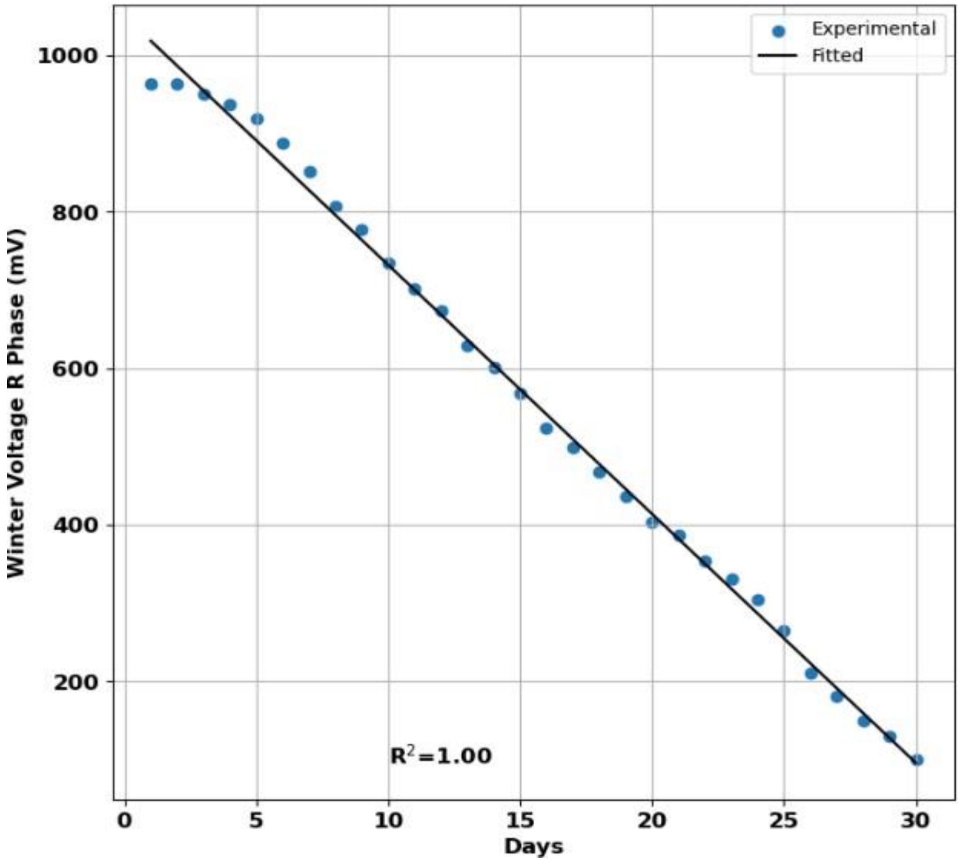

Figure 4.14 Voltage produced in Ripening phase of Winter Experiment

4.5.6 Current Generated in Nursery Phase

As the definition, current is the flow of ordered directional electrically charged particles (electrons). Such flow of electrons is termed as electricity flow in that specific circuit. In coherence with voltage, current produced in nursery phase was very less. All essential pre-requisite activities occurred were noticed like seed germination, coleoptile formation and first seminal root emergence occurred. Therefore, very few electrons released into the soil leading to low current flow. In summer experiment after emergence of primary roots little root exudation was noticed which means that less electron released and minimum current flow. Current showed sharp increasing trend over the month. The maximum current generated in nursery phase during summer experiment was 272.7 mA.

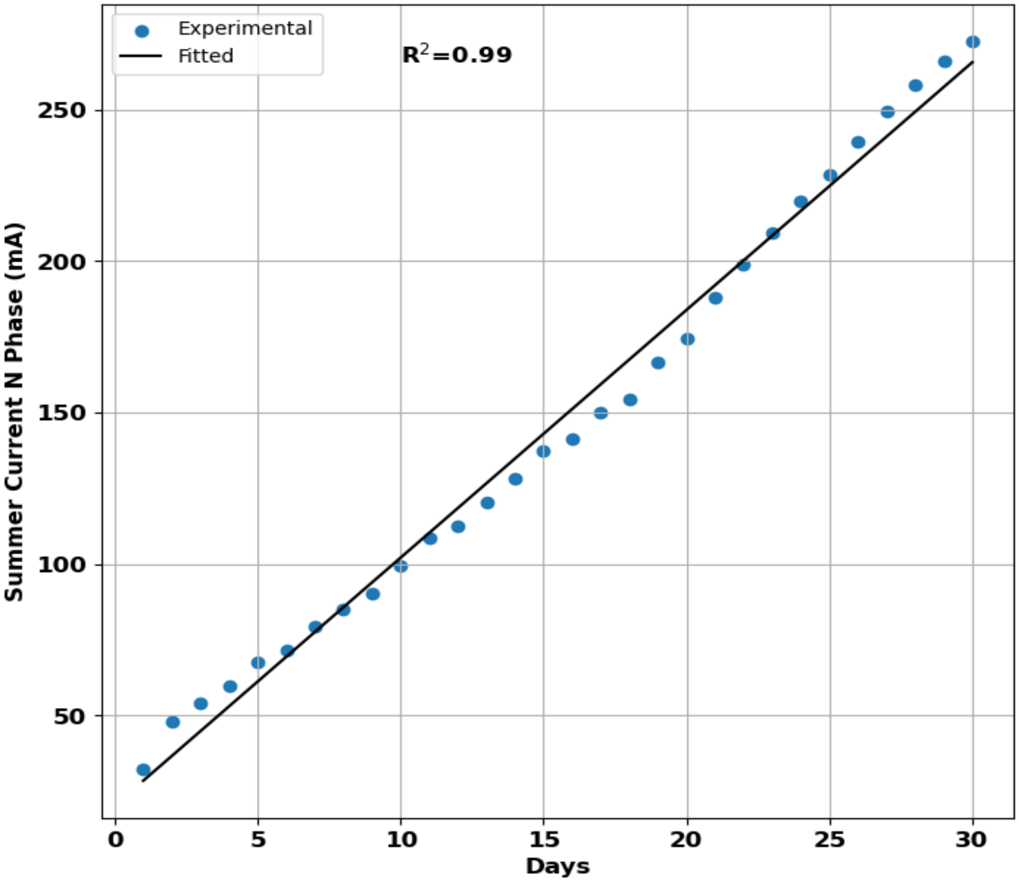

Figure 4.15 Current produced in Nursery phase of Summer Experiment

In comparison to Summer current, current generated in Winter experiment was almost insignificant due to insignificant driving factors rate at this stage. Climatic conditions such as less solar radiance, short day time, relatively cold temperature and change in water temperature affected the overall generation rate of PMFC in winter. Generally delayed increased was observed in all essential activities linked to efficiency of cell. Less electron flow means very little current in the cell. Current showed delayed increasing trend over the month. The maximum current generated in nursery phase during Winter experiment was 62.1 mA which is only 22.7% of summer current.

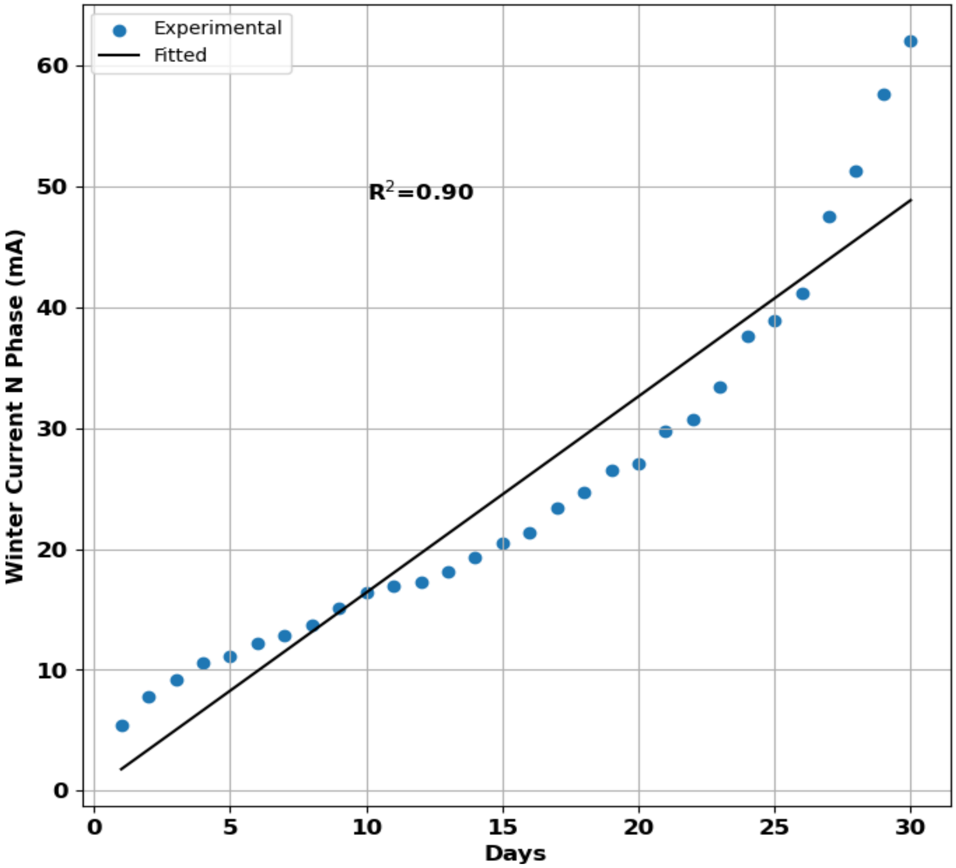

Figure 4.16 Current produced in Nursery phase of Winter Experiment

4.5.7 Current Generated in Transplantation Phase

Potentially gained voltage in transplantation phase was documented in Summer and Winter experiment respectively. Similar to Summer voltage, current breakdown was occured after the transplantation of nursery in crop rotation. After the adaptation of transplanted plants into the new growth medium, little rise in flux to almost constant electron stream flow was maintained from mid until completion of phase. Resultantly current flow in a complete circuit was logged. Current supply discontinue for one week after transplantation due to no potential. Current showed regular rising curve over this phase. The maximum current generated in transplantation phase during summer experiment was 483.5 mA.

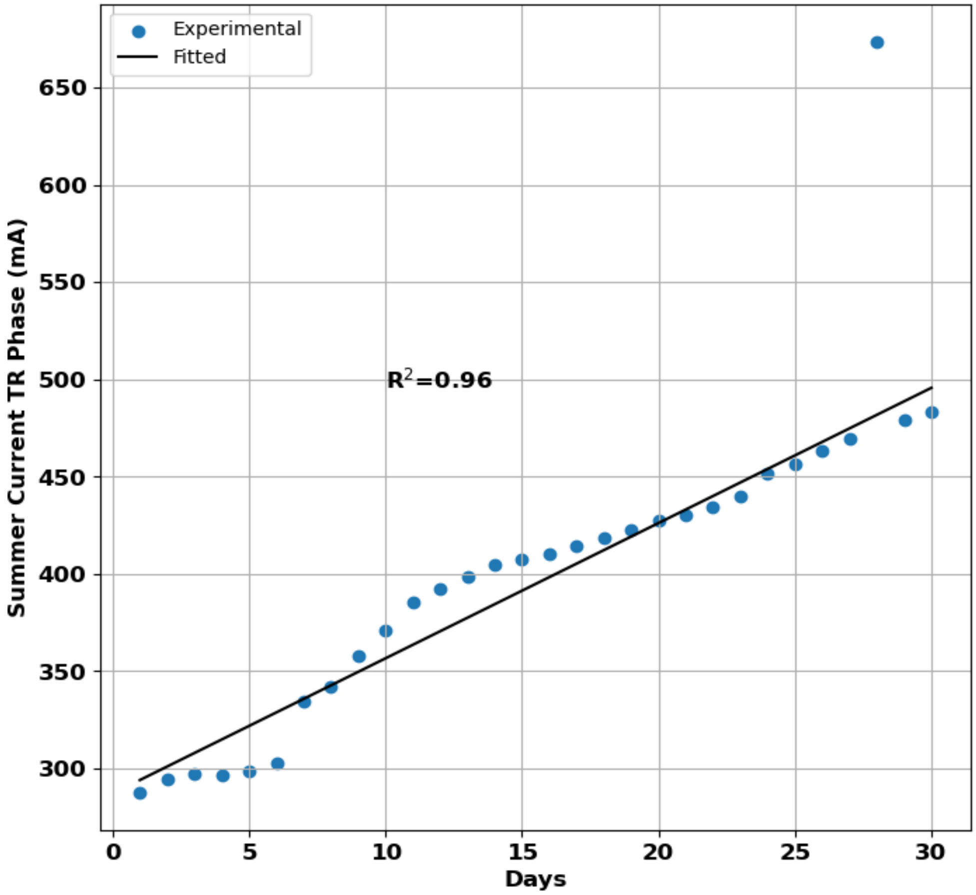

Figure 4.17 Current produced in Transplantation phase of Summer Experiment

The results collected in Winter experiment were slight deviate from normal trend followed in summer experiment. An outlier was logged, it could be due to any natural or anthropogenic factor involved while noting the observations. There was continuously flowing electron stream as a result of continuous voltage generation across the electrodes. Continuity in electron release leads to almost equal amount of current to flow in PMFC. Electron flow was irregular at the start of the phase. Current showed rising curve over this phase. The maximum current generated in transplantation phase during winter experiment was 475.8 mA which is nearly equals to the current produced in summer experiment.

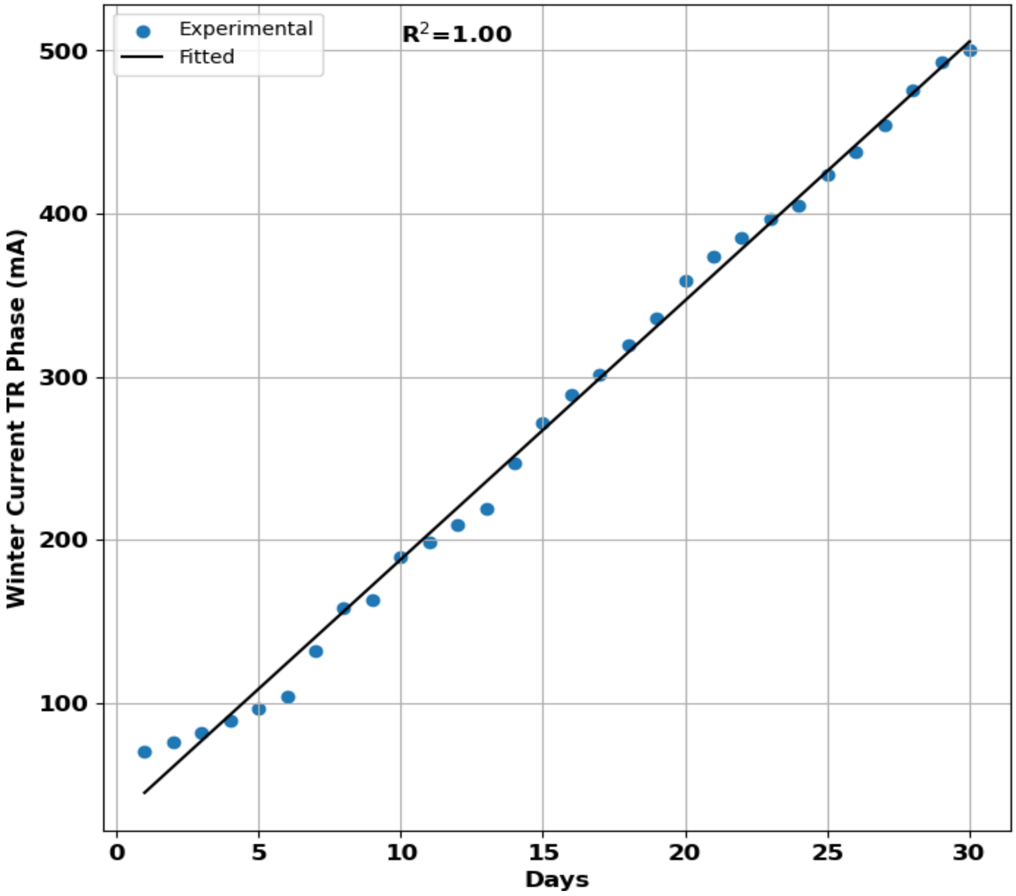

Figure 4.18 Current produced in Transplantation phase of Winter Experiment

4.5.8 Current Generated in Tillering Phase

Root elongation and horizontal-vertical root distribution along with tiller formation was noticed in this phase. The current generated in tillering phase is proportional to the occurring developments in plant and roots. Rate of oxidation process defined by the number of root exudated and relative microbial abundance. In summer experiment high electron presence was detected leading higher current generation in PMFC. Current showed sharp uprise curve over this phase. The maximum current generated in tillering phase in summer experiment was 931.3 mA.

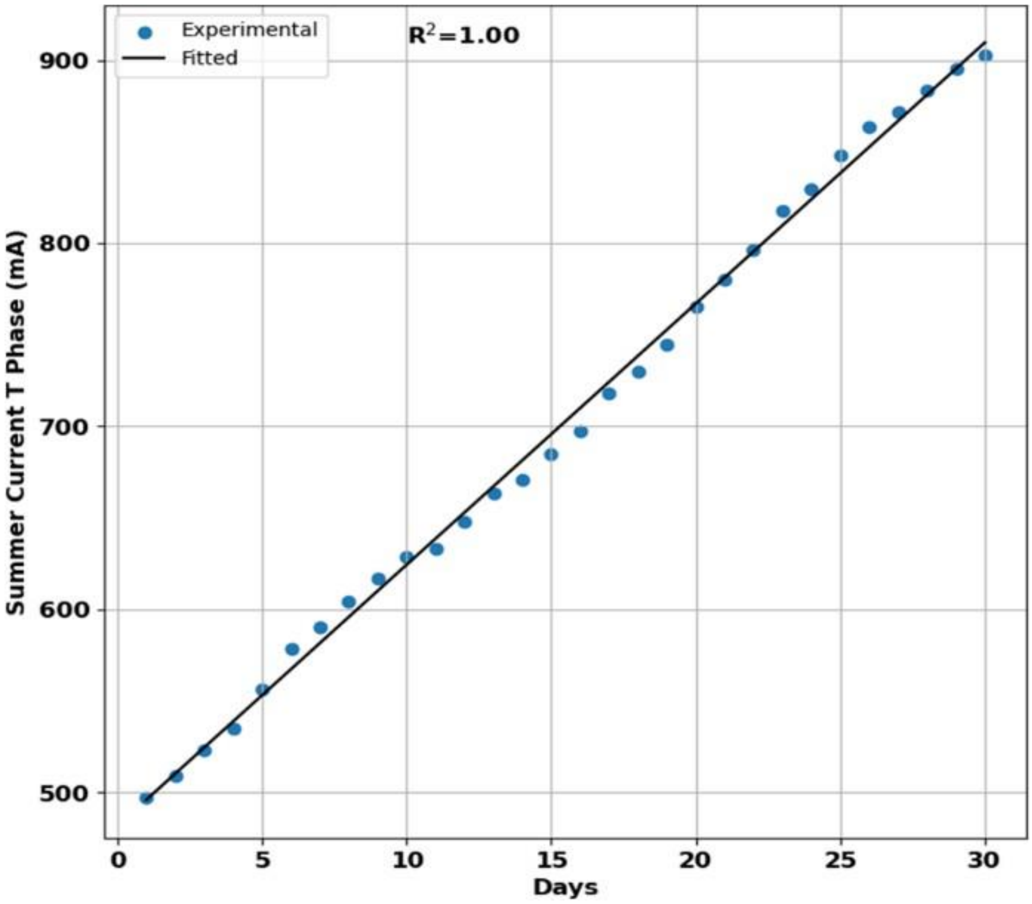

Figure 4.19 Current produced in Tillering phase of Summer Experiment

Seasonal oscillation in current generation was reported in Winter experiment. This was attributed to the difference in rhizodeposition during Summer and Winter. This shows the influence of climatic variability with season variation. During Winter experiment current showed irregular increasing curve over the tillering phase. Interruptions occurred with overall change in prevailing conditions. The maximum current generated in tillering phase during winter experiment was 806.7 mA which is only 86.6% of summer current.

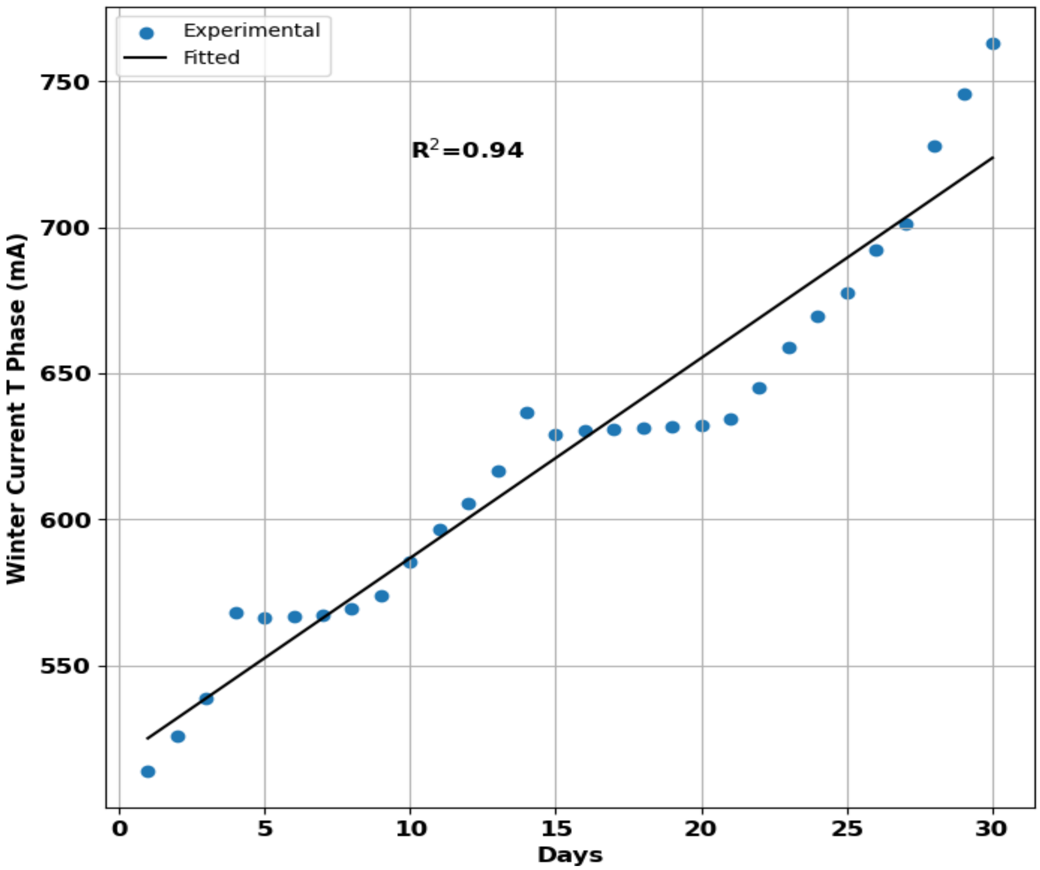

Figure 4.20 Current produced in Tillering phase of Winter Experiment

4.5.9 Current Generated in Panicle Phase

The current generated in panicle phase revealed high generation rate as compared to other phases. The trend was similar to voltage trend in panicle phase due to direct relationship between the potential gradient and electric potential. The improved and efficient electron flow was observed with highest generation rate. Highest root exudation means highest electron released and maximum current flow. Current showed sharp increasing trend over this phase. The maximum current generated in panicle phase during Summer experiment was 1376.6 mA.

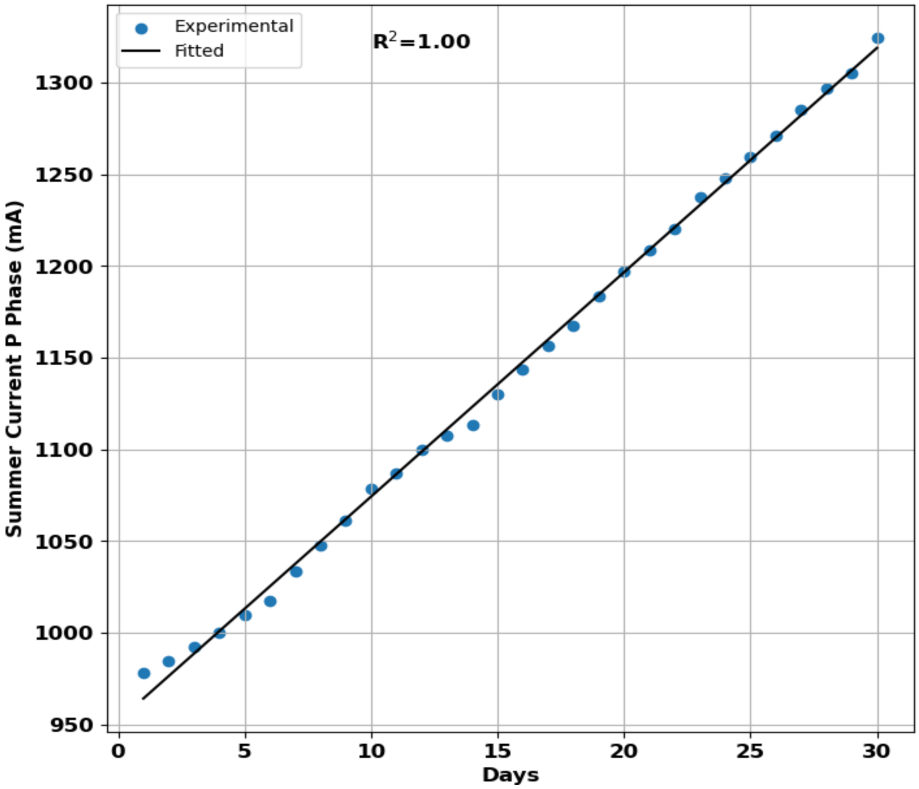

Figure 4.21 Current produced in Panicle phase of Summer Experiment

The results obtained in Winter experiment re-established the impact and inter-dependence of current generation and the growth phase of paddy crop. Similar to voltage, current rate increased till the mid of phase and afterwards, a dip with irregular fluctuations was observed in second half period of panicle phase. First 18 days increasing trend of current showed while the last 12 days declining trend with fluctuation was observed. The maximum current generated in panicle phase during Winter experiment was 1037.6 mA which is 75% of Summer current.

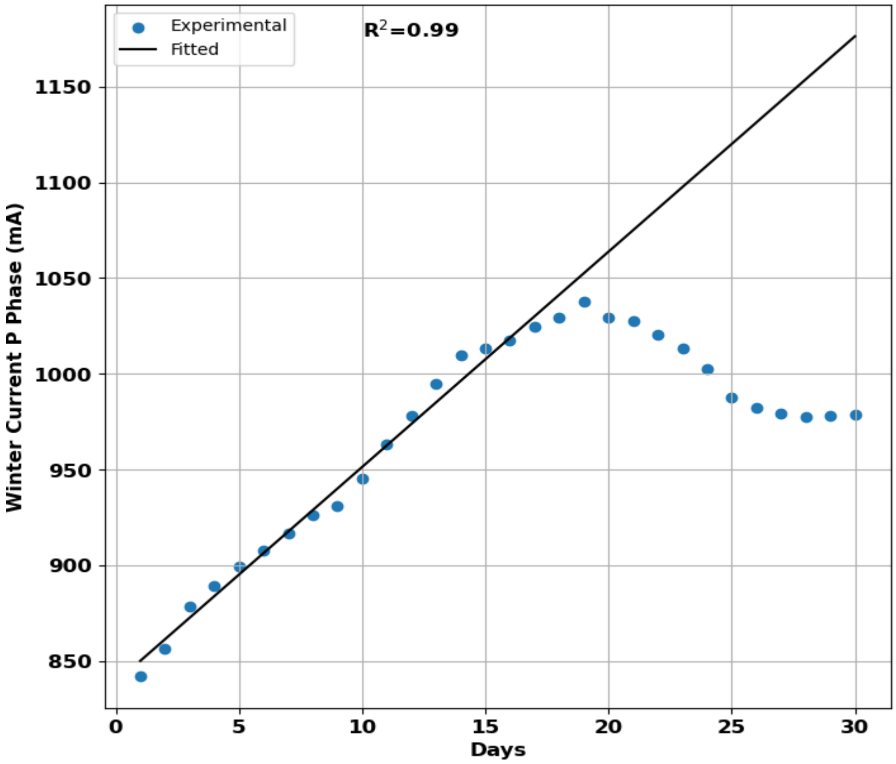

Figure 4.22 Current produced in Panicle phase of Winter Experiment

4.5.10 Current Generated in Ripening Phase

Current generation is the function of PMFC performance. Current rate gradually decreased till the crop was harvested. The available substrate completely oxidized as per requirement of plant. PMFC attained peak current generation in previous phase and subsequently at the onset of last phase declining trend was recorded. Least root exudation means least electron released and minimum current flow. Current showed sharp declining curve over the phase. The minimum current attained at the end of ripening phase during Summer experiment was 73.2 mA.

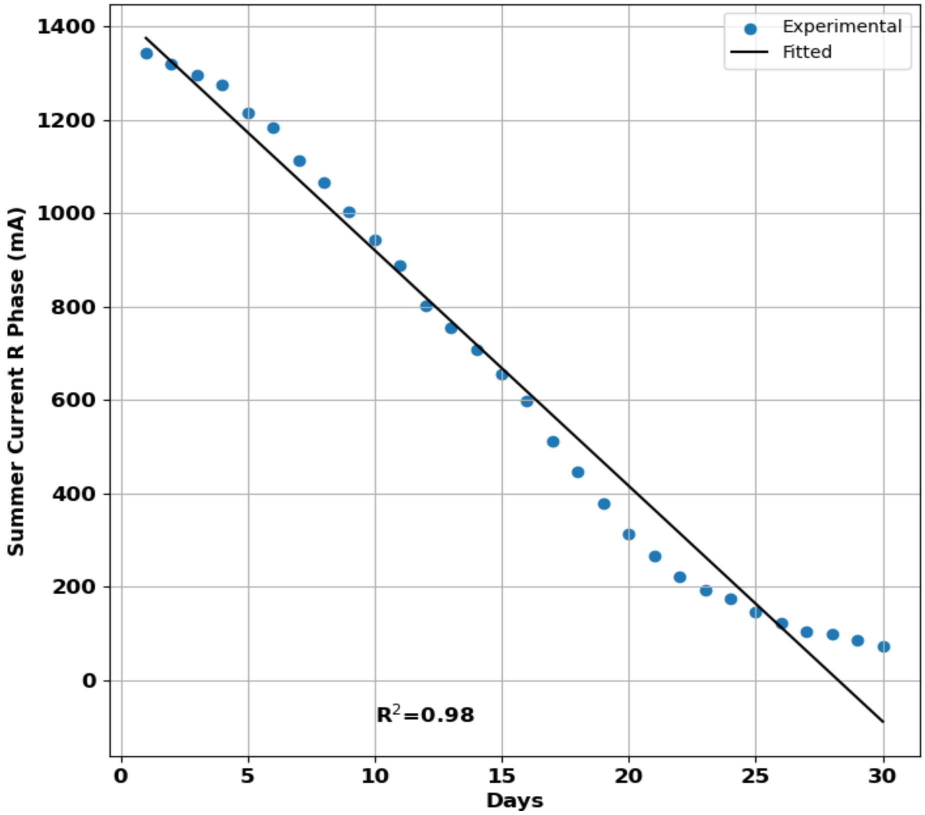

Figure 4.23 Current produced in Ripening phase of Summer Experiment

The average rate of current generation in Winter experiment was relatively less than the current generation rate in summer experiment. Multiple driving factors were responsible for this variation including soil and water temperature, plant growth with duration of solar radiance and light hours in both seasons. Therefore, in Winter experiment overall efficiency of PMFC is far less than the summer PMFC under natural conditions. In winter experiment electron flow reduced with passage of time during the last phase which is proportional to amount of current in cell. Current showed sharp decreasing curve over this phase. The minimum current at the end of ripening phase during Winter experiment was 94.3 mA which was only 22.4% of Summer current.

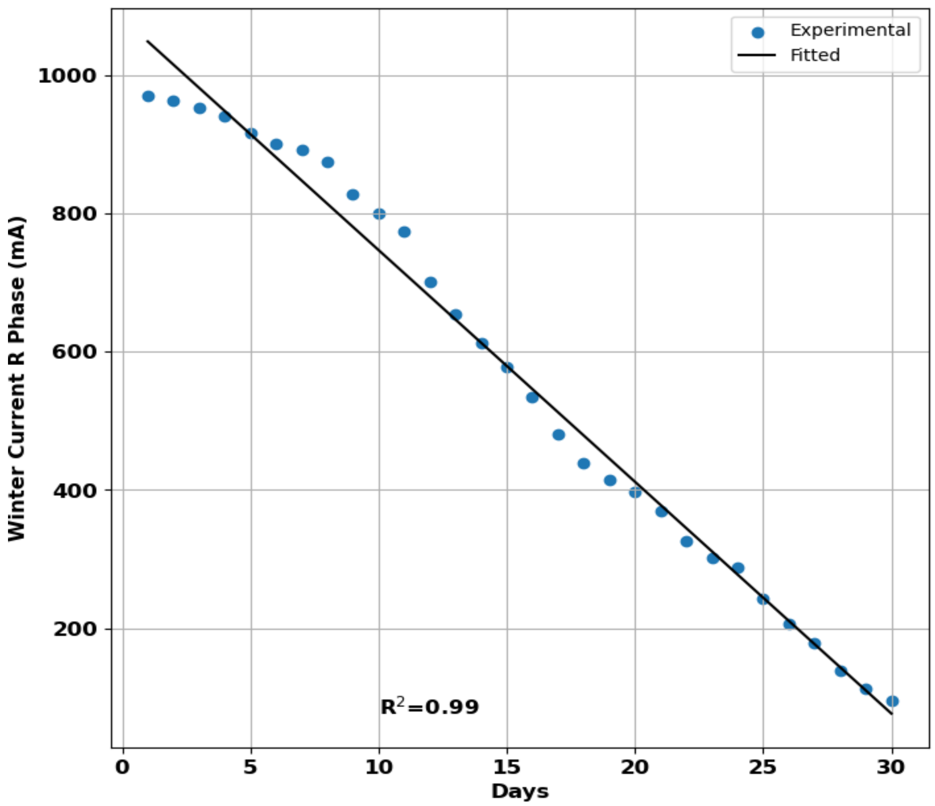

Figure 4.24 Current produced in Ripening phase of Winter Experiment

4.5.11 Overall Voltage in Paddy Growth Phases

Results showed that at establishment and initial growth phases electrochemical parameters were at low pace. After the establishment plant growth rate was the function of soil nutrient status. The number of root exudates discharged by roots of rice plant at multiple growth phases vary from specie to specie and directly influenced the power output from paddy based PMFCs at their respective growth phases. The degree of root exudation in paddy plant was least at seedling stage, a gradual increase till panicle phase followed by subsequent decrease in ripening phase. In Summer experiment current waved clearly regular and sharp rising trend in first four phases and subsequently decreasing curve in the last phase was noticed. Summer paddy growth phases voltage figure illustrating constant increase after defined interval of 5 days from nursery phase to panicle phase while a dropped trend line dissecting curve line of all phase was studied. However, the Winter paddy growth phases voltage figure demonstrating voltage produced in first four phases was in increasing order trend line with little delayed faced in power generation due to multiple driving factors. The current displayed irregular broadly distant increasing trends in first four phases and entirely decreasing curve was observed in last phase. The maximum energy was harvested in the panicle phase while lowest energy was harvested in ripening phase.

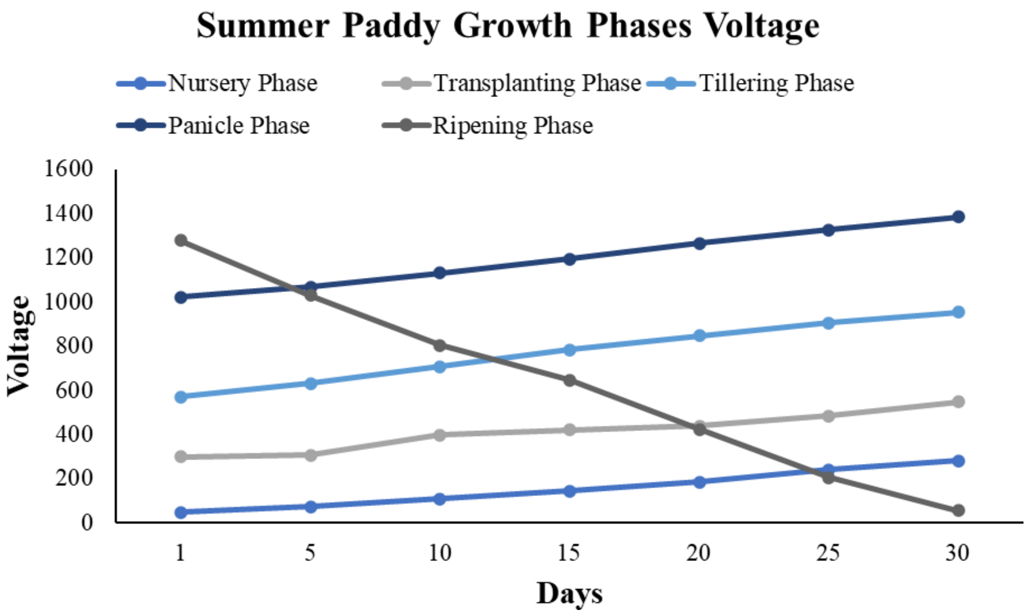

Figure 4.25 Voltage curves of all Paddy phases in Summer Experiment

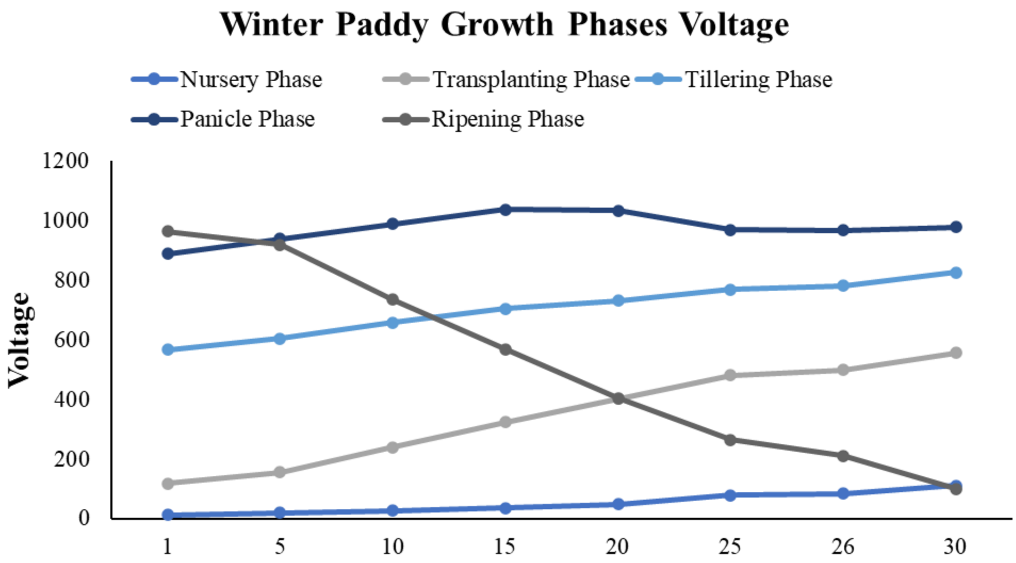

Figure 4.26 Voltage curves fo all Paddy phases in Winter Experiment

4.5.12 Overall Current in Paddy Growth Phases

The results of current in each paddy growth phase are in same order with the outputs of voltage at different phases of rice plant. The electrochemically active bacteria could have an effect on current generation within paddy fields. Current curves were drawn against each phase with formation of crust and trough. The current produced in Summer experiment was relatively higher than the current produced in winter. It may be attributed to more availability of rhizodeposits in different rice phases. In Summer experiment current showed the sharp increasing trends in first three phases with broad gap in trend during phase four while decreasing trend in the last phase. Panicle phase showed the highest deviation from the normal trend of first three phases. However, ripening phase followed the same sequence trend as the voltage formed. Winter experiment current flashed delayed rising trends from narrow to broad gap in first four phases and declining trend was observed in last phase.

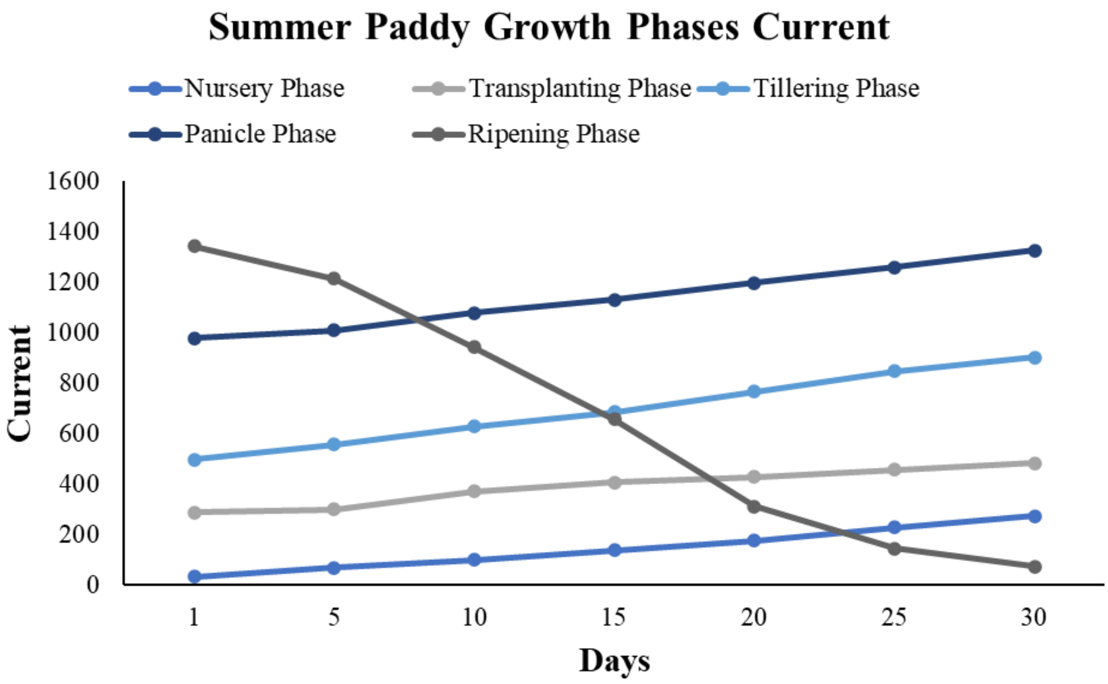

Figure 4.27 Current Curves of all Paddy phases in Summer Experiment

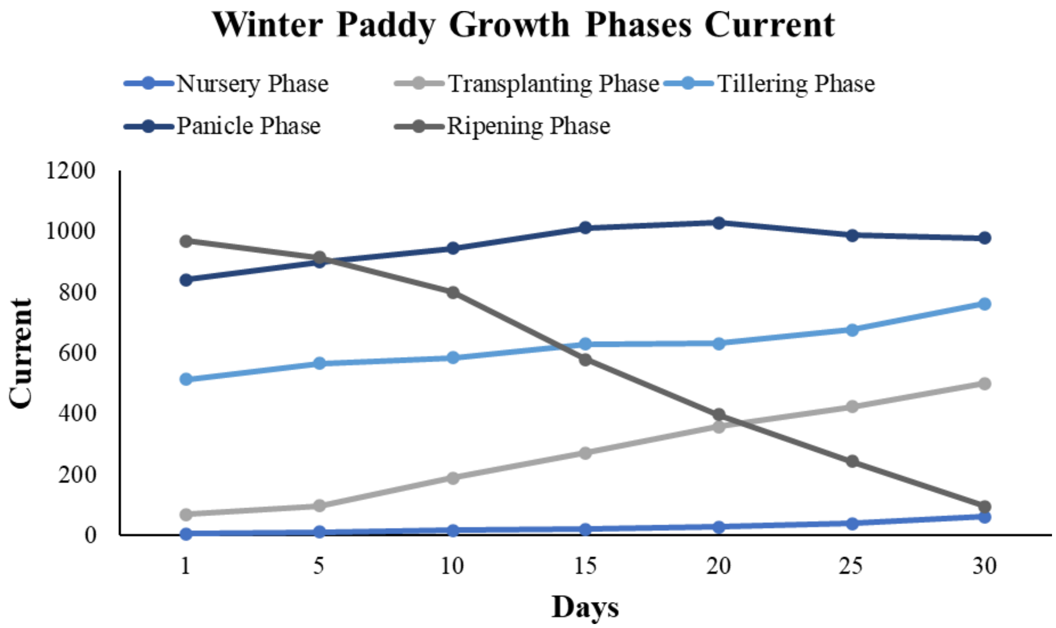

Figure 4.28 Curren Curves of all Paddy phases in Winter Experiment

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

i. With development of traditional PMFC and its monitoring in different rice growth phases, first research objective was accomplished, and achieved second research objective as the microbial communities from anode sample were successfully isolated and studied their morphological characteristics with abundance of each specie.

ii. The PMFCs performance was recorded in terms of voltage, current and power in both summer and winter experiments. The voltage produced in Summer was 40 % more than voltage produced in Winter. Similarly current generated in Summer was 30 % higher than current generated in Winter whereas power produced in Summer was 44 % more than power produced in Winter.

iii. The maximum electricity was harnessed during late active tillering phase to panicle phase while minimum energy was recorded in ripening phase of paddy growth. Growth phases of paddy are found to be highly correlated with generation rate of voltage, current and power in both summer and winter experiments.

iv. Active electrogenic microbial communities was successfully isolated from anodic sample. Their tight attachment at surface of anode in the form of mature biofilm indicates that these are most active group of bacteria mainly responsible for electricity generation in PMFC. Their abundance is directly proportional to power generation rate.

5.2 Recommendations

i. It is required to import proton exchange membrane (PEM) to formulate PMFC which is expensive. Therefore, it is recommend to use locally available materials as electrodes and membrane in order to make it accessible technology.

ii. The results revealed that to use PMFC for future use in year-round crops, there is need to continue research on improvement and formulation of PMFC in order to cut the cost electricity generation from plant products.

iii. Other parameters and variables, such as weather, should be tested to understand their effect on PMFC performance in economic crops which mayoffer substantial opportunities in the energy, water and food nexus.

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