Bioelectricity Production through PMFC using Bryophyllum Pinnatum in Association with Microbes

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Abstract: ***Natural resources such as oil may get depleted due to the exploitation of natural resources and so human has to find an alternate source of energy for the survival. As a result, various kinds of discoveries and ideas for developing greener and more efficient energy producing methods are progressively being mentioned around the world. A Microbial Fuel Cell (MFC) is capable of generating electricity directly from a large variety of organic or inorganic compounds, using a microbe as a catalyst. A plant microbial fuel cell (PMFC), a biological cell that converts the solar energy into the bioelectricity with an aid of the microbes at the rhizosphere region of plant, seems to be an emergent source of sustainable energy. In thisstudy Azotobacter was chosen to influence the increase in electricity production. The parameter for study is Variation in pH, Variation of voltage with time, Variation of voltage in difference in solar radiation, Variation of voltage influence of temperature. PMFC with and without Acetobacter was analysed. The performance of the PMFCs was then compared to MFC. This study reports on the use of Bryophyllum sp in PMFC.***

Keywords: PMFC, Bryophyllum sp, Azetobacter, MFC

1. Introduction

"Incidentally, the word "energy" is the same as the Greek word for "challenge. " "Thinking about our federal energy crisis in that light, I believe there is a lot to learn, " Thomas Carr. Natural resources like oil will soon become scarce and we need modifications in our current lifestyles if we wish to stretch the remaining oil reserves. As a result, various kinds of discoveries and ideas for developing greener and more efficient energy producing methods are progressively being lauded around the world. (Lal, 2013).

One of such approachesis the use of microbes for the production of electricity. The first report that bacteria can generate electricity appeared almost a hundred years ago (Potter, 1911). However, the work did not gain any major coverage at that time andonly in recent years that this ability of microbes has been rediscovered. The reason for this renewed interest, as mentioned above, is the need for new resources of energy and better understanding of the microbial system in relation to the electron transport and eventually, the development of Microbial Fuel Cells. A Microbial Fuel Cell (MFC) is capable of generating electricity directly from a large variety of organic or inorganic compounds, using a microbe as a catalyst (Marcus *et al.*, 2007). Microbial fuel cells (MFCs) are bio - electrochemical cells that convert microbial reducing power into electrical energy that is green. Plant MFC (PMFC) with living plants is also a way to get green energy. In PMFCs, plant roots directly fuel the electrochemically active bacteria at the anode by excreting rhizodeposits (Nitisoravut *et al*, 2017).

Recently, research related to PMFC has received great attention all over the world. Moqsud et al. (2015) conducted PMFCs with rice plant, but rice plant PMFCs need special care to grow and life time is short. So to find out the best plant to conduct the PMFC is still needed as the plants grown in different weather conditions are different and the ability of generating the bioelectricity is also different. In this study, water plants were used to generate bioelectricity with MFCs that are easily found in Japan to compare the bioelectricity generation of fresh and marine water plant MFCs and to observe the environmental factors those influence on bioelectricity generation. Current study is done with Miracle plant, *Bryophyllum pinnatum*, also known as the air plant, cathedral bells, life plant, and Goethe plant is a succulent plant native to Madagascar, which is a popular houseplant and has become naturalized in tropical and subtropical areas.

A plant microbial fuel cell (PMFC), a biological cell that converts the solar energy into the bioelectricity with an aid of the microbes at the rhizosphere region of plant, seems to be an emergent source of sustainable energy. Concurrent bioelectricity and biomass production make PMFC an appealing choice for the future green energy (Timmers *et al.*, 2010; Helder *et al.*, 2010; Arends *et al* 2014). PMFCs can generate continuous energy without competition for food and can be operated at any location. Mild operating conditions make PMFC more attractive than these traditionally viewed alternative sources of energy. However, there are some challenges that need to be met before its real application. PMFC was purposed with the idea of incorporating a plant at an anode region as the source of substrates for bacteria (Strik *et al*, 2010). This technology comprises multidisciplinary areas ranging from the study of microbes, plants, electrochemistry and different engineering fields. Therefore, exploration of these fields in PMFCs seems to be an essential to understand the relationship that exists among them. The best way to holistically understand the interrelationships that exist among these factors is to view a PMFC system as a biosystem that comprises biotic and abiotic components for production of biomass and bioenergy. Biosystems comprise living and non - living components, interconnected to achieve a unified purpose with respect to food production, environmental preservation, economic development, and technological advancement. The photosynthetic process of converting solar radiation into carbohydrates and, finally, biomass is a series of interconnected transformations (Alocilja *et al*, 2010) and a classical example of a biosystem.

PMFC arrests the root exudate from photosynthesis and converts it to bioelectricity with an aid of microbial metabolisms (Schamphelaire *et al*, 2010; Kaku *et al*., 2008). Plants are the primary producer of an ecosystem. Being autotrophs, plants utilize solar energy to produce biomass with the aid of a special pigment called chlorophyll in the green part of its leaves. However, 40% of their energy is consumed by the plants itself, with exudation of the remaining half to the rhizosphere. Microbe populations present in the soil around the rhizosphere break down the organics to yield electrons. PMFC exploits this phenomenon and traps the electrons released by the microbes in the anode region. When the electrons pass through a load 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 *et al*., 1990). A PMFC can be viewed as an open loop type of biosystem and can be divided into two major structures: the bio - control and bio - process structures. The bio - control structure (plant) receives the external input signal (sunlight) to achieve voltage. The bio - process structure (microbial population) takes material resources (root exudates) and acts on them to produce the outputs (voltage). This structure is subject to disturbances, which may cause variations in the output and in the process, itself. In open - loop biosystems, the actuating signal (sunlight) can be altered thereby affecting the outputs

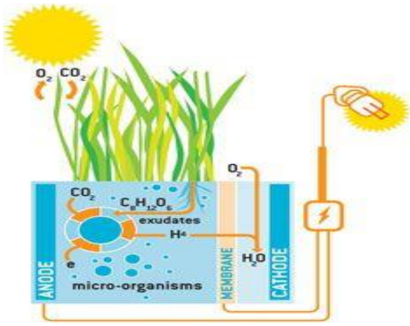


Figure 1: Sunlight on photosynthesis and its influence in fuel cells

Plant–microbial fuel cells (PMFCs) are emerging derivative devices of MFCs. Organic substrates are mostly supplied by the root deposits of aquatic plants in PMFCs and they are degraded by using bacteria on the anode surface to generate electricity. The classical configuration of a PMFC is shown in Figure 1. The anode is submerged in a support matrix near the plant roots to obtain the exudates as fuel, and the cathode.

With the consideration of expanding researches in MFCs, few works have been reported in the field of PMFCs. Review papers with different flavours were delivered in recent years in terms of MFC technologies, such as methodology (Logan *et al.*, 2006) configuration (DU *et al*., 2007), substrates (Pant *et al*., 2007), microbes, applications (Logan *et al*., 2006), micro fluidic cell (Kjeang *et al*., 2009), cellulosic MFC (Logan *et al*., 2009), constructed wetland (Doherty *et al*., 2015), domestic wastewater treatment (Lefebvre O et al, 2011), and phototropic organism in an MFC (Xiao *et al*., 2014). However, only a couple of review papers were reported for PMFCs (Deng *et al*., 2012; Strik *et al*., 2011). A cross section of PMFC is depicted below in fig 2

Figure 2: Cross section of a PMFC

Differences in time for achievement 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 bybacteria and release of the electrons. 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 convert the photosynthetic matters in root exudates with the simultaneous absorption by the microbes are well suited to PMFCs since enhanced bioenergy harvest can be achieved. Nevertheless, identification of the optimum light intensity for an efficient photosynthesis, optimum microbial activity, and higher rhizodeposition are the factors that need to be researched intensively within PMFCs. For our present work Azotobacter is chosen to influence the increase in electricity production. The parameter for study are Variation in pH, Variation of voltage with time, Variation of voltage in difference in solar radiation, Variation of voltage influence of temperature, Polarization curve of the PMFs, Polarization curve is plotted between the Current density (mA/cm^2) and Cell voltage (mV) in which control, PMFC with and without Acetobacter will be analysed. PMFC without Acetobacter will have natural microbes present in the water and plant roots.

The performance of the PMFCs was then compared to MFC. This study reports on the use of Bryophyllum sp in PMFC. These results would be useful for the development of projects on the production of bioelectricity with succulent plants of similar to this variety.

2. Material and Method

Thestudy plantBryophyllum pinnatum was collected from a nursery. The plant were transferred in required number to the pots when required.

Plant used for PMFC construction

Figure 2: Bryophyllum pinnatum

Bryophyllum pinnatum, also known as the **air plant**, **cathedral bells**, **life plant**, **miracle leaf**, and **Goethe plant** is a succulent plant native to Madagascar, which is a popular houseplant and has become naturalized in tropical and subtropical areas.

Construction of PMFC

Bryophyllum pinnatum plants of 3 replicates are used for the study. Each replicates contains 6 trials to be studied.3 for control with different light intensity and 3 for trials with microbes with different light intensity.

Experimental set - up

Six Pots (Figure - 3) illustrate the test set up for the PMFCs in all cases. The set - up is made as follows:

Figure 3: Experimental set up of PMFC –control and test

Soil was patted down in MFC up to 1cm to make a smooth surface and anode was placed on the top of the soil, finally soil sample was added up to 4 cm line. The cathode was placed on the top of the soil and the setup was closed using a lid. Electricity production was measured using digital multimeter.

Power Calculation (Potential differences)

The voltage across the external resistor in an MFC can be measured using a multi meter. Voltage measurements are converted to current values using Ohm's law:

$$V = I \times R$$ Where V = voltage, V, I = current, A, R = resistance, Ω

Trials on Microbial association and Sunlight intensity with plant on efficacy:

PMFC 1: 25˚C **Control** - Pot, plant loaded on thermocoal, Soil (growth medium), Carbon rods, copper wire, Multimeter

PMFC 2: 25˚C **M - P - C -** Microbe, Pot, plant loaded on thermocoal, Soil (growth medium), Carbon rods, copper wire, Multimeter

PMFC 3: 35˚C **Control -** Pot, plant loaded on thermocoal, Soil (growth medium), Carbon rods, copper wire, Multimeter

PMFC 4: 35˚C **M - P - C -** Microbe, Pot, plant loaded on thermocoal, Soil (growth medium), Carbon rods, copper wire, Multimeter.

PMFC 5: 50 % shade **Control -** Pot, plant loaded on thermocoal, water (growth medium), Carbon rods, copper wire, Multimeter.

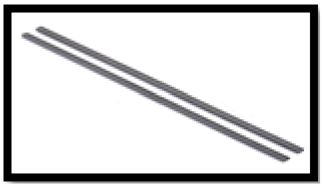
PMFC 6: 50% shade **M - P - C -** Microbe, Pot, plantloaded on thermocoal, water (growth medium), Carbon rods, copper wire, Voltmeter.

Where, control is only Plant fuel cell that has light intensity of about 23000 - 40000 lux during 11 am to 1 pm everyday with temperature 25 - 27 ˚C and trial PMFC - 2, 4 and 6 will be Microbial Plant fuel cell that has light intensity of about 23000 - 40000 lux during 11 am to 1 pm everyday with temperature 25 - 27 ˚C.

In brief, 30cm in length and 30 cm in diameter plastic pots were used for the PMFCs. Three replications were used for this experiment. The soil used in the experiments was collected from local area where the top soil is mixed with 1 ft below soil equally and it is analysed for classification.

It is classified as sandy loamy soil (pH 7.51 and Organic matter content 7.5 %). The Bryophyllum plants were planted in the soil.

The carbon electrode is not only good at conducting electricity with an electrical resistance of 5 ohm but also durable and favorable materials for soil environment (Moqsud et al.2013). The anode area covers around $125cm^2$ inside the soil of the PMFCs. The carbon fiber used in this study has a density of 1.75 g/cm3 as per the label.



Carbon rod with Density 1.75 g/cm3 as anode. The anode was set approximately 5cm below the surface of the soil, while the cathode was placed immediately above the soil surface, but under the water. These electrodes were connected via epoxy - encapsulated wires, and the circuit was completed using an external resister of 100 ohm. The electricity depends on the organism and density of biofilm coating the anode over the period of time.

Figure 4: Experimental setup of PMFC

Based on the procedure, the MFC was constructed as shown below and electricity was produced.

In this set up, the PMFC trials containing Micro Organism Azotobacter which is inoculated at the rate of 5% of the volume of the soil that are in PMFC 2, 4 and 6. The viable microorganism being adhered to anode, i. e. carbon rod determines the quality of current and density of current.

Isolation of Viable microorganisms from anode surface

After the experiment was over, anode graphite fiber was removed from the PMFC and was kept for incubation in phosphate buffer solution for 1 hour in shaker and serial dilution was done by adding 1ml of phosphate buffer in 99ml of sterile water.0.5ml aliquots of each serial dilution were transferred to agar plates by spread plating technique and were incubated at 37°C for 24 hours. After incubation, colonies were checked for the confirmation of biofilm formation by Azotobacter in the PMFC in appropriate media within the cathodic chamber. The MRS broth in the anode was supplemented with 20 mM indigo carmine to serve as the mediator for electron transfer. Electrical output and voltage were measured continuously for 24 hours.

Electricity productionby Plant Microbial fuel cell with soil as medium in a plastic container which is very cheap when compare to the other fuel cells. Soil MFC is capable of producing electricity more than 90 days continuously. Initially, when soil sample is inoculated in PMFC no electricity production has been obtained and after incubation for 48hours we observed increase in electricity production continuously. Finally based on the procedure the microbial fuel cell was constructed and electricity was produced. The MFCs were operated for 400 hrs and the voltage produced using agricultural soil increased after 50 hrs and then began to decrease after 360 hrs. Variation in pH, Variation of voltage with time, Variation of voltage in difference in solar radiation, Variation of voltage influence of temperature, Comparison of Power Output between all 7 days i. e 400 hours were studied

3. Result and Discussion

Variation of voltage with time and association of *B. pinnatum* plants with Azatobacter

Table - 1 illustrates the influence of solar radiation on the variation of voltage generation with time in *B. pinnatum*. PMFCs in the presence and absence of *A. croococcum*. It was observed that the voltage values were higher in the presence of microbe *A. croococcum* where the pot had kept under the temperature of 35^0C than the pots kept under temperature of 25^0C and under the 50% shade (By comparing pot 4 with remaining 5 pots). Plants continuously provide an input of organic matter to the soil throughout their plant life (Strik *et al*.2011). Whear as the present study was performed with bryophyllum pinnatum in associated with azotobactor

The general trend of voltage generation was that it increased gradually in the initial stage before becoming constant. There was a gradual increase in the voltage in the plants which were associated with Azatobacter and it was almost constant and it was able to produce electricity upto 90 days. However, the voltage generation in the case of *B. pinnatum* plants without Azatobacter was almost constant in all the stages.

A small amount of voltage was generated due to the potential difference between the anode and cathode and also probably the phenomenon of organic matter decomposition in the soil. Thus, *B. pinnatum* MFC is an ecological solar cell in which plant photosynthesis is coupled to the microbial conversion of azatobacter into electricity.

Variation of voltage with time and influence of solar radiation

Solar radiation has effects during the experiment. When the solar radiation (35^0C) was high the voltage generation was also high. The voltage generation was low when the solar radiation was low (25^0C and 50% shade). This type of phenomena was probably due to the food (glucose) produced in the green leaves due to the photosynthesis being used by the plants rather than being discharged into the soil and also due to the bacterial activity inside the soil (Mosqud *et al.*, 2014). The readings of day 1 is noted and subsequently everyday the readings were noted. Till Day 6 it was on log phase and later slowly on Day 7, the Power output started declining. Day 6 was ideal and the calculations are given below.

Table 1: Current and Power generation with respect to association of *B. pinnatum* with Azatobacter on day 1 and day 6.
= V/R

CHAMBER	Day 1 - Power I (amps)	Day 6 - Power I (amps)
PMFC 1	0.002	0.004
PMFC 2	0.005	0.007
PMFC 3	0.001	0.003
PMFC 4	0.007	0.004
PMFC 5	0.002	0.002
PMFC 6	0.004	0.002

Highest Peak Voltage arrived On Day 6 and values are tabulated below.

Current density= V / (Area of anode x Resistance)

So, for PMFC 1, Current density= 429/ (125 x100) = 0.034

Table 2: Voltage generation under temperature

CHAMBER	Peak Voltage (mV)	Density mW/cm^2
PMFC 1	429	0.034
PMFC 2	712	0.056
PMFC 3	390	0.031
PMFC 4	463	0.037
PMFC 5	292	0.023
PMFC 6	295	0.023

The peak voltage generated in our study was around 700 mV in Azatobacter PMFC with *B. pinnatum* plants under temperature of 35^{0}C (Table) and the peak electricity generated was 0.0056 watts which is sufficient to charge mobiles (Mosqud *et al*., 2014).

In the present study the power generation with respect to association with of bryophyllum pinnatum with azotobacter in different time interval ie from day 1 to day 6 at two temperature 35® & 25® revealed that bryophyllum pinnatum in association with actobacter at 35® showed maximum voltage values, Strike et al., 2008 reported such study but there are no studies made with bryophyllum pinnatum. Soil MFC is capable of producing electricity more than 90 days continuously. Initially, when soil sample is inoculated in SMFC no electricity production has been obtained and after incubation for 48hours we observed increase in electricity production continuously (Raj *et al.*, 2013).

In conclusion, we found that soil MFCs constructed from the Industrial effluent soil are sustained in active, highly electrogenic bacterial anode community are present and capable of producing electricity continuously for 650 hours, whereas soil MFCs from agricultural soil produce electricity only 400 hours. These results shows the importance of soil type in MFC bacterial communities and this work will behelpful for the further research on soil MFC for long term electricity production. Interestingly, construction of MFC is important to achieve high power production using MFC technology thus Keego Technologies LLC resolved the problem of MFC construction. More research on industrial effluent soil and agricultural soil to study the exact mechanism in MFC is required.

The peak voltage generated in our study by PMFC 2 - was around 712 mV in Azatobacter PMFC with *B. pinnatum* plants under temperature of 35^{0}C that is full light which is 22000 - 28000 lux at 1.30 pm everyday and the peak electricity generated was 0.0056 watts which is sufficient to charge mobiles (Mosqud *et al*., 2014). Subsequently the PMFC 1 chamber 429 mV with same conditions as PMFC 2.

Also the study confirms that the light intensity is most important factor in bioelectricity generation by plant itself as well as with microbial association. Although the plant itself produce good power output, in association with microbes, the output of PMFC is 80% higher than only PFC.

MFC has been considered as a sustainable technology for energy production and wastewater treatment. However, energy consumption was necessary to maintain MFC operations. Algal photosynthesis provides an option to eliminate or maintain minimum energy consumption in MFC technology by omitting aeration. Therefore, aeration was not required and the greenhouse gas (CO_2) emitted from the anode chamber was self - sequestrated in the MCCs, making real green systems for energy generation. However, it is notable that energy production of the photosynthetic algal MFCs is currently quite low compared with conventional MFCs. Light intensity was proved to be one of the critical parameters in affecting their performance. Other parameters such as reactor configurations, algal species, and electrode materials should be improved in the near future for targeting high - performance algal photo MFC systems.

In this study, PMFCs containing microbial association in full light system has produced higher voltage. It was observed that some of the planet’s tiniest inhabitants might help address one of the society’s biggest environmental challenge *ie*., energy crisis. Association of Azatobater with *B. pinnatum* have proved to be a good way to get electricity generation. A small amount of electricity is also necessary for electricity - scarce populations (25% of the world’s population are deprived of electricity). Small amounts of electricity can be used for lighting light - emitting diode lamps or just to charge a mobile phone in a particular household by cultivating *B. pinatum* in association with Azatobacter in open condition. *Bryophyllum pinnatum* is a popular houseplant and has become naturalized in tropical and subtropical countries and many of them are suffering from lack of electricity. Consequently, to use food products such as rice, corn and soybean to produce bio - fuel is not a good idea as it is unnecessary waste of precious food products for the millions of poor people in these areas.

MFC, as an energy - saving technology, may well wean us away from the dwindling oil resources. But there are many technical challenges that must be overcome before it can be used for renewable energy production. Nonetheless, the technology might open the door to a new method for renewable and sustainable energy products.

Applying the PMFC as large scale electricity generation technology will make an electricity plant look like a wetland. Again the aesthetic value is high and there is an opportunity of adding economic value to natural areas that currently only hold implicit value. It can be expected that the P - MFC will be socially acceptable. Economic feasibility was assessed based on three cases: the Green Electricity Roof, decentralized electricity production in remote areas and large - scale electricity production in wetlands.

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