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Polymer Degradation by Soil Microorganisms and Efficacy in Production of Soil and Plant Microbial Fuel Cell

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Abstract
The present society is facing many catastrophic scenarios in public health care due the poor liquid and solid waste management. The increased amount of plastic use and its disposal have also slowed the degradation and management of waste. This paper examines the study of polymer degradation by the soil microorganisms and efficacy in production of microbial fuel cell from plant and soil. According to the recent research soil microorganisms are responsible for polymer degradation and through respiration of microorganism's electrons are released where chemical energy is converted into electrical energy. The specific size of polymer sheets is cut dried and weight and placed inside the soil for in-situ degradation and the microbial population in the soil is determined by using plate count method on nutrient agar plate and basal medium incorporated with polymer. The open circuit voltage was measure before and after plantation because of the unique plant-microbe relationship at the rhizosphere region of a plant and converts solar energy into bioelectricity. This is done to check whether the plantation is responsible for increasing the voltage in the circuit. This research could show a new way in producing bioelectricity by plantation and also an ecofriendly method in polymer degradation.

Keywords
Polymer, Microbial fuel cell, Open circuit voltage, Bioelectricity.

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INTRODUCTION
Plastic is a synthetic polymer, strong, durable, moisture resistant, light weight polymers of carbon along with hydrogen, nitrogen, sulfur, and other organic and inorganic elements and are manufactured from fossil fuel. Plastics are extensively used because of their stability and durability. Due to the absence of efficient methods for safe disposal of these synthetic polymer they often end up accumulated in the environment posing an ever increasing ecological threat to flora and fauna. As per a case study, plastic contribute about 8% of the total solid waste generated. At present, there are principally three ways to get rid of plastic, inceration, dumping in landfills and

recycling. But of three methods are not proficient to manage the existing bulk of plastic waste generated. The three basic steps through which synthetic plastic can degraded – photo oxidation, thermo oxidation degradation, and biodegradation. From the above three methods most acceptable method is biodegradation since it is cheap and environmentally friendly.

Biodegradation of a polymer depend upon its molecular weight which determines various physical properties and its chemical composition. Degradability is inversely proportional to molecular weight. Biological biodegradation is generally considered a phenomenon of biological transformation of organic compound by living organisms particularly microbes. It is considered as a natural process in the microbial world as a carbon and energy source for their growth and takes a key role in recycling of materials in the natural ecosystem. Degraded plastic is further processed by microorganisms by two metabolism method-aerobic and anaerobic metabolism which results in the formation of carbon dioxide and water and electrons the end product in both the metabolism.

MICROBIAL FUEL CELL (MFC)

Microbial fuel gained much attention because of its ability to generate power from organic or inorganic compound via microorganism. Around one hundred years ago the technology of generating electricity through bacteria was found but it did not gain much attention. Due to its ability to convert chemical energy to electrical energy MFC's have many potential applications such as electricity generating, bio hydrogen production, waste water treatment and biosensor. Many studies are focused in discovering the mechanisms of how bacteria transfer electrons. Some researcher has used MFC for the treatment of phenol, chromium and pentachlorophenol. In addition, Microbial fuel cell have been tested for the production of Hydrogen. The microbial fuel cell is device that convert chemical energy directly into electricity. Materials with the large population of microorganisms and high content of organic matter have been used to generate power. MFC fabrication include marine segment, sewage sludge, industrial /domestic waste water[1]

SOIL MICROBIAL FUEL CELL

Soil can be used to generate electrical power in microbial fuel cell which converts energy from soil organic compounds into electricity via catalysis by soil source exoelectrogenic microorganisms. The process of soil generation into several potential applications. Firstly, the pollutant toxicity and soil microbial activity could be monitored by the generated electrical signals of the MFCs, such as peak voltage, quantity of electrons and start-uptime. Secondly, the use of MFCs would lead to the elimination of soil pollutants including phenol, petrol and oil. Thirdly, the operation of MFCs methane emissions from paddy soil and sediment[1]. MFCs do not need energy input, instead, a small amount of electrical power is generated. Therefore, MFCs are considered a sustainable technology. The performance of these MFCs is largely related to the magnitude of electrical current generated by the exoelectrogenic bacteria in soil. However, little is known about the character of power generation and the diversity of exoelectrogenic bacteria in different soils.

PLANT MICROBIAL FUEL CELL

Plant microbial fuel cell (PMFC) is a promising modification of microbial fuel cell that exercises the unique plant-microbe relationship at the rhizosphere region of a plant and converts solar energy into bioelectricity. In-situ bioelectricity and biomass production, rather than the supply of external substrates, make this technology different from traditional MFCs. Thus, designing and understanding PMFCs should be viewed from a bio systems engineering perspective rather than only through MFC methodology. Plant-microbe harmony at the soil interface, driven by rhizodeposition coupled with efficient engineering, ultimately directs towards its real applications. The three main paradigms Firstly, effects of plants in PMFC via rhizodeposition and photosynthetic activity are explored. Secondly, the role of microbes driven by soil physiochemical and biological characteristics are shown. Thirdly, the engineering aspects involved in designing an efficient configuration are revealed and an attempt is made to interpret the PMFC with bio systems principles. Furthermore, an overview of a PMFC system is done, along with the future perspectives and challenge.

Although MFC have been studied as an alternative energy source their application are presently limited to certain niche area. With further improvements in design cost effective based on these near team application, it would possible to scale up and the use MFC as a renewable energy resource. MFC technology is used for the waste water treatment, powering under water monitoring devices, power supply to remote sensing, BOD sensing and hydrogen.

MATERIALS AND METHOD

Sample collection.

The garden soil was collected from Hindusthan gardens, Coimbatore, Tamil Nadu. The soil sample was brought to the laboratory for further examination and was preserved in laboratory conditions.

Physical parameters of soil.

The soil sample was tested for various physical parameters, which are pH, temperature, moisture content and microbial population.

In-situ degradation of polymer bags in the soil.

In this, the polymer bags were cut into small squares (8cm*8cm), thoroughly rinsed with tap water and then with the distilled water and dried under room temperature. The initial weights of squares of polymer bags were noted and they were incubated in containers containing selected soil samples. After a specific period of time (2 months) the films were removed from the soil samples. These films were rinsed under tap water and then immersed in distilled water and dried. Weights of the polymer were measured.

Agar plate with the emulsified polymer.

Polymer degrading microorganisms were obtained from agar medium emulsified with the HDPE (High density polyethylene) polymer. 2g of HDPE powder was added into the 1l of mineral salt medium containing yeast extract; 100 mg; $(NH_4)_2SO_4$; 1 g; $MgSo_4 7H_2O$, 200 mg; NaCl, 100 mg; $CaCl_2.2H_2O$, 20 mg; $FeSo_4.7H_2O$, 10 mg; $Na_2MoO_4.2H_2O$, 0.5 mg, $Na_2WO_4.2H_2O$, O.5 mg, $Na_2WO_4.2H_2O$, 0.5 mg; $MnSO_4$, 0.5 mg; K_2HPO_4, 1.6g; KH_2PO_4, 200 mg. Then 0.1 ml of appropriate dilution of soil sample was inoculated into the medium by spread plate technique and incubated at 37ºC for 24 - 72 hours[3]

Plate count method.

The total number of microorganisms and plastic degradation microorganisms in the sample were counted by plate count respectively. One gram of soil sample was dispersed in 9ml sterile water in a test tube plugged with cotton.

A series of dilutions were prepared from 10^{-2} to 10^{-6} by adding 1ml of soil suspension to 9 ml sterile water. A 0.1ml aliquot of the appropriate dilution was spread aseptically onto agar plate. The plates were sealed with the plastic tapes and incubated at 37ºC.new colonies were counted periodically and were expressed as colony forming unit.

Microscopy observation.

The bacterial and fungal isolates were presumptively identified by means of microscopy examination. Gram staining and lacto phenol cotton blue staining was done for identification of bacteria and fungi.

Microbial fuel cell setup.

Six MFC reactors constructed in grow bags with the size of 9*12. In each reactor, the anode and cathodes were zinc and copper plate with an area of 0.48m2 and 0.42 m2 respectively. The thickness of zinc and copper rod was 0.01cm. The anode was positioned close to the bottom of the reactor and embedded with the first layer of soil while cathode was fixed on the top layer of the soil which was further embedded with the soil.to study the microbial population of the soil along with the growth of the plant which is further generate electricity three reactor was assigned.

GROUP 1: Soils were cultivated with finger millet crop and maize crop (2:1) with HDPE polymer sheet

GROUP 2: Soils were cultivated with maize crop and finger millet crop (2:1) with HD-HDPE polymer sheet.

GROUP 3: Soils without plantation was kept as control.

Pretreatment of electrodes.

Copper and zinc was used as electrodes to collect the electrons in both anode and cathode with copper wire connection at the other bag was sealed with tape. These electrodes were relatively inexpensive and available easily. The electrodes were first washed soaked in 100%ethanol for 30mins. After this the electrodes were washed with 1 HCL followed by 1M sodium hydroxide each for 1 hour to remove possible metals and inorganic contaminants and to neutralized them. They were stored in distilled water before use.

Analysis of bacterial count

Total bacterial Count was calculated from all the groups (group 1, group 2, group, group 3) of the soil for every 7 days by using standard colony count method. Generation of electricity was calculated based on the bacterial growth and voltage obtained.

Generation of electricity

The MFC's was operated for 60 days at room temperature of 28-30ºC maximum amount of electricity was gained by connections in parallel and series connection to give higher electricity. The voltage generation was noted. Electricity generation was assessed based on the microbial count and groups of MFC.

RESULTS AND DISCUSSION

The present study reveals that the microorganisms present in the planted soil get to be a source of electrical energy. The physical parameters such as pH, temperature of the soil were calculated in every 7 days of interval and the microbial count was also counted the pH of soil was about 7.76 (neutral pH) in all the three groups and the temperature was around 20-28ºc.

The biodegradation of polythene is relatively faster and earlier than that of the plastics. The surface of materials has turned from smooth to rough with cracking. This may be due to the compounds secreted extracellular by the microbes that may break the complex molecular structure of plastics. These microbes were separately allowed to degrade the polythene and plastics inside the laboratory. The degradation of polythene and plastic films was compared by the weight loss of the samples inside the soil. This study shows that the degradation rate is faster in the laboratory condition

The electricity was generated more after plantation when compared to before plantation. The results indicated that the bio-process structure (microbial population) takes material resources (root exudates of the plant) and acts on them to produce the outputs (voltage).

The electricity was even compared by connection the MFC setup in parallel connection and in series connection. The series connection of the MFC setup showed to produce more voltage (above 1.0v) compared to the parallel connection (less than 0.86).

Table -1

ANALYSIS OF MICROBIAL LOAD V/S ELECTRICITY GENERATION

(Parallel connection)

INCUBATION DAYS	GROUP 1		GROUP 2		GROUP 3	
	Microbial load	**Voltage (mV)**	**Microbial load**	**Voltage (mV)**	**Microbial load**	**Voltage (mV)**
1 - 7 days	234	0.6	245	0.65	234	0.6
7 - 14 days	287	0.65	265	0.7	287	0.64
14 - 21 days	296	0.7	300	0.7	310	0.7
After Plantation						
21-28 days	TNTC	0.8	TNTC	0.8	TNTC	0.78
28 - 35 days	TNTC	0.9	TNTC	0.9	TNTC	0.79
35 - 42 days	TNTC	0.9	TNTC	0.9	TNTC	0.79
49 - 56 days	TNTC	0.9	TNTC	0.9	TNTC	0.8

Comparing all the three groups, voltage was noted and it showed similar results. Seedlings of plants were done after 21 days and the voltage generated was compared with before and after plantation. The voltage lend to increase day by day after plantation and it means that the roots of the plants were able to release electrons which lead to increase the voltage reading

Table-2

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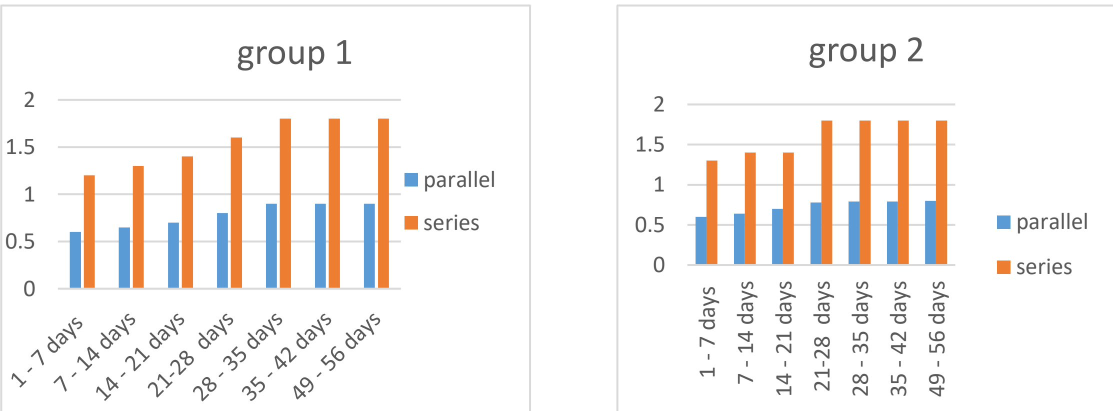
ANALYSIS OF MICROBIAL LOAD V/S ELECTRICITY GENERATION.
(Series connection)

INCUBATION DAYS	GROUP 1		GROUP 2		GROUP 3	
	Microbial load	**Voltage (mV)**	**Microbial load**	**Voltage (mV)**	**Microbial load**	**Voltage (mV)**
1 - 7 days	234	1.2	245	1.3	234	1.2
7 - 14 days	287	1.3	265	1.4	287	1.28
14 - 21 days	296	1.4	300	1.4	310	1.4
After Plantation						
21-28 days	TNTC	1.6	TNTC	1.8	TNTC	1.56
28 - 35 days	TNTC	1.8	TNTC	1.8	TNTC	1.58
35 - 42 days	TNTC	1.8	TNTC	1.8	TNTC	1.58
49 - 56 days	TNTC	1.8	TNTC	1.8	TNTC	1.6

The microbial fuel set up was connected in series connection to increase the voltage generation and on comparing with the parallel connection the voltage was found to be doubled so this indicated that series connection can produce more voltage

Figure1

Graphical representation of the Comparison of the 3 groups MFC setup when connected into series and parallel connection.



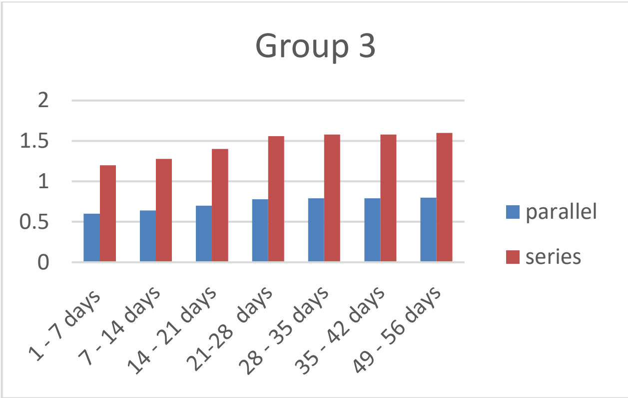


Figure2

Graphical representation of the Voltage reading of MFC 's setup of all the 3 groups when it is connected in parallel connection.

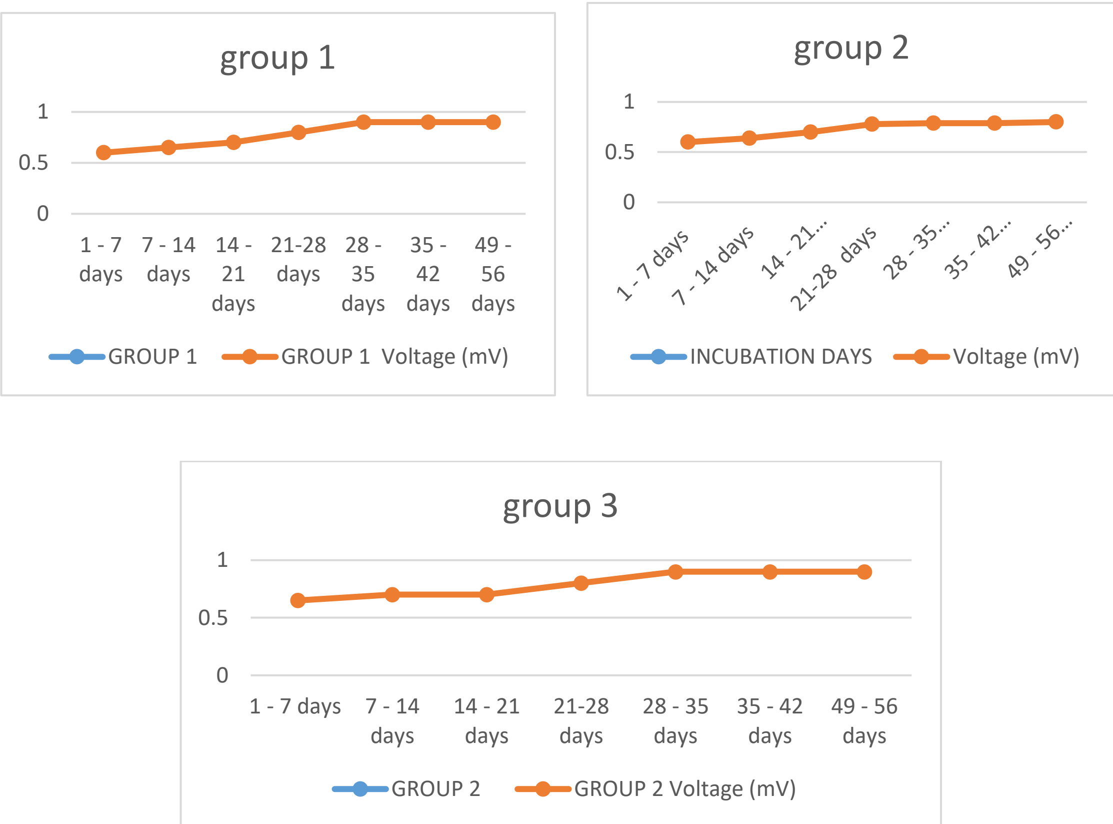


Figure 3

Soil sample collection

Bags with soil sample and connected in parallel connection.

Table3

Physical parameter test of soil

Physical parameter	Value
pH	7
Temperature	31ºC
Moisture content	4.721

Figure4

Agar plate emulsified with polymer

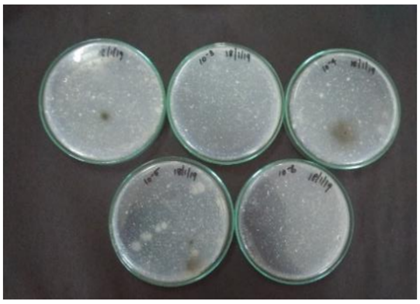


Table4

Colony forming unit of mineral salt medium emulsified with polymer.

S.NO	DILUTION	CFU
1	10^{-2}	120
2	10^{-3}	108
3	10^{-4}	88
4	10^{-5}	82
5	10^{-6}	52

Microscopy observation

Figure5

gram staining of the bacteria observed on the on the mineral salt medium emulsified with polymer

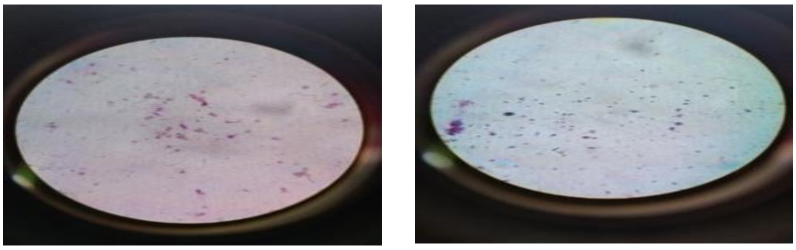


Figure6

Lacto phenol cotton blue staining of the fungus observed on the mineral salt medium emulsified with polymer.

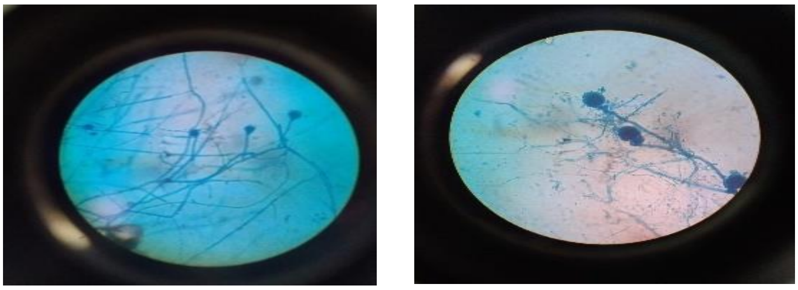


Figure7
Microbial fuel setup

Group 1 Group 2 Group 3

Group 1, Soils were cultivated with finger millet crop and maize crop (2:1) with HDPE polymer sheet.
GROUP 2, Soils were cultivated with maize crop and finger millet crop (2:1) with HD-HDPE polymer sheet.
GROUP 3, Soils without plantation was kept as control.

Figure8
Pretreatment of the electrodes

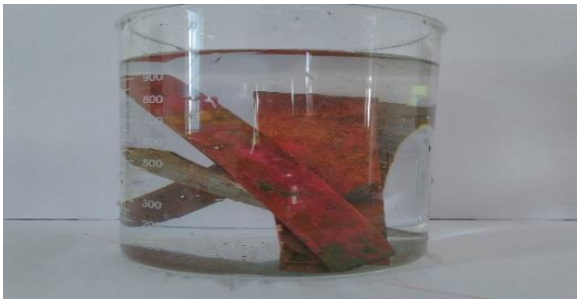


Figure9
Zinc and copper electrode

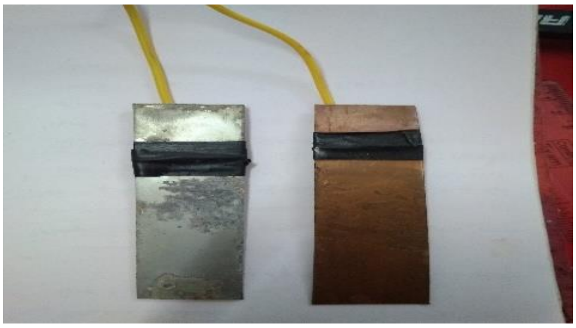


Figure10
Roots of millet crop

Figure11

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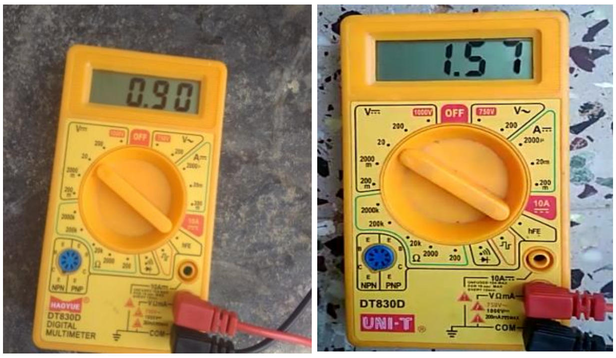
Roots of maize crop

Figure12
initial and final weight of the HD-HDPE polymer

Figure13
initial and final reading of HDPE polymer

Figure14
Voltage reading of MFC setup connected in series and parallel connection.



Parallel connection Series connection

DISCUSSION

Microbial fuel cell technology has emerged as a promising & challenging technology. MFC are considered to be an alternative sustainable technology to meet increasing energy needs, simultaneously accomplishing efficient waste water treatment and electricity generation. Microbiologically catalyzed electron liberation at the node and subsequent electron consumption at the cathode when both processes are sustainable are the defining characteristics of MFC[2]. MFCs represent a potential alternative for activated sludge in waste water treatment systems because energy is produced in the form of electricity or hydrogen gas rather than using energy for aeration or for other treatment processes. The applications of MFCs for waste water treatment have been documented in many reports.

Soil has been used to generate electrical power in microbial fuel cells (MFCS) at exhibited several potential applications. This study aimed to reveal the effect of soil properties on the generated electricity and the diversity of soil source exoelectrogenic bacteria.

The biodegradation of polythene is relatively faster and earlier than that of the plastics. The surface of materials has turned from smooth to rough with cracking. This may be due to the compounds secreted extracellular by the microbes that may break the complex molecular structure of plastics. These microbes were separately allowed to degrade the polythene and plastics inside the laboratory. The degradation of polythene and plastic films was compared by the weight loss of the samples inside the soil. This study shows that the degradation rate is faster in the laboratory condition because individual microorganism inoculated with sample which may help in the degradation of polythene and plastics.

CONCLUSION

Microbial fuel cell can be as an alternative source of electrical energy. From this current study it indicates that by maintaining soil moisture content in the microbial fuel setup which will leads to the production of more voltage because water acts as an electrolyte chamber were electrons transfer from anode to cathode. This study also indicates that the roots of the plants are also able to produce electrons which helps in the increase of voltage and also conclude that soil microorganisms are responsible for degradation of polymer.

REFERENCE

1. Arends JB, Speeckaert J, Blondeel E, De Vrieze J, Boeckx P, Verstraete W, et al. Greenhouse gas emissions from rice microcosms amended with a plant microbial fuel cell. ApplMicrobiolBiotechnol 2014; 98:3205–17.
2. Kamble Asmita, Tanwar Subhamsingh and Shanbhag Tejashree., 2015, Isolation of plastic degradation microorganism from soil sample collected at various loacation in vironment Mumbai, India, vol.4(3),77-85, Int.Res.J.Environment Sci.
3. Tansengo, Y. Tokiwas,2014 Thermophilic microbial degradation of polyethylene succinate, 2014, World Journal of Microbiology & Biotechnology 133-138.