PRODUCTION OF ELECTRICITY FROM AGRICULTURAL SOIL AND DYE INDUSTRIAL EFFLUENT SOIL USING MICROBIAL FUEL CELL

Samuel Raj. B[1], Jebakumar Solomon. R.D[2], Prathipa.R[3], Anis Kumar.M[4]
[1&2]*Department of Molecular Microbiology, School of Biotechnolgy, Madurai Kamaraj University, Madurai Tamilnadu* ***jsolomon_mrna@yahoo.com, jarleumas@gmail.com***
[3&4] *Department of Biotechnology, PABCET, Tamilnadu, India.* ***anik_biotech10@yahoo.com***

Abstract

Microbial Fuel Cells (MFCs) or biological fuel cells are biochemical system that drives energy by mimicking bacterial interactions found in nature. It converts chemical energy into electrical energy without any combustion reactions being carried out. In our research, soil MFC was constructed and bioelectricity was harvested from two different types of soil samples such as agricultural soil and dye industrial effluent soil. The production of electricity was measured by using power measurements and it was compared for both the soil samples. The dye industrial effluent soil produced 0.93V of electricity continuously for 650 hours whereas the agricultural soil produces 0.82V for 400 hours.

Key words: *Microbial Fuel Cells (MFC's), agriculture soil, dye industrial effluent soil, power measurement, electricity.*

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1. INTRODUCTION

Nowadays, Energy plays an important role in our life. Fossil fuels are depleted and the demand for alternative energy generation has an increasing trend. Renewable energy may be a suitable alternative for existing energy sources. Power generated from microbial fuel cell (MFC) is considered as renewable energy. MFCs provide new possibility for production of bio-energy from organic and inorganic sources [30]. The organic matters are converted to hydrogen in the presence of active biocatalysts in anode chamber under anaerobic condition. Generally, MFC consists of two compartments: an anaerobic anode and aerated cathode compartments in single chamber MFC. Microorganisms are used in MFCs to convert organic and inorganic compounds into bioelectricity. Pure or mixed culture of microorganisms can be used as biocatalyst in anaerobic anode chamber [21]. The concept of bioelectricity production was introduced in past few decades. Recently soil MFCs are effectively used in waste treatment and degradation of pollutions but application of MFC technology required more research in MFC construction and operation. In this work, applicability of soil MFC operation was checked in different soil sample to confirm the influence of soil in MFC technology.

2. MATERIALS AND METHODS

2.1 Sample collection

Two different types of soil samples were collected from two different sources namely S_1 soil sample collected from agricultural land and S_2 sample collected from dye industrial effluent soil near Madurai.The two samples were immediately sent to the laboratory for the inoculation of Soil MFC.

2.2. Soil testing

Soil testing has been accepted as a unique tool for rational fertilizer use. Soil testing is conducted to calculate the availability of nutrients to the plants, and to know the physical and chemical properties of the soil. The two soil samples S1 & S2 were send to agricultural laboratory.

2.3 Construction of SMFC

In this work, Mudwatt Microbial fuel cell was used for the electricity production for both the S1 and S2 samples. (Keego Technologies LLC, Stanford, USA) 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.

2.4 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

The power output from an MFC is calculated as

$$P = I \times V$$

Where P = power, W.

2.5 Isolation of Viable microorganisms from anode surface

After the experiment was over, anode graphite fiber was removed from the Soil MFC 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, 20 different colonies were selected based upon colony morphology.

2.6. Identification and characterization of organism

2.6.1. Colony Morphology

The size and morphology of the bacterial colonies was determined by the help of an ocular and stage microscope.

2.6.2 Gram staining

Smear of the 12 colonies were made on slides and gram staining techniques was carried out.

2.6.3. Genomic DNA isolation

Genomic DNA was isolated by Sharma and singh method 2005. All the DNA sample was checked by running the sample in 0.8% agarose gel.

2.6.3 16S rRNA gene amplification

16S rRNA gene was amplified using two specific universal primers 27F 5' - AGA GTT TGA TCC TGG TAC CTC AG - 3' and 1492R 5' – GGT TAC CTT GTT ACG ACT T – 3' by PCR and the amp icons were resolved by electrophoresis in 1% agarose gel. The fragments were visualized under UV transilluminator.

2.6.4. Polymerase Chain Reaction

The 16S rRNA gene was amplified with 50 µl reaction mixture containing 1X reaction buffer (10 mM Tris [pH 8.3], 50mM KCl, 1.5 mM MgCl2), 200 µl dNTPs, 0.05 U Taq DNA polymerase enzyme (Sigma, USA), 0.5µM each primer and 1 ng template DNA. The thermal cycling conditions were: 5 min at 94°C for initial denaturation; 31 cycles of 30 sec at 95°C, 1 min 30 sec at 54°C, 2 min at 72°C, and a final extension for 5 min at 72°C.

2.6.5. Phylogeny identification of bacteria by 16S rRNA gene typing

The sequences were compared with the 16S rDNA sequences available in the public databases from a BLAST search, and identified to the generic level. The sequences generated from the materials in this study, and retrieved from GenBank, were initially aligned using the CLUSTAL X program and the alignment then refined manually using version 3.0 of the PHYDIT program. A bootstrap analysis, using 1,000 replications, was performed to assess the relative stability of the branches.

3. SEM ANALYSIS

The morphologies of the anode and cathode surfaces were studied by using scanning electron microscope (SEM) (Hitachi, S570; Japan). The anodic samples were collected and fixed overnight with 2.5% paraformaldehyde and 1.5% glutaraldehyde in a buffer solution (0.1 M cacodylate, pH 7.5) at 4◦C, and then washed twice followed by stepwise dehydration in a gradient series of water/ethanol solutions (25, 50, 70, 85, 95, 100%), and then was critical-point dried (carbon dioxide). Samples were finally coated with Au/Pt before SEM observation

4. RESULTS AND DISCUSSION

4.1 Soil testing

The two soil samples S1 and S2 were sent to soil plant analytical advisory centre, Department of soil and environment, Agriculture College and Research Institute, Tamil Nadu Agriculture university, Madurai and the result were shown in the table 1(a) and 1(b).

Table- 1(a): Soil testing report for agricultural soil

Sample ID	Parameter	Unit	Value	Interpretation
S1	pH	-	8.12	Normal
	EC	ds/m	0.05	Harmless
	$KMnO_4$	Kg/ha	210	Low
	Olsen-P	Kg/ha	80	High
	$NNNH_4$-Ac-K	Kg/ha	1255	High
	Organic carbon	%	0.29	Low
	DTPA Fe	ppm	3.44	Deficient
	DTPA Mn	ppm	1.76	Deficient
	DTPA Zn	ppm	2.09	Sufficient
	DTPA Cu	ppm	1.20	Sufficient

Table-1(b): Soil testing report for dye industrial effluent soil

Sample ID	Parameter	Unit	Value	Interpretation
S2	pH	-	7.80	Normal
	EC	ds/m	0.04	Harmless
	$KMnO_4$	Kg/ha	73	Low
	Olsen-P	Kg/ha	20	Medium
	$NNNH_4$-Ac-K	Kg/ha	588	High
	Organic carbon	%	0.26	Low
	DTPA Fe	ppm	5.62	Sufficient
	DTPA Mn	ppm	6.89	Sufficient
	DTPA Zn	ppm	2.17	Sufficient
	DTPA Cu	ppm	2.14	Sufficient

4.2 Microbial Fuel Cell Design and construction

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

Fig-1: Single Chamber Microbial Fuel Cell

4.3 Electricity production

Soil Microbial fuel cell was designed by Keego Technologies LLC, 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 SMFC 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. (Fig-2) The dye industrial effluent soil was considered to result in the electrode potential and then an increase after 520 hrs. (Fig-3)

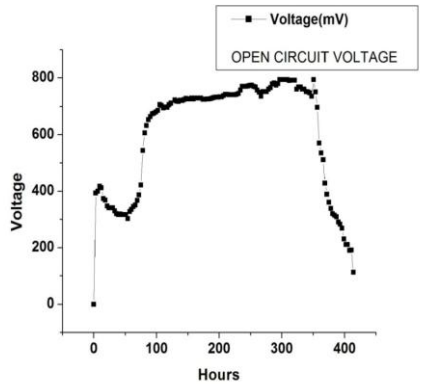


Fig-2: Graphical representation of voltage reading in agricultural soil sample

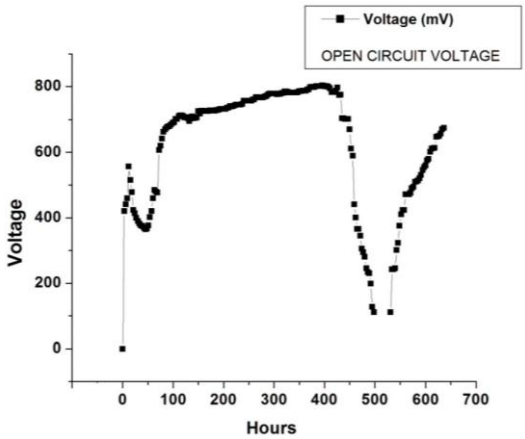
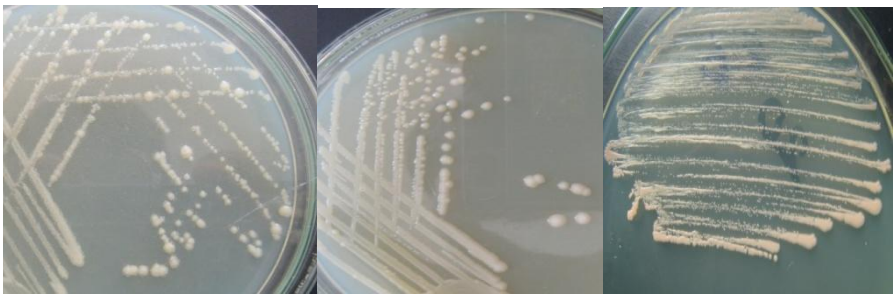


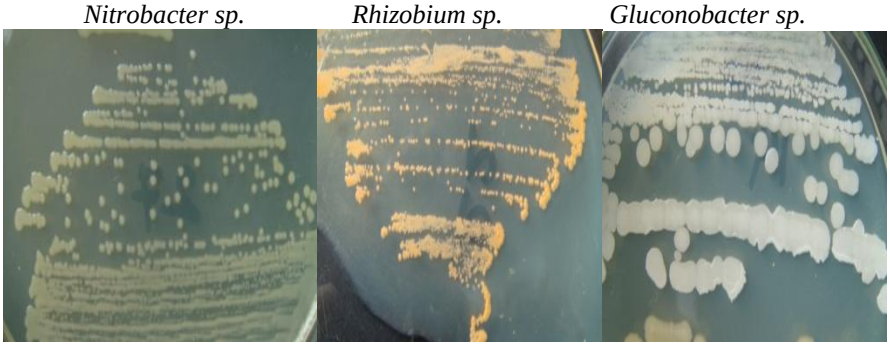
Fig-3: Graphical representation of voltage reading in dye industrial effluent soil sample.

4.4 Isolation of microorganisms from the anode surface

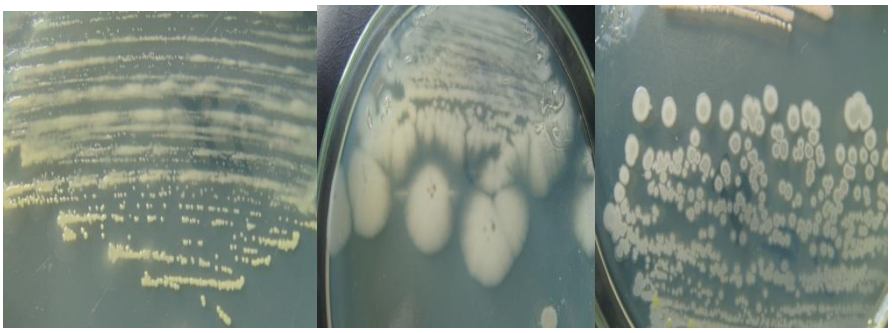
Isolated microorganisms grown on nutrient agar plates were sub cultured. The 12 different bacterial species were identified as *Nitrobacter sp., Rhizobium sp., Gluconobacter sp., Bacillus sp., Stenotrophomonas sp., Escherichia sp., Proteus sp., Aeromonas sp., Azotobacter sp.,* and *Pseudomonas sp*.



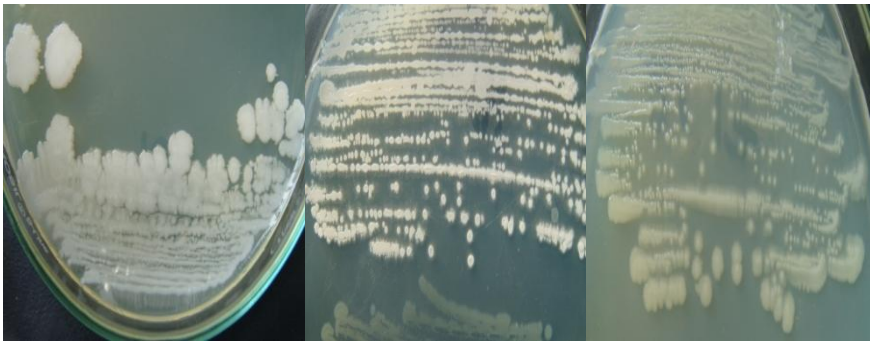
Nitrobacter sp. *Rhizobium sp.* *Gluconobacter sp.*



Bacillus sp. *Stenotrophomonas sp.* *Escherichia sp.*



Proteus sp. *Aeromonas sp.* *Azotobacter sp.*



Pseudomonas sp. *Pseudomonas sp.* *Pseudomonas sp.*

Fig-4: Isolation of viable microorganisms from anode surface

4.5 Gram Staining

Gram staining was done for the 12 different bacterial species and the results were obtained as shown in table 2.

Table -2: Gram's staining of 12 bacterial species.

SPECIES	SHAPE	TYPE	BACTERIA
Nitrobacter sp.	Rod	Gram negative	Facultative anaerobic
Rhizobium sp.	Rod	Gram negative	Aerobic
Gluconobacter sp.	Rod	Gram negative	Obligate aerobic
Bacillus sp.	Rod	Gram positive	Obligate aerobic
Stenotrophomonas sp.	Rod	Gram negative	Obligate aerobic
Escherichia sp.	Rod	Gram negative	Facultative anaerobic
Proteus sp.	Rod	Gram negative	Facultative anaerobic
Azotobacter sp.	Rod	Gram negative	Aerobic
Aeromonas sp.	Rod	Gram negative	Facultative anaerobic
Pseudomonas sp.	Rod	Gram negative	Facultative anaerobic
Pseudomonas sp.	Rod	Gram negative	Facultative anaerobic
Pseudomonas sp.	Rod	Gram negative	Facultative anaerobic

4.6 Genomic DNA isolation

Genomic DNA was isolated by Sharma and singh method 2005. All the DNA sample was checked by running the sample in 0.8% agarose gel. (Fig. 5).

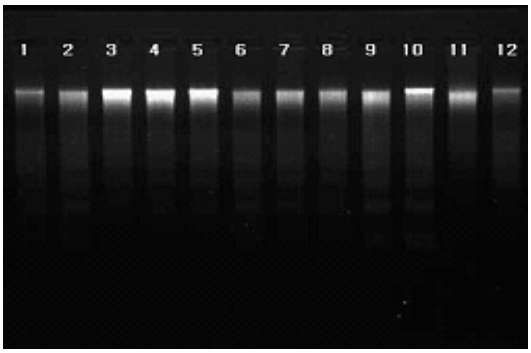


Fig - 5: Agarose gel showing the band of extracted genomic DNA from the 12 bacterial species.

4.7 PCR amplification with Phylogenetic marker gene primer

PCR amplicons were resolved by electrophoresis in 1% Agarose gel to confirm the expected size of the product of 1500bp. The gel picture (Fig. 6.) shows all three templates were amplified of 1500bp size.

FP: 27F 5'-AGA GTT TGA TCC TGG CTC AG-3'

RP: 1492R 5'-GGT TAC CTT GTT ACG ACT T-3'

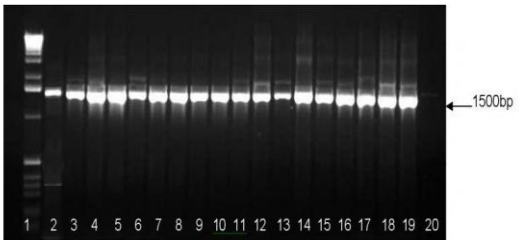


Fig-6: Agarose gel showing the band of amplified 16SrDNA of the 12 bacterial species and their size is 1500bp.

4.8 Phylogeny identification of bacteria by 16S rRNA gene typing

The taxonomy of the 12 different bacterial species was identified and the results were shown as follows.

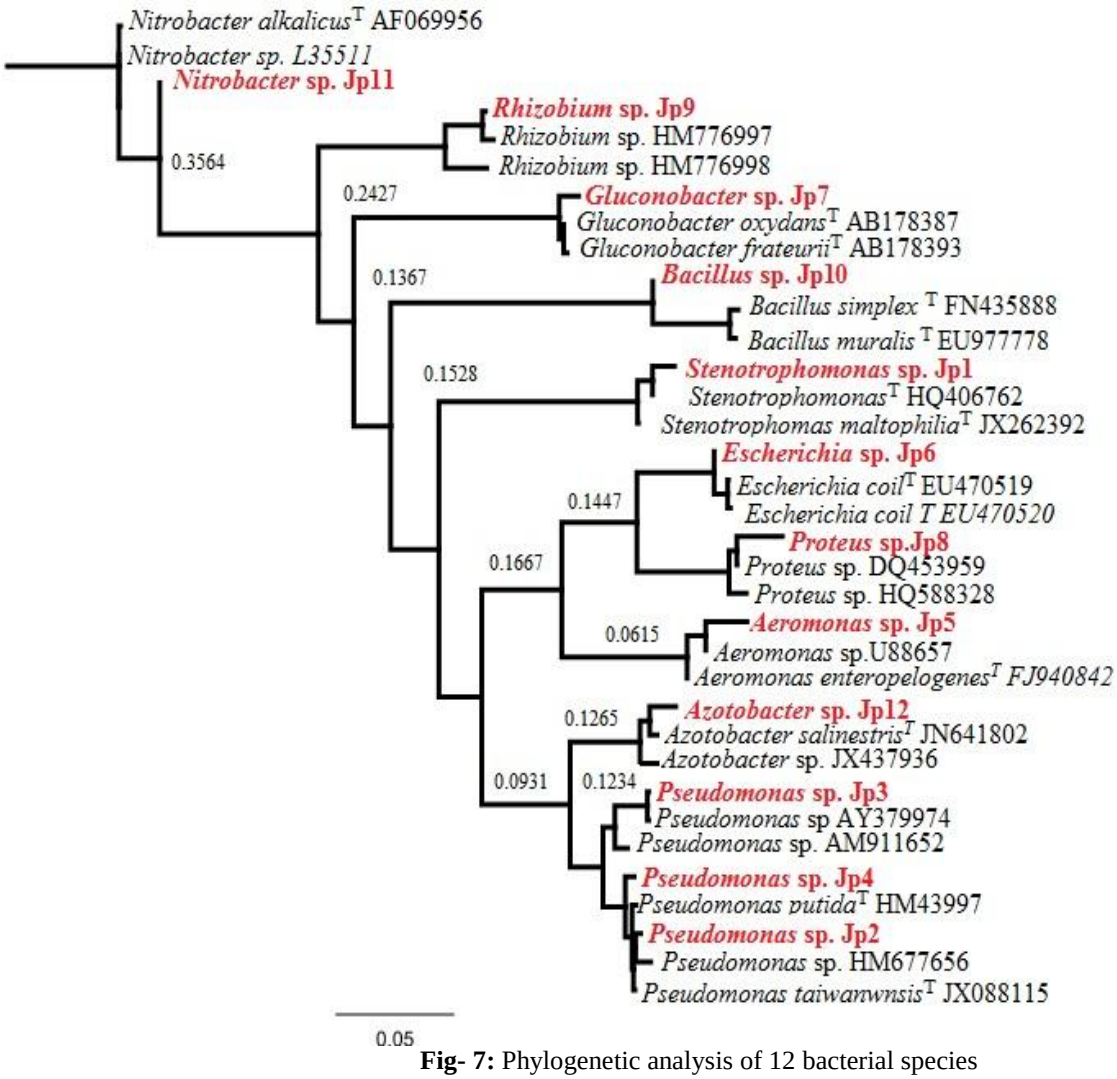
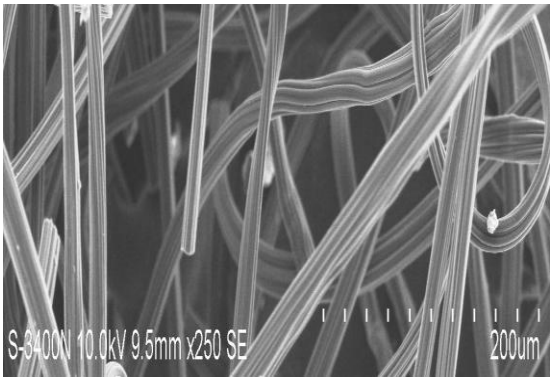


Fig- 7: Phylogenetic analysis of 12 bacterial species

4.9 Analysis of SEM images

The surface images of the graphite electrode were successfully obtained by SEM. The image from the surface of graphite electrode before and after experimental run was taken. The sample size was 1.5 × 1.5 cm for SEM analysis. Figure 8.a. and 8.b. showed the outer surface of the graphite electrode prior to and after use in the MFC respectively. These images demonstrated that microorganisms were grown on the graphite surface as attached biofilm.



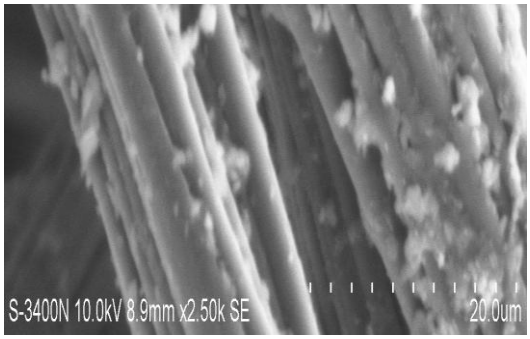


Fig-8: Scanning electron micrographs on carbon-based materials before and after biofilm formation. **(a)** Graphite fiber without biofilm **(b)** Graphite fiber with biofilm.

CONCLUSION

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 be

helpful 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.

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