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A Preliminary Study on Comparing the Efficacy of Anaerobic Inocula and Facultative Inocula for Application in Microbial Fuel Cells

Gunaseelan K[1], Gajalakshmi S[2] & Kamaraj Sathish-Kumar[3]

[1&2]Sustainable Fuel Cells Technology Lab, Centre for Pollution Control & Environmental Engineering, Pondicherry University, Puducherry, India.

[3]Instituto Tecnológico El Llano (ITEL)/Tecnológico Nacional de México (TecNM), Laboratorio de Cultivo de Tejidos Vegetales, Aguascalientes. Km 18 Carretera Agua ascalientes-San Luis Potosí, El Llano Ags., México.

C.P. 20330.

[1]gunsie3@gmail.com, [2]dr.s.gajalakshmi@gmail.com, [3]sathish.bot@gmail.com

Abstract - In the present work, for the first time, a preliminary study on the possibility of assessing the efficacy of two microbial inocula sources namely the anaerobic and facultative as the biocatalyst in the microbial fuel cells for treating synthetic wastewater was attempted. The efficacy of both the inocula was evaluated through Coulombic efficiency, COD removal, Power density, electrochemical kinetics studies like Cyclic voltammetry and Electrochemical Impedance spectroscopy. The performance of reactor with both the inocula was evaluated for a period of 30 days under the batch mode of operation.

The Coulombic efficiency of anaerobic inocula (MFC-S) was higher when compared to the facultative inocula (MFC-P), due to the higher organic substrate's oxidation, free electrons generation and rapid microbial biofilm formation over the surface of the graphite felt anodes. The anaerobic inocula from septic tank sludge (MFCs-S) reactors has shown higher power density of 1296.5 ± 17.3 mW/m^3 which was higher than the MFC inoculated with the facultative inocula from Panchagavya solution (MFCs-P) reactors under the same operational conditions. The maximum redox current of 4 mA during cyclic voltammetry was noticed in the anaerobic inocula (MFC-S) as compared to facultative inocula (MFC-P) with 2 mA which reveals that a greater number of free electrons were available in the anodic chamber along with an accelerated Extracellular Electron Transfer (EET) mechanism.

Keywords - Anaerobic Inocula, Electro kinetics, Facultative Inocula, Microbial fuel cells, Power generation, and wastewater treatment.

I. INTRODUCTION

Microbial fuel cell (MFC) is a biological electrochemical system with the ability of converting chemical energy into electrical energy due to the bio-catalytic process of exo-electrogenic bacterial communities under anaerobic conditions [1], [2].

MFC reactors can be fabricated by using several materials and various configurations. The MFC system functions mainly on the basis of anodic oxidation and cathodic reduction reactions. The anode compartment assists the generation of protons and electrons **(Fig.1)**. The generated protons reach the cathode through the proton exchange membrane (PEM) and result with the developed potential difference.

In the earlier of the nineteenth century, it was considered that only a few microorganisms namely exoelectrogens can be employed to produce the electricity. With advancement in research the potential of various diverse microorganisms as a biocatalyst in MFC has been reported. The earliest MFC theory was proved by Potter in 1910 with the *Escherichia coli* and *Saccharomyces sp.* cultures for generating electricity along with the platinum electrodes [3]. Nevertheless, due to not drawing appreciations and attention till the 1980s, this MFC concept drew a huge response only after the advent and use of electron mediators for improving the electricity generation with many folds [4].

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In general, the bacteria are used to generate electricity in MFC reactors by while degrading organic substances or substrates or wastes [4], [5].

Several categories of wastewaters have been treated successfully and reported by employing MFCs, which includes brewery, domestic, tannery, animal, agro and food processing wastewaters [6] , by the mechanism of removing the organic pollutants in wastewaters along with the valuable forms of energy like electrical power or hydrogen gas [4], [7] or valuable algal lipid extraction [8] for biodiesel production.

In the recent past, Microbial Fuel Cell Technology has witnessed advancement in electricity generation and removal of pollutants from the wastewater thereby achieving wastewater treatment. Yet, there are several limitations encountered during the operation of the MFCs in the real-time with special context to the microbial inocula handling and high performance. With the ultimate motto of assessing the efficacy of the two different inocula sources to overcome all the existing challenges, this study was conceived.

II. MATERIALS AND METHODS

A.Sources of Inocula and Substrates

The anodic chamber of MFC (triplicates) namely MFCs S (1-3) was started with anaerobic sludge from the septic tank and the anodic chamber of MFC (triplicates) namely MFCs P (1-3) was inoculated with facultative Panchagavya inocula [9]–[11] after a thermal treatment at 100°C for the time duration of 15 minutes [12]. This is to ensure the proper suppression of methanogenic bacterial colonies as testified in the previous literature [13]. As an inocula, 20 mL of this pre-treated inocula consortia sludge like UASB and Panchagavya source was used in the anodic compartments of the two variants of triplicate MFCs named MFC S (1-3) and MFC P (1-3) respectively.

As a source of the substrate in the anodic chamber, the synthetic wastewater with the pH of 7 comprising sodium acetate as the major source of carbon with chemical oxygen demand (COD) of about 1000 mg/ L was used in this work. The sodium acetate medium was made from 1281 mg/ L CH3COONa, 250 mg/ L $CaCl_2.2H_2O$, 318mg/L NH_4Cl, 27 mg/L K_2HPO_4, 1500 mg/ L $NaHCO_3$, 9 mg/L KH_2PO^4, 64mg/ L $MgSO_4.7H_2O$ and other trace minerals like Fe, Zn, Ni, Co, Mn, Mo, and Cu [12], [14].

B.Dual Chamber MFC construction and Operation

For this study triplicates of identical dual -chambered MFCs were employed to study the performance of the two inocula, the reactors were fabricated using the Polyacrylic sheet (Plexiglas) materials with an anodic chamber volume and a cathodic chamber volume of 90 ml each. Both the compartment of the MFC reactor were provided with two ports at the top of the reactors for electrode wire and the other for the multiple utilities like the addition of influent, sampling and for reference electrodes.

In all the MFCs, graphite felt with the effective surface area of 20 cm^2 was used both as the anode and cathode. The Nafion$^{®}$ membrane of projected size 4x 4 cm was inserted between the two compartments of the MFC reactor to ensure the migration of H + ions from anodic compartment to cathodic compartment reaction interface exchange through a tightly packed poly acrylic sheet frame.

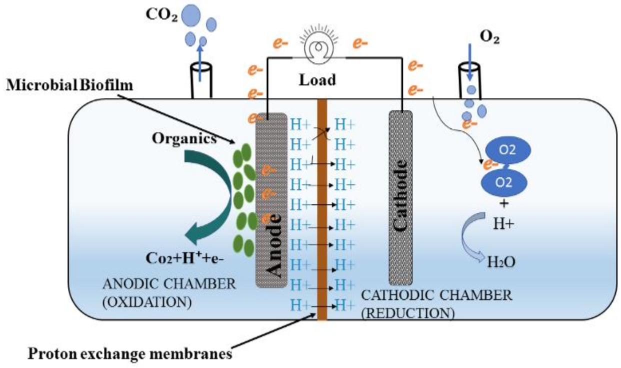

Figure 1. Dual chamber Microbial Fuel cell

Both the compartments and the electrode membrane assembly (EMA) of the whole MFCs were held together tightly with nut-bolt joints to ensure zero leakage. Graphite felt anode and cathode of the MFCs were connected with a high-grade concealed copper wire's joints sealed with silica gel to ensure oxidation and also with an external load of 1000 Ω resistor after the first five days reaction cycle.

These fabricated triplicates were designated as MFC U-1, MFC U-2, MFC U-3, MFC P-1, MFC P-2 and MFC P-3 based on the selection of the inocula in the anodic chambers, which were used namely septic tank sludge inocula reactors (MFC S 1-3) and facultative Panchagavya inocula reactors (MFC P 1-3) respectively.

These triplicates MFCs were operated under batch mode for one month with a fresh feeding interval of 5 days reaction cycle under the ambient temperature varying from 33 to 37 °C. The preliminary observation studies were taken for multiple numbers of reaction cycles in triplicate, henceforth the reliability in the performance of MFCs can be obtained.

C.Chemical Analysis and Calculation

During the MFCs operation, the influent and effluent synthetic wastewater's parameters namely pH and COD concentrations were measured according to APHA 2005.

The performance of MFC with the two different inocula was evaluated in terms of Open circuit voltage, power density, COD removal, Coulombic efficiency and

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electrochemical kinetics studies with cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

Open circuit voltage (OCV) and Operating voltage (OV) over 1000 ohms resistance were measured with a digital multimeter unit (CIE, Model no.122) after reaching the stable output value.

To compare the performance of the different MFC reactor output, power is very often adopted and used as a key characteristic of the reactors. The Power density (**P_{An}**, mW/m^2) can be determined with the effective surface area of the anode (**A_{An}**) and the volumetric power (**$P_{v,\,m}$**W/m^3) with the normalized reactor's anodic volume [15]–[19].

Coulombic efficiency (CE) is known as the ratio of total Coulombs got transported from the substrate to the anode region, to the peak probable Coulombs due to produced current by overall substrate removal [14], [15].

$$CE = \left(M \int_0^{tb} I\, dt\right) / \left(FbVAn\ \Delta COD\right)$$

The overall Coulombs gained can be calculated by integrating the current over time, consequently. The Coulombic efficiency for an MFC run in batch mode. **CE** can be estimated over a period of time **t_b** [15], [20], [21] where **M**=32, the molecular weight of oxygen, **F** is Faraday's constant (96485 C/mol), **b**=4 is the number of electrons replaced per mole of oxygen, **V_{An}** is the volume of liquid in the anode chamber , and **ΔCOD** is the difference in the influent and effluent COD over time **t_b.**

Cyclic voltammetry (CV) is the most widely adopted technique to analyze the electrochemical kinetic parameter namely the redox reaction in an MFC system. The direction of potential get swiped amongst two fixed points and the subsequent current is recorded by this cyclic voltammetry technique [22]. A full cell MFCs along with a three-electrode set-up comprising of a graphite felt working electrode (WE), Ag/AgCl reference electrode (RE), and a graphite felt counter electrode (CE) were used to perform the cyclic voltammetry (CV) experiment from -1 to +1 V at a slow scan rate of 5 mV/s and the generated current–potential polarization curves where recorded by using SQUIDSTAT PLUS Potentiostat (Admiral Instrument, USA).

Electrochemical Impedance Spectroscopy (EIS) is one of the powerful tools for exploring the created internal resistance, several chemical, and physical processes in solutions, at solid-solid interfaces and at solid-liquid interfaces, due to the phenomenon of different voltage loss separation [23], [24].

EIS analysis was carried for the MFCs in a frequency range of 100 kHz to 1 MHz with an AC excitation amplitude of 10 mA. EIS was performed by using SQUIDSTAT PLUS Potentiostat (Admiral Instrument, USA). The Nyquist plot and equivalent circuit model fitting were performed to calculate the variuos forms of created resistance within the MFC setup.

III. RESULTS AND DISCUSSIONS

A. VOLTAGE AND POWER PRODUCTION

Among the MFCs of two inocula sources, the reactors with anaerobic inocula reactors (MFCs-S) resulted with the highest operating voltage of 332.06 ± 2.26 mV across a 1000 ohms external resistance at the 25th day of operation after achieving a stable OCV of above 700 mv. The facultative inocula reactors (MFCs-P) resulted with the lower operating voltage of 261.955 ± 1.88 mV across a 1000 ohms resistance. Both the triplicates of both the inocula sources of MFCs reported with a similar average open circuit voltage (OCV) from the range of 401.30 ± 1.77 mV till the entire 30 days of operation **(Tab.I)**. The generation of power was recorded after the 25th day by introducing a 1000 ohms resistance across anode and cathode of the MFCs. The anaerobic inocula reactors (MFCs-S) performed with the generation of the higher volumetric power density of 1296.5 ± 17.3 mW/m^3 than the facultative inocula reactors (MFCs-P) with 806.26 ± 11.9 mW/ m^3.

Table I. Voltage generation in MFCs with two Inocula Types

Reactor Name	Inocula Type	Avg. OCV (mV)	Avg.OV (mV)
MFCs-S	Anaerobic (Septic tank)	401.30 ± 1.77	332.06 ± 2.26
MFCs-P	Facultative (Panchagavya)	295.22 ± 1.30	261.955 ± 1.88

The difference in the variation of volumetric power densities among the MFCs with different inocula sources might be due to the difference in organic matter oxidation, free electrons generation, better Extracellular Electron Transfer (EET) and stable biofilms over the graphite felt anode. From this present study, the anaerobic inocula from septic tank sludge (MFC-S) resulted with the improved operating voltage (OV) and the increased power density outputs.

B. COD REMOVAL AND COULOMBIC EFFICIENCY

The reactors with anaerobic inocula (MFCs-S) have shown higher average COD removal efficiencies of 69.68 ± 0.90 % than the reactor with facultative inocula reactors (MFCs-P) of 54.31 ± 0.72 %**(Fig.2).**

However, the COD removal efficiencies of the facultative inocula reactors (MFCs-P) was lower, which may be due to the less capability to oxidize the provided organic loads in the synthetic wastewater and challenges in adapting to the anaerobic condition in the anodic compartment rather than a facultative condition.

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Table II. Evaluation of Performance in MFCs with different Inocula Types

Reactor Name	Inocula Type	COD Removal (%)	Coulombic Efficiency (%)	Power Density	
				mW/m^2	mW/m^3
MFCs-S	Anaerobic Sludge (Septic tank)	69.68 ± 0.90	21.39 ± 0 .77	55.1 ± 0.73	1296.5 ± 17.3
MFCs-P	Facultative (Panchagavya)	54.31 ± 0.72	11.11 ± 0.23	34.26 ± 0.50	806.26 ± 11.9

A linear trend was reported in CE along with the volumetric power generation in the case of anaerobic inocula reactors (MFCs-S) with an average CE of 21.39 ± 0 .77 % **(Tab.II),** which is the highest than the other type of MFCs with 11.11 ± 0.23 %.

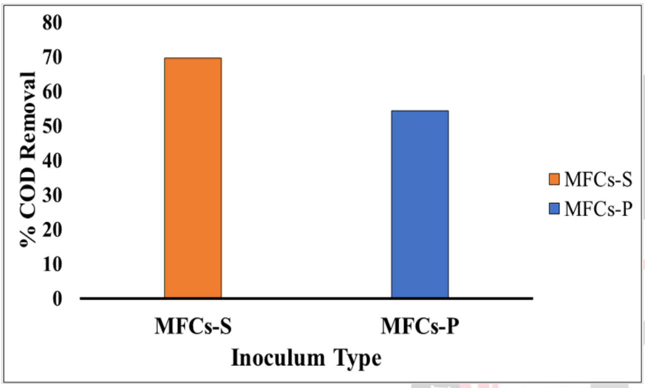

Figure 2. COD removal efficiency of the two Inocula

From this study, the COD removal and CE was analyzed which directly determines the efficacy of the two sources of the inocula. Even though the CE of the anaerobic inocula reactors (MFCs-S) was lower than the previously reported studies it has shown promising results than the facultative inocula reactors (MFCs-P) used in the present study.

C. ELECTROCHEMICAL KINETICS

1) CYCLIC VOLTAMMETRY

Cyclic voltammetry (CV) is used for the study of the extracellular electron-transfer of electroactive microbial biofilms (Harnisch and Freguia 2012).

In the present study, CV analysis revealed that the substantial redox peaks were perceived with the scanning voltage from -1 to +1 V. The highest oxidation current of 4 mA and 2.5 mA were achieved by anaerobic inocula reactors (MFCs-S) and the facultative inocula reactors (MFCs-P) respectively **(Fig.3 & Fig.4)**. The maximum redox current was observed in case of MFCs-S as compared to MFC-P essentially due to faster extracellular electron transfer (EET) and increase in electrochemically active microbial biofilm's surface area.

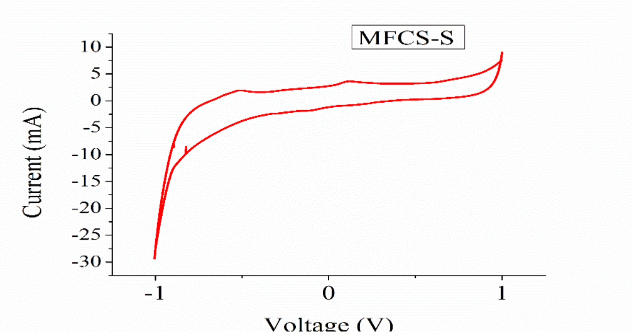

Figure 3. CV Plot for anaerobic inocula MFCs-S

The noteworthy increase in maximum current produced by anaerobic inocula reactors (MFCs-S) reveals that the number of free electrons was available due to their bioelectrocatalytic- metabolic activities either instigated by a developing biofilm density over the anode surface, or by an intensification of membrane-bound electron transfer proteins in each of the bacterial cells [26]–[29].

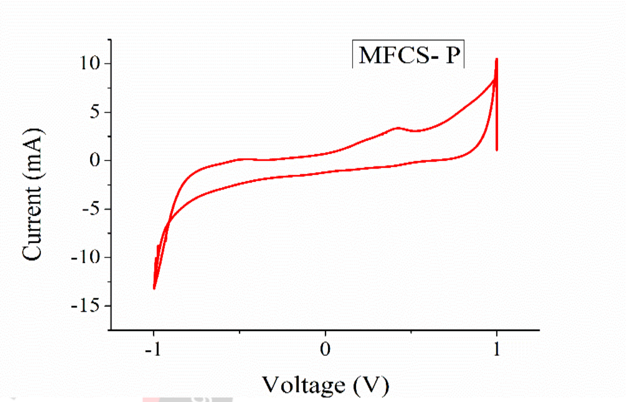

Figure 4. CV Plot for facultative inocula MFCs-P

2) EIS

Electrochemical Impedance Spectroscopy is a non-destructive and powerful technique which can provide numerous information about all the capacitive, resistive, and inductive contributors of an MFC system in a very short time of operation by fitting with the suitable equivalent circuit model [22], [30]–[32].

The Nyquist plot contains a semicircle and a linear portion line which representing charge-transfer resistance (R_{ct}) and Warburg diffusion resistance (W), respectively(Wang et al. 2009). the Solution resistance (Rs) was calculated by measuring the difference between the values from the origin to the initial start of the semicircle.

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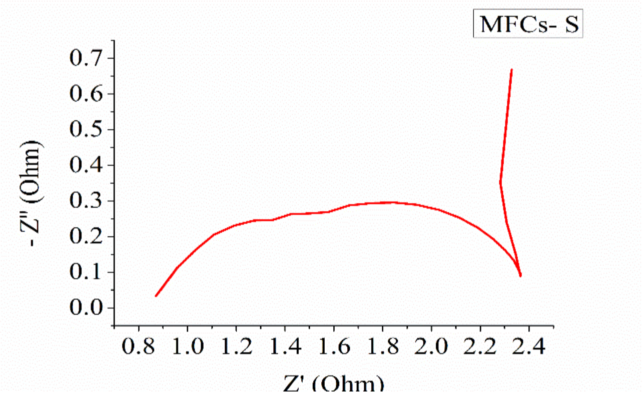

Figure 5. EIS Plot for Anaerobic Inocula MFCs-S

Through the Nyquist plot and suitable equivalent circuit model, the highly reliable Solution resistance (Rs) and charge-transfer resistance (R_{ct}) was found **(Fig. 5 & Fig. 6)**. The R_s and R_{ct} values for the reactors namely MFC-S {0.9 and 1.6} and MFC-P {0.8 and 2.8} respectively.

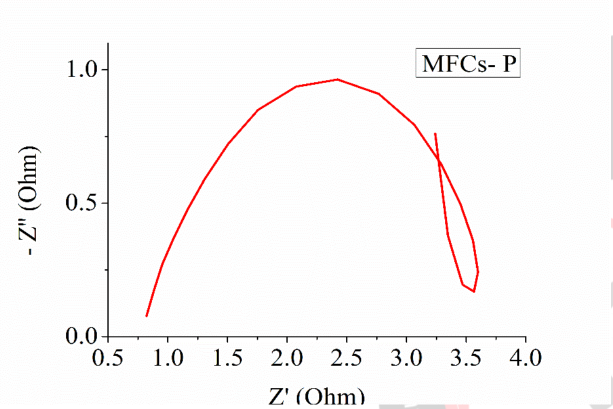

Figure 6. EIS Plot for Facultative Inocula MFCs-P

The polarization resistance was less in MFC-S, due to the fall in over-potential loss in this MFC reactor. Warburg diffusion resistance (W) of MFC-S was on the higher side than MFC-P which indicates that has been maximum mass transport loss in the anode [34]. Thus, the MFC with the anaerobic inocula resulted with better power output which is due to a fall in the overall internal resistance of the cell.

IV. CONCLUSIONS

The preliminary study on assessing the efficacy of the two microbial inocula sources namely the anaerobic and facultative as the biocatalyst in the microbial fuel cells for treating the synthetic wastewater shows that the reactors with anaerobic inocula (MFCs-S) has exhibited higher power density, Coulombic efficiency, COD removal, and maximum redox current than the MFC inoculated with the facultative inocula (Panchagavya) under the same operational conditions. This finding provides huge scope of using the anaerobic inocula reactors (MFCs-S) as a better source of the biocatalyst to ensure the optimum performance in MFCs for treating the wastewater.

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