Abstract

As a way of enhancing the potential of electrodes to harness sustainable electricity, Multi walled Carbon Nano Tubes (CNTs) coated stainless steel mesh-200 (SSM) electrode was fabricated and compared with commonly used graphite plate electrode. The efficiency was ascertained in terms of output voltage over an external resistance of 100 Ohms for a period of 30 days and by Electrochemical impedance spectroscopy study. The output voltage was higher in MFC fitted with CNT coated SSM electrode than MFC with graphite electrode. Electrochemical impedance spectroscopy study indicated lesser internal resistance in MFC with CNT coated SSM electrode generated higher voltage than graphite electrode. SEM images of CNT coating and bacterial colonies on electrodes are presented in support of the findings. This study is first in its kind, involving Electrochemical Impedance Spectroscopic studies in a cow-dung fed Microbial fuel cells. From this study, it is demonstrated that CNT coated SSM electrode is the better choice for cow dung slurry-fed Microbial fuel cell to harness sustained electricity generation.

Keywords: Microbial fuel cell - cow dung slurry- carbon nano tubes (CNT) – Scanning Electron Microscope (SEM) - Stainless Steel Mesh (SSM) - electricity generation.
Introduction

In the recent past, Microbial fuel cell technology experiences a new momentum in its advancement as it is an interesting but challenging field of research focussing on green as well as renewable energy. Efficiency of Microbial Fuel Cell (MFC) depends on the kinetics of the electrode reactions within the fuel cell. The surface area of electrodes greatly influences the output power from MFCs. The easiest way to decrease the resistance is to increase the effective surface area (Rismani-Yazdi, 2008). By improving the active surface area of the electrode, the internal resistance of the system can be reduced. A wide range of materials has been employed to improve the performance of MFCs. More specifically the anode material significantly influences the biofilm formation and the electron transfer between the microorganism and the electron acceptor. It is well studied that microbial communities that constitute biofilm are the energy-producing nano-factories in MFCs. Therefore, it is more important to choose an efficient and biocompatible electrode material for a perfect system. In that pursuit, carbon-based nano-materials have emerged as promising materials for the purpose of both anode and cathode.

Carbon-based materials are widely used because of their high conductivity, biocompatibility and chemical stability as well as low cost (Mustakeem, 2015). Composite materials have also shown to have the potential to become materials of choice for electrode manufacturers (Zhang et al 2015). A variety of compost materials have been tried in MFCs including carbon rods, carbon cloth, carbon fibers, and stainless steel mesh. The glassy carbon electrode was coated with multiwalled CNTs and used as electrode in anode chamber. It was then reported an increase in current density by a factor of 82 compared with a bare glassy carbon electrode using the same experimental conditions. Additionally, it was found that the multiwalled CNTs increase the ability of the endogenous mediators like cytochrome present in the bacterial membrane (Peng et al 2010). Ultimately, carbon nanotubes (CNTs) and allotropes of carbon, happened to be promising alternative material for MFC electrodes because of their unique electrical conductivity, chemical stability, biocompatibility, high specific area with catalytic properties. It is reported that CNTs have strong cell adhesion, cell attachment, and growth properties. CNTs over the carbon cloth to form a highly conductive anode of MFC with a large surface area and found that the maximum power density was improved by 250%. Microbes grown over CNTs result in an excellent charge transfer characteristics due to π–π stacking between the carbon atoms of graphite and the pili.
cellular outgrowth) of microorganisms. In order to assess the potential of the CNT coating on an SSM electrode, an experiment was carried out to compare the potential of this nano-composted electrode with graphite plate.

Materials and methods

2. Materials and Methods

A traditional "H" shape twin-chambered MFC fabricated from inexpensive materials which includes two polycarbonate container (500ml), PVC pipe, adhesive glue, (PEM-7001S) having 0.45mm thickness, graphite plate electrodes, Cow dung slurry as a substrate, Resistor 100ohms, and a digital multi-meter.

2.1 Fabrication of MFC

Two polycarbonate containers (500mL) were used as an anode and cathode chambers. A PVC pipe having the diameter equivalent to that of PEM (7001S) derived from Membrane International USA has been embraced to the chambers using an adhesive which makes the module leak proof. The membrane was made up of polyvinyl alcohol sulphisuccinic acid. The membrane with 4 cm diameter was soaked in saline water overnight to make the pores to open appropriately and dried in the shade. Two holes were made on the cap of the containers to insert copper conductor/wire which holds the electrodes. A digital multimeter was used to record the generated voltage on external resistance of 100 ohms. This experiment was extended for a period of thirty days. The designed and fabricated H-shaped twin-chambered MFC-II and III are shown in figure 1 &2.

Preparation of anolyte (cow dung slurry) and Catholyte (KMnO₄)

Fresh cow dung weighing about 1kg was collected directly from the nearby dairy farm and allowed to dry under shade for three days. After three days the top rigid layer of the cow dung was removed about 50 grams of the cow dung below with wetness was separated and made into slurry in 500ml of water and used as anolyte in both MFCs.

The efficiency of MFC also depends on catholyte and its concentration. KMnO₄ and K₂Cr₂O₇ are commonly used as catholyte, but potassium ferrocyanide is reported to produce higher electricity than KMnO₄. Although the standard redox potential of ferricyanide is not as high
as that of oxygen, it has much lower potential, which results in a faster reaction rate with much higher power output (Rabaey et al, 2003, Schröder et al, 2003, Aelterman et al, 2006). However, use of ferrocyanide outside the laboratory is not practical due to its high toxicity, and it requires frequent replacement as it is insufficient for re-oxidation by Oxygen in the cathode chamber (Rabaey et al. 2005), Logan and Regan, 2006). Therefore, in this study, the cathode chamber was filled with 500ml of water which contains 2% potassium permanganate solution which is a powerful oxidizing agent.

Preparation of electrodes

The electrode must be conductive and chemically non-reactive in the MFC/reactor. The commonly referred electrode material is carbon, which is available in the form of graphite plates, rods or granules. Two graphite plates (8.0cm× 4cm × 0.5 cm), with a surface area of 76 cm²) were used as electrodes. The electrodes were boiled in de-ionized water and soaked overnight in 1M of HCl solution and rinsed in de-ionized water for activation. Copper wires are fused with the electrodes and sealed with epoxy resin.

Surface modification in SSM with CNT coating

The surface of electrode has been modification as described by Zhang et al. (2013). The SSM electrode for CNTcoating was etched in a mixture of sulphuric acid and Nitric acid, followed by repeated washing in distilled water till acidity of washings become neutral. Then, the electrode was oven dried for one hour at 40°C. Multiwalled carbon nanotubes are the products of Sigma Aldrich, Mumbai. For anodic modification, carbon ink using CNT was prepared by dispersing 20 mg of multiwall CNTs (MW-CNTs) with an average diameter of 50 nm, a length between 10 and 20 μm, in 95% ethanol (10 mL). Ethanol with MW-CNTs was ultrasonicated till a homogeneous solution was obtained. The etched SSM (3 cm X 4cm) with an average hole diameter of 40 μm, thickness 100 μm, and wire diameter 50 μm) was then dipped into the carbon ink ( CNTs dispersed in ethanol). Finally, it was baked at 150°C for one hour to obtain SSM coated with MW-CNTs. (Zhang, et al. 2013). This SSM with CNT coating was fitted in the MFC-III as anode and graphite at the anode in MFC-II. The total surface area of SSM electrode was 46cm² and graphite electrode was 76cm².

Execution and data collection

Both systems MFC-II and MFC-III were operative for a period of thirty days. Data of generated voltage were recorded using Multimeter everyday and fed into Origin Pro8.5
software for obtaining graphs depicting all electrical parameters of MFCs. The system was connected to an external resistance of 100 ohms. Current, Power, Current density and Power density were calculated. All these data are graphically presented.

**Electrochemical Impedance Spectroscopy (EIS)**

To understand the status of oxidation-reduction reactions in terms of impedance, both systems were subjected to electrochemical impedance spectroscopic study. The impedance of the MFC, are normally expressed in terms of CPE as,

\[ Z = R_s + R_a/(1 + Q_a(j\omega)^{-\alpha_a}) + R_c/(1 + Q_c(j\omega)^{-\alpha_c}) \] ---- (1)

The CPE parameters \(\alpha\) and \(Q\) are independent of frequency. Here \(Q\) has the numerical value of the admittance and \(\alpha\) is the exponent which indicates the capacitive or resistive character of the system and the value of the exponent varies in between \((0 - 1)\). The lower limit suggests that the system is purely resistive and the upper value shows complete capacitive nature.

The effective capacitance are calculated using the following equation,

\[ C = (Q \times R)^{(1/n)} / R \]

The Nyquist plot and Bode plot for MFC-II and III were generated and the fitted parameters and effective capacitance were found out.

**Scanning Electron Microscopic (SEM) studies**

SEM images of SSM mesh before and after CNT coating, a bacterial film on both anode (CNT coated SSM) and graphite(MFC-II) as an anode were obtained using SEM ((Jeol SEM (JSM-T20). EIS study was carried out at Central Instrumentation Facility (CIF), Pondicherry University. Bacterial species present in samples are identified by the method of Metagenomics and MALDI TOF MS

**Result**

MFC-II and MFC-III set up used in the study were given in (Fig.1 & 2). Data collected on generated output voltage in each MFC-II were reported as: Maximum voltage was recorded (1.12V) on the 13th day, From 11th day to 21st day it showed 1.0V and above. More or less a sustained output voltage of 1.12 was recorded for 7 days (from 12th to 17th day) in a 30 days cycle. In MFC-III with SSM electrode, there was an early shoot up in
output voltage and reached a maximum 1.17V on 8th day itself. From 4\textsuperscript{th} day to 21\textsuperscript{st} day it showed 1.0V and above. More or less a sustained voltage of 1.1V was noticed from (6th to 12th day) in a 30 days cycle (Fig.3). The calculated current density was 1.4711A/m\textsuperscript{2} and Power density was 1.6443w/m\textsuperscript{2} for MFC-II and 1.5395A/m\textsuperscript{2} and 1.8012W/m\textsuperscript{2} respectively for MFC-III which is depicted in (Fig.4 & 5).

Fig.1. MFC-II

Fig.2. MFC-III

Fig.3. Generated voltage in MF- II and MFC-III in a 30 days cycle
Fig. 4. Output voltage and calculated current density & power density in MFC-II

Fig. 5. Output voltage and calculated current density & power density in MFC-III
Table 1  The fitted parameters and effective capacitance

<table>
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<th>S.No</th>
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<td>1</td>
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MFC-II with graphite electrode; MFC-III with CNT coated SSM electrode
Surface morphology of bare graphite electrode under microscope and SEM images of the SSM electrode before and after coating with the CNT are given in (Fig. 8 - 14). Similarly the SEM images of graphite electrode with bacterial colonies in (MFC-II) and on CNT coated SSM electrode in (MFC-III) were represented in (Fig 15&16).
Fig. 8. Graphite electrode surface

Fig. 9. Stainless steel mesh

Fig. 10. Microscopic view of CNT coating on SSM

Fig. 11. SEM image of SS Mesh

Fig. 12 SEM images of CNT coating on SS mesh

Fig. 13. SSM SEM

Fig. 14 SEM images of CNT coating on SS mesh
Microbial screening using 16S V3-V4 Metagenome using region-specific targeting proprietary primers at Genotypic Technology Pvt. Ltd., Bangalore, India revealed that bacterial community present in the cow dung slurry represented four major phyla (above 10%) viz. Bacteroidetes (33.175); Firmicutes (29.8%); Proteobacteria (21.47%); Tenericutes (10%); 64 species under 13 genera; RFN20-sp. and SMB53 sp (P2534-1885.BA008)- are the unclassified but showing similarity with strains present in the sample. Shannon Index is a
measure diversity of species and Simpson Index as the measure of species richness. Both indices were found statistically significant. Bacterial species present in cow dung and their families are represented in pie-charts (fig.19 & 20). Bacterial species present in biofilm on electrode were Lysinibacillus sphaericus, Bacillus pumilus, B.cereus, B.mojavensis, Pseudomonas aeruginosa belonging to four genera. Though cow dung supported many species (60 and above), in the biofilm on the electrode (SSM) there were only 7 bacterial species and 5 species in graphite electrode surface.

![Fig.19. Pie chart shows the phyla of bacterial species](image1)

![Fig.20. Pie chart showing microbial species present in cow dung slurry.](image2)
Discussion

For improving interactions between biofilm and the electrode surface in Microbial fuel cells (MFCs), the anode electrodes must have biocompatibility with biofilm. Recently, nanocomposite based electrode catalysts were assembled with MFC for the usage of wastewater treatment and green energy power production. Carbon nanotubes (CNTs), as a new class of carbon nanomaterials, have been evaluated as a potential anode in MFCs due to its unique properties such as higher active surface area, excellent conductivity, and biocompatibility (Zhang et al 2013).

Therefore, in the present study stainless steel mesh coated with Multiwalled CNT is used as an anodic electrode replacing graphite electrode for the first time in cow dung fed MFC. Surface modification was carried out in SSM for two reasons: firstly, mere SSM electrode exhibited lesser electrochemical activity (Dumas et al. 2007, Zhang et al. 2012) than traditional graphite electrode. In this study, the CNT coated SSM electrode is compared with traditional graphite. From figure 3 it is quite obvious that output voltage on load (100 ohms) are higher in MFC with CNT coated SSM electrode than the graphite electrode. The CD and PD also exhibited a similar trend (Fig.4 & 5). The output voltage revealed that a sustained increase in a 30 days cycle whereas MFC-II with graphite electrode exhibits an early rise and fall in output voltage (Fig.3).

Higher potential of MFC-III with CNT coated SSM is attributed to two factors, CNT coated SSM provides a three-dimensional space for biofilm formation and growth. It is obvious in Figures 15 & 16 that bacterial growth is more on CNT coated SSM than in graphite electrode. Though surface area of graphite electrode was higher (76cm$^2$) than CNT coated. SSM electrode (46cm$^2$), it is noted that CNT coated SSM electrode is having three-dimensional network of SSM electrode and further Carbon-nanotubes coated on the wire...
mesh improves the more space for the biofilm formation. It is more clear from figure 18 that each nanotube has the surface morphology of 25micrometer length and less than 50nm diameter; each tube provides extra space for the microbial growth). Eventually, the space available for biofilm development is higher than in graphite. Further, CNTs have strong cell adhesion, cell attachment and growth properties (Correa-Duarte et al. 2004, Heister et al. 2013). All these factors facilitated the growth of more bacteria both in terms of quantity and quality in the CNT coated electrode.

Further, it is also noticed from microbial screening studies in the biofilm that eventhough there were more than 60 bacterial species present in cow dung slurry (anolyte), only seven bacterial species were present in the biofilm from CNT coated SSM electrode and five species from graphite electrode in MFC-II. Such variation in microbial diversity between the biofilm from both systemsis considered a valid reason for higher output voltage and Current density and power density exhibited by MFC-III with CNT coated electrode. These findings are further supported by SEM images of biofilm on the anode surface. For the CNT-SSM, as shown in Fig.18, electrode surface was covered by thick biofilms and it was also obvious that each carbon nanotube supported more than one bacterial colony and showed a thick biofilm on the CNT-SSM anode. The biofilms-CNTcomposite ensured a rapid transfer of electrons from biofilm to the electrode surface. These observations are in conformity with the reports of Malvankar and Lovley (2012).

To understand variation in voltage potential of two systems (MFC-II & MFC-III), data from EIS were analysed and fitted to circuit parameters using the non-linear square method with chi-square (X2) values around 10^-4. The Nyquist plots of experimental data (Fig.2 &3) and Table 1 indicate lesser internal resistance in MFC-III than MGC-II. It is further clear from Table 1 that the anodic polarisation resistance is manifoldtimes lesser in
MFC-III (fitted with CNT coated SSM electrode). Internal resistance was 5013 ohms and 364 ohms in MFC-II and MFC-III respectively. Thus, anodic polarisation resistance, internal resistance, solution/electrolyte resistance, and cathode resistance are lesser in MFC-III than MFC-II. All these impedance parameters are in support of results obtained in the experimental studies. Such low internal resistance in MFC-III is attributed to the CNT coated SSM electrode with more biofilm formation which facilitates the effective transfer of electrons and proton without accumulation in the anode chamber. Moreover, microorganisms on the CNT-SSM biocathode play a major role in oxygen reduction reaction (ORR) and execute better catalytic activity toward ORR than that of the graphite electrode. Similar reduction in the internal resistance in MFC with CNT compost electrodes were in conformity with previous reports that SSM coated with CNT exhibits higher efficiency as indicated in EIS, due to reduced mass transfer losses and accelerated electron transfer between biocatalysts and electrode. These findings are in Ren et al (2012) reported similar reduction in the internal resistance in MFC with CNT compost electrodes. It is in conformity with the reports of Ren et al (2015), Zhang et al., (2013), Wang et al., (2015) and Bharghavi et al., (2018) that SSM coated with CNT exhibited higher efficiency due to reduced mass transfer losses and accelerated electron transfer between biocatalysts and electrode.

An Attempt was also made to find out the internal (endogenous) mediator responsible for the transfer of electrons from biofilm to the electrode surface. Electron transfer from bacterial cell to electrode surface is done in three ways: 1. generated electrons are transferred to anode surfaces directly by producing microbial pili, microbial nanowires or soluble mediators like cytochrome complex (Watanabe et al. 2009, Malvankar and Lovely 2012, Toores et al. 2009; 2. bacteria produce soluble protein matrix that transfer electrons from bacteria cell to electrode surface; 3. Electrons are picked up by substances produced by
bacteria present in the anolyte (other than those in biofilms) and transfer to the electrode surface.

In the presently operated MFCs, attempts were also made to find out the type of natural endogenous mediator involved in electron transfer. Samples taken from the surface of the electrode (biofilm) was subjected to FTIR and UV-Vis spectroscopy (Fig.22). The spectrum showed fingerprints of cytochrome C (a soluble protein fraction secreted by the microbes). Spectroscopic location at 418 nm in the UV-Vis spectrum and 560–cm in FTIR spectrum (Fig.17 & 18) are the fingerprints of cytochrome c present in the sample taken from the electrode released by the microbes for transfer of electrons from biofilm to electrode surface.

Therefore, it is demonstrated from an experimental study that the CNT coated SSM is highly efficient in generating stable and sustainable electricity generation from cow dung slurry-fed MFCs than bare graphite electrode. The electron transfer from the bacterial cell to the electrode surface is done by cytochrome complex present in the bacteria.

References


