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ORIGINAL RESEARCH ARTICLE

Production of Microbial Fuel Cell from Marine Sediment

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ABSTRACT

The present work aimed to study the bioelectricity is produced by using Sediment microbial fuel cell technology. In this study, four individual colonies were isolated from the electrode. Based on this biochemical test, the cultures A1- A3 isolates which are identified as may be *Bacillus sp* and A4 as a *Micrococcus sp*. Analysis of pH in a three different SMFC by varying catholytic environment by using pH meter (pH Tester 30). The proteins molecules are separated according to their different electrophoretic motilities by using SDS PAGE. Biofilm formation observed under Scanning Electron Microscopy (SEM). There is no significant improvement between air purged cathode and normal cathode which is higher than the algae cathodes from polarization study. Light source, temperature and pH are necessary for algae growing in sea water. The above mentioned parameter is essential to get the more power production in SMFC.

Key words: Microbial fuel cell (MFC), Marine sediment, Sea water, Electrodes, Graphite, Sea algae.

1. INTRODUCTION

The increasing demand for energy combined with decreasing supplies of fossil fuel has lead to great interest in alternate energy production technologies [1].The ongoing use of petroleum-based fossil fuel is now also widely considered to be unsustainable due to both diminishing supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment [2]. One hundred years ago it was well known that bacteria could generate electricity [4,2]. But only in the past few years has this capability become more than a laboratory novelty. The reasons for this recent interest in using bacteria to generate electricity are a combination of the need for new sources of energy, with discoveries about microbial physiology related to electron transport, and the advancement of fuel cell technologies [6]. Microbial fuel cell (MFC) is a bio-electrochemical system that drives a current by mimicking bacterial interaction found in nature. In a microbial fuel cell (MFC), bacteria are separated from terminal electron acceptor at the cathode so that the only means for respiration is to transfer electron to the anode. A microbial fuel cell is in essence, a system that harvests electrons produced during microbial metabolism and channels them for the generation of electric current[5,1] Microbial fuel cell using microorganisms as catalysts in fuel cell have been explored since the 1970s. Microbial fuel cell is device that converts chemical energy to electrical energy by the catalytic active microorganism [1].The electron flow to the cathode, as a result of the electrochemical potential between the respiratory enzymes and the electron acceptor at the cathode. Electron transfer from the anode to the cathode must be matched by an equal number of protons moving between these electrodes, so that electron neutrality is preserver [7,9]. The present study, Sediment microbial fuel cell an energy conversion device that can efficiently convert and use the power of hydrogen is a key to making it happen. This can be used for the backup power, power for remote locations, distributed power generation, and treat waste while generating electricity. In recent year, current generating bacteria have been documented from freshwater [8], estuarine, and salt marsh sediments, as well as marine sediment, In future, Marine waste used as a good source for the electricity generation.

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2. MATERIALS AND METHOD

2.1. Collection of samples

Marine sediment was collected from mandapam sea shore, Ramanathapuram district, at the depth of 100cm. Sea water was collected from mandapam sea shore. Then the samples were stored at -4°C until to use. These samples were stored in an ice box and transported for microbiological characterization to EEC laboratory, CECRI at Karaikudi, India for further analysis.

2.2. Isolation of power generating bacteria

One traditional microbiological method for the isolation of single colonies from mixed cultures incorporates the plating of serial dilutions in nutrient agar plate used for isolation; unwanted population can be eliminated while the growth of desired species can be enhanced by further plating [3].

2.3. Serial dilution method

0.1ml of samples ware scratched from anode single cell chamber microbial fuel cell on running stage. The sample collection was done aseptically to avoid contamination. It was collected in sterile plates. After collecting the sample, it was serially diluted as follows; 0.1 ml of sample was mixed with 0.9ml of sterile Millipore water. Again from this,0.1ml of solution taken and it dissolved in 0.9 ml of sterile fresh Millipore water and vice versa (10^{-1}-10^{-9}). After sterile dilution, from each tube 0.1 ml of sample were taken and spread plate Techniques were done in the Petri plates. Before spread plate technique were done in the Petri plates. Before spread plating, the plates and medium autoclaved and the medium was poured in the plates for solidification. The plates were incubated at 37°C for 48 hrs, and the colonies were observed after incubation time.

2.4. Partial Biochemical Characterization of the Isolates

Morphologically dissimilar dominating isolated colonies were selected randomly, further streaked on B4M agar plates and purified. The pure cultures were maintained in B4M agar slants at 4ºC to keep the microbial strain viable. The dissimilar aerobic bacteria isolated from medium were identified according to Bergey's Manual of Determinative Bacteriology. The isolated bacterial cultures were identified up to genus level by their morphological and partial biochemical characterization using the following tests shown in Table:1 : (1) Gram staining, (2) Catalase test, (3) Oxidase test, (4) Pigment production, (5) Nitrate reduction test, (6) Indole production, (7) Methyl red test, (8) Voges- proskauer test, (9) Citrate utilization test, (10) Mc Conkey test, (11) Urease activity, (12) Starch hydrolysis test, and (13) Carbohydrate fermentation test. In addition, citrate agar was used to detect the iron-reducing activity of the isolates. Bio-chemical characterization of the isolates was carried out employing Himedia biochemical test kit (Mumbai) according to the manufacturer's instructions.

2.5. Epifluorescence Microscopy

Epifluorescence microscopy (EFM) is a method of fluorescence microscopy that is widely used in life sciences. The basic task of an Epifluorescence microscope is to get the sample excited. To do this, the excitatory light is passed from above through the objective lens and then onto the specimen instead of passing it first through the specimen. The fluorescence in the specimen gives rise to emitted light which is focused to the detector by the same objective that is used for the excitation. Since most of the excitatory light is transmitted through the specimen, only reflected excitatory light reaches the objective together with the emitted light and this method therefore gives an improved signal to noise ratio. An additional filter between the objective and the detector can filter out the remaining excitation light from fluorescent light. Because of the key features of an Epifluorescence microscope, it is now becoming rapidly used in the biological and medical field. This microscopy technique has made it possible for people to determine and identify the cellular components of each sample with a high amount of specificity. For example, impurities and disease conditions can now be studied effectively by using Epifluorescence microscopy.

2.6. pH analysis

Analysis of pH in a three different SMFC by varying cathode environment by using pH meter (pH Tester 30).

2.7. Electrodes

Graphite's plates were used as a current collector (both anode and cathode) with a total surface area of $60x10^{-4}m^2$. The graphite electrodes were pretreated with acetone for 5min and HCl for 5min and then followed by deionized water for 15min.

2.8. Sediment Microbial Fuel cell (SMFC) set up and operations

The three different SMFC were operated for nearly 95^{th} days by varying the catholyte environments.

The configuration of catholyte as follows,

a. SMFC with real sea water.
b. SMFC with overlying water with purging oxygen.
c. SMFC with the overlying water covered with sea algae

2.9. Operations of SMFC with real sea water (Normal Atmosphere)

SMFC with real sea water as catholyte. It consists of an inert cylindrical perspex tube 5cm in diameter and 14cm height. The volume of cylinder is 263.7cm^3 and made up of perspex tubes. The anode graphite was placed on the bottom of the cell in vertical position and filled by fresh marine sediment up to a height of 7cm. The cathode was immersed in the marine water which was placed perpendicular to the anode. Then the anode and cathode were connected to an external electric circuit through the digital data logger connect in Personal computer at room temperature.

2.10. SMFC with overlying water with purging oxygen

To saturate the level of oxygen on the catholyte, a controlled air bubbles were purged over the surface of the catholyte by using fish tank aerated kit. The laboratory set up of SMFC with surface air floating seawater as catholyte.

2.11. SMFC with the overlying water covered with sea algae

In this configuration of catholyte, two different species of marine micro algae were inoculated (Sea grass, *Phyllospadix scouleri*).*Phyllospadix scouleri*, or Scouler's surf grass, is a flowering marine plant in the family Zosteraceae. The laboratory set up of SMFC with surface algae [45] in seawater as catholyte.

2.12. Polarization study

2.12.1. Voltage

Voltage is on informal term for electric potential difference and also called electric tension voltage is measure of the energy of electricity. Specifically, it is the energy per unit charge. A voltage may represent a source of energy are it may represent lost of stored energy (potential drop) Voltage is always measured between two points since it is not usually practical to measure the absolute electric potential of a point of a point. Voltage can be measured by a voltmeter. The unit of measurement is the Volt. The voltage between two ends of a path is the total energy required to move as all electric charge along the path divided by the magnitude of the charge.

2.12.1. a. Open circuit voltage (abbreviated as OCV or V_{OC})

The difference of electrical potential between two terminals of a device when there is no external load connected, i.e. the circuit is broken or open. Under these conditions there is no external electric current between the terminals, even though there may be current internally (e.g. self-discharge currents in batteries). It is sometimes given the symbol V_{oc}.

2.12.1. b. Closed circuit Voltage

It is considered to be closed when electricity flows from an energy source to the desired end point of the circuit. Closed circuit voltage test under Load and it is used to create a Voltage. The closed circuit potential between Cathode and anode [14, 15] across a resister were measured using a Data acquisition and recorded on a personal computer.

2.12.2. Current density

Current density is measure of the entity of flow of a conserved charge. Usually the charge is the electric charge, in which case the associated current density is the electric current per unit area of cross section, but the term current density can also be applied to other conserved quantities.

Where,

V is the voltage

R is the external resistance.

Current (I) was calculated as I (mA) =V (mV)/R (Ω).

Power (P) in miliwatts was calculated as P (mW) = I^2 (mA)/ R (Ω).

Current and power densities were normalized to the surface area of the electrodes [37].

Current density is calculated by formula = current (I)/$60\times10^{-4}\times 1000$.

2.12.3. Power density

Power density (or volume power density or volume specific power) is the amount of power (time rate of energy transfer) per unit volume. Power is calculated from a voltage and current. Polarization curve should be plotted using the current and potential measured for each resistance value.

Power density is calculated by the formula = power/$60\times10^{-4}\times1000$.

2.13. Characterization of microbial fuel cell

2.13.1. Scanning electron microscopy (SEM)

SEM is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition, another properties such as electrical conductivity The types of signals produced by an SEM include

secondary electrons, back-scattered electrons (BSE), characteristic X-rays, light (cathode luminescence), specimen current and transmitted electrons. Secondary electron detectors are common in all SEMs, but it is rare that a single machine would have detectors for all possible signals. The signals result from interactions of the electron beam with atoms at ornery the surface of the sample. In the most common or standard detection mode, secondary electron imaging or SEI, the SEM can produce very high-resolution images of a sample surface, revealing details less than 1nm in size. Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample. The graphite anode was removed from a SMFC, after decanting the sediment, the anode was cut into 1×1 square pieces were cut from the anode. Then, it was treated with 1% of acetone and 2% phosphotungstic acids and dried for 15minutes and biofilms was analyzed formation under the Scanning Electron Microscopy.

2.14. (SDS – PAGE) Protein profiling Sodium Dodecyl Sulphate – Polyacrylamide gel electrophoresis

SDS-PAGE is the most widely used method for analyzing protein mixtures qualitatively; it is particularly useful for monitoring protein purification. The method is based on the separation of proteins according to size. It can also be used to determine the relative molecular mass of proteins**.** SDS-PAGE is the most widely used method for analyzing protein mixtures qualitatively. The method is based on the separation of proteins according to size. It can also be used to determine the relative molecular mass of proteins. The crude enzyme was subjected to Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS – PAGE). Molecular weight of the enzyme samples was determined by [21] using 10% acrylamide.

SEPARATING GEL (12%)

Acryl amide	- 4 ml
(pH – 8.8) Buffer	- 2.6 ml
Ammoniumpersulphate	- 0.1 ml
TEMED	- 0.004 ml
Distilled water	- 3.3 ml

STACKING GEL (5%)

Acryl amide	- 0.83 ml
(pH – 6.8) Buffer	- 0.68 ml
Ammoniumpersulphate	- 0.05 ml
TEMED	- 0.005 ml
Distilled water	- 3.4 ml

The glass plates, combs, spacers were cleaned with acetone and the plates were fixed using spacers. It was assured that the plates were not leaking. 12 % separating gel was prepared and poured between the glass plates and allowed to polymerize. After polymerization of separating gel, a 5% stacking gel was poured and the comb was inserted into it and allowed to polymerize. After polymerization the comb was removed without distorting the shapes of the well. The wells were cleaned with tris hydrochloric acid of (pH - 6.8) and the samples were loaded into the wells. The gel was run at a constant rate of 50 - 100 volts till the dye reached the bottom. The gel was stained with 25 % staining solution over night and following day it was subsequently detained to visualize the protein band. The molecular weight of protein was calculated by comparing with the distance traveled by hand. The gel was stored in 10 percent acetic acid and photographed. The PMW - M molecular marker (Genei) was as standard.

3. RESULTS AND DISCUSSION

In this study, the marine sediment used for the generation of electricity, was taken from mandapam sea shore (Ramanathapuram District). The bioelectricity is produced by using Sediment microbial fuel cell technology. Marine sediment microbial fuel cell (MFC) utilizes oxdisable carbon compounds and other components present in sediments to produce power. Electricity can be generated by bacteria growing on carbon electrodes (anodes) in anaerobic sediments when connected to electrodes in the toxic overlying water (cathodes) [11,12].

3.1. pH analyses in three different SMFC were operated by differing the catholyte conditions

Analysis of pH in a three different SMFC by varying catholytic environment by using pH meter (pH Tester 30). Generally, bacteria require a pH close to neutral for their optimal growth; while oxygen reduction on the cathode electrode results in an alkaline pH. In normal conditions, Initial pH almost equal to 8(pH in real sea water) pH 7.9(air floated sea water) pH 7.8(algae 1) pH 7.8 (algae 2).End of 160 hr under OCP , the pH of SMFCs were 8.9, 9, 7.9, 7.8.After 3 days incubation, (72hrs), the result indicates as in the case of Algae1 and Algae 2 based cathode getting slowly lowering the pH from 8.6 to 8.9.In addition to that at end of 7th day the pH of algae based cathode is slightly slowly that cathodes **(Fig 1 and Table 4)**.

3.2. Epifluorescence Microscopy

A small piece of graphite (anode) was taken from SMFCs and then 3 drops of 0.01% aqueous solution of acridine orange and ethidium bromide was added. It was incubated for 15 min; the excess stain was washed with sterile distilled water and examined by the Epi-fluorescence microscope. Model: Nikon Epi-fluorescent microscope (E200, Nikon, and Tokyo, Japan) Live cells and death cell were observed in SMFCs under the Epifluorescence microscopy. Appearance of Green color indicating rod shape bacteria in a live condition and orange color indicating the death conditions of cell. So, this image indicating the presence of live cells around the graphite was helped in the enhancement of current generation **(Fig 2)**.

3.3. Operations and polarization of SMFC with real sea water (Normal Atmosphere)

Sediment fuel cell constructed with marine sediment and operated at 37^0C without added energy sources or synthetic electron carrying mediators. The (Open circuit voltage) OCV increased gradually to 0.581V **(Fig 3)**. From the polarization the maximum power density (10.2mW/m^2) at current density (91.6 mA/m^2) under the applied external resistance of 200Ω **(Fig 6)**. The Power was produced through the microbial activity at the anode in conjunction with, principally oxygen reduction at a graphite cloth at cathode. Steady state polarizations gave maximum power densities of 55 mW m^{-2} sing carbon sponge as the anode; the latter material typically gave power densities of around 20 mW m-2. In single chamber microbial fuel cells (MFCs).Maximum power densities obtained using either single cycle or multiple cycle methods were 0.98 W/m2 (277 W/m3) using *C. vulgaris*, and 0.76 W/m2 (215 W/m3) using *U. lactuca* . [19] After a period of stabilization, open circuit voltages up to 700 mV were observed from most of cells. Addition of cellulose reduced the output power from SMFC at the beginning of experiments, while the output power was found to increase after adding cellulose to sediments on day 90 of operation. [19] An examination of marine sediment from temperate waters [8] proved to be a good source of thermophilic electrode reducing bacteria.

3.4. SMFC with overlying water with purging oxygen

Sediment fuel cell constructed with marine sediment and operated at 37°C without added energy sources or synthetic electron carrying mediators. The OCV increased gradually to 0.676V **(Fig 4)**. [4] From the polarization the maximum power density (10.2mW/m^2) at current density (23.8 mA/m^2) under the applied external resistance of 3000Ω **(Fig 7)**.

3.5. Electricity generated by using Algae (Sea grass)

In this study, the electricity generated from an algae [16] SMFCs was evaluated Based on the result, Sediment fuel cell constructed with marine sediment [20] and operated at 37^0C without added energy sources or synthetic electron carrying mediators.. The OCV increased gradually to 0.257 **(Fig 4)**. From the polarization maximum power density (7.3mW/m^2) at current density (77.5mA/m^2) under the applied external resistance of 200Ω **(Fig 8)**.

3.6. Biofilm formation

Biofilm formation [5] observed under Scanning Electron Microscopy. A group of microbes occurred on the graphite electrode. These groups of microbes, which is responsible for the generation of power. **(Fig 9)** Show the image representation of Biofilm formation on graphite electrode. The size and range of the microbes were 100μm.

3.7. (SDS-PAGE) Protein separation

The samples were loaded on the SDS-PAGE Gel and it was run under the electrophoresis tank. After the sample reaching the end, it was removed and stained using coomassie blue. A different type of protein was observed under UV transilluminator, and it moved according to their molecular weight. Proteins molecules are separated according to their different electrophoretic motilities by SDS PAGE based on the molecular weight using protein marker. These proteins of molecular weight separated and it's visualized through band formation. All the proteins molecular weight is near to 60 kDa **(Fig 10)**.

In this study, current generation at constant resistance in the case of air purged cathode shows slightly higher performance with a fluctuated response compared to other cathodes. In the case of algae cathode shows decreasing performance at constant residence. In SMFC, oxygen is widely used as the electron acceptor for the cathodic [21] reaction due to its high redox potential. The power generation is very low for algae growing in sea water, because the pH [6,1] was decreased in a normal temperature before 7days. Light source, temperature and pH are necessary for algae growing in sea water. The above mentioned

parameter is essential to get the more power production in SMFC.

Table 1: Overview of current and power result obtained with SMFCs

S.No	Types of samples (Sediment)	Type of Electrodes	Power density (mWm^{-2})	Current density (mAm^{-2})	Voltage(V)	Reference
1	Marine sediment	Anode with Anthraquinone1,6 disulfonic acid (AQDS) or1,4 naphthoquinione(NQ) Plain graphite anode Mn^{2+} and Ni^{2+}	98 20 105	560 66 350	0.24 0.30 0.35	Tender *et al.*(2006)
2	Marine sediment	Graphite	10 -20		0.75‡0.03	Tenter *et al.*, 2002
3	Estuarine sediment	Platinum mesh electrode	1-4	7.4		Reimer *et al.*(2001)
4	Marine sediment	Graphite	29-43	209-254		Mathis *et al.*(2008)
5	Marine sediment	Anode: Carbon cloth. Cathode: Platinum coated	29-49	160	0.73	Zhen He *et al.* (2007)
6	Marine sediment	-	10-20	-	-	Lowy *et al.*(2006)
7	Marine sediment	-	100	-	-	colleagues *et*
8	Marine sediment	-	233	-	-	Nielsen *et al.*(2007)
9	Lake sediment	Graphite plate and felt electrode	4.08	20	-	Seok Won Hong *et al.*(2008)
10	Marine sediment	Stainless steel electrodes Graphite electrodes	23 4	140	0.05	Dumas *et al* (2007)
11	Marine sediment	Carbon cloth electrodes	20-27	60-90	650-700	Scott *et al.*, 2008
12	Marine sediment	Anode with anthroquinone 1,6 disulfonic acid	105			Lowy & Colleagues *et al.*(2006)
13	Deep sea	Unpolished Graphite		0.28		Wei and Zhang
14	Marine sediment	Graphite cloth	68		700	K Scott *et al*(2008)
15	Marine sediment	Graphite disk	16			Bond *et al.*(2002)
16	Marine Salt marsh Fresh Water			20 7		Holmes *et al.*(2004)
17	Ocean cold seep	Vertical graphite rod	34	82		Reimers *et al.*(2006)
18	Sea water	Pillow shapes carbon cloth filled with chitin 80	51	184		Rezaeis *et al.*(2007)
19	Fresh water matrix planted with rice plant	Graphite felt	33	55		De Schamphelaire *et al.*(2008)
20	Fresh water rice paddy field	Graphite felt	3	15		Kaku *et al.*(2008)
21	Salt marsh	Vertical graphite plate	387	1105		Tenter *et al.*(2008)
22	Marine algae	Anode: carbon rod. Cathode: Stainless steel rod			0.792V	Ramanathan *et al.*, (2011)
23	Marine algae	Graphite	0.98	0.25		Sharon *et al* (2009)

Table 2: Preliminary test for identifications of isolates

Species	Gram staining	Spore staining	Motility	Shape	Appendence of colonies
A1	Gram positive	Positive	Positive	Rod	Milky white
A2	Gram positive	positive	Positive	Rod	Irregular white
A3	Gram positive	positive	Negative	Rod	colonies
A4	Gram positive	Negative	Negative	Cocci	White dry colonies Light yellow appearance

Table 3: Biochemical Tests

Name of the Test	Culture A1	Culture A2	Culture A3	Culture A4
Catalyst	+Ve	+Ve	+Ve	+Ve
Oxidase	-Ve	+Ve	+Ve	+Ve
Indole	-Ve	-Ve	-Ve	-Ve
Citrate test	+Ve	+Ve	-Ve	+Ve
Methyl Red	-Ve	-Ve	+Ve	-Ve
VP Test	+Ve	+Ve	+Ve	-Ve
Starch hydrolysis	+Ve	+Ve	+Ve	-Ve
TSI test	+Ve	+Ve	-Ve	-Ve
Gelatin hydrolysis	+Ve	+Ve	+Ve	-Ve
Macon key agar Test	+Ve	+Ve	+Ve	+Ve
NO_3 Reduction	+Ve	-Ve	+Ve	-Ve
Urease test	-Ve	-Ve	-Ve	-Ve

Note: Based on this biochemical test, the culture A1, A2, A3 may be *Bacillus sp* and A4 as a *Micrococcus sp*

Table 4: pH analysis of SMFC operated with three different cathodic conditions

Time interval (Hours)	pH in sea water	pH in air floated seawater	pH in algae growing 1	pH in Algae growing 2
0	8.0	7.9	7.8	7.8
8.0	8.2	8.0	8.0	8.1
16	8.3	8.5	8.1	8.3
24	8.4	8.8	8.3	8.5
48	8.6	8.7	8.4	8.4
72	8.5	8.6	8.6	8.6
96	8.6	8.8	8.2	8.4
108	8.7	8.8	8.0	8.6
120	8.8	8.7	7.9	8.3
156	8.9	8.7	7.8	8
156	8.9	9	7.9	7.8
160	8.9	9	7.9	7.8

Fig 1: pH of SMFC operated with three different cathodic conditions Vs. Time (hrs)

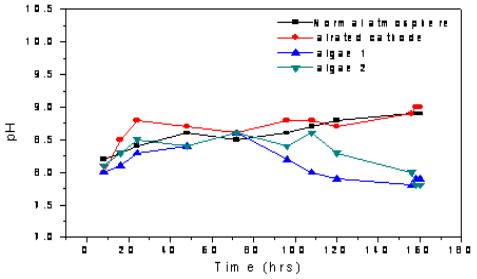


Fig 2: Epifluorescence image of bacterial cells attached on graphite anode

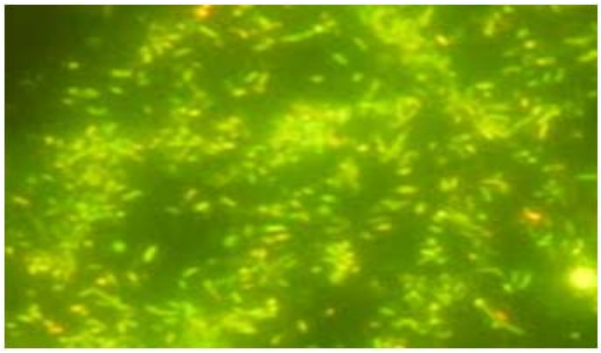


Fig.3: OCV and CCV (at 1000Ω) vs. Time (hrs) for ordinary sea water

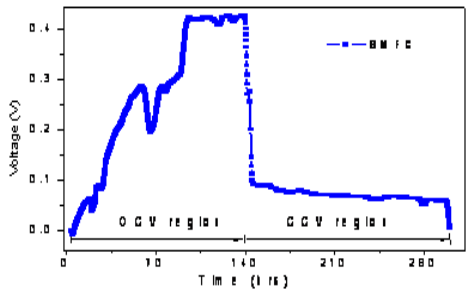


Fig 4: OCV and CCV (at 1000Ω) vs. Time (hrs) for air floated sea water

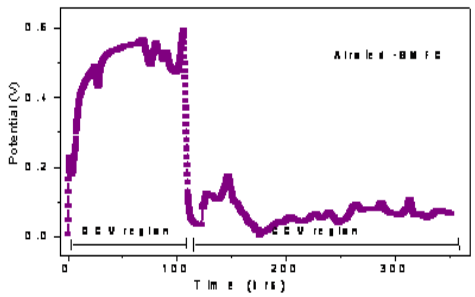


Fig 5: OCV and CCV (at 1000Ω) vs. Time (hrs) for SMFC operated with growing algae in sea water

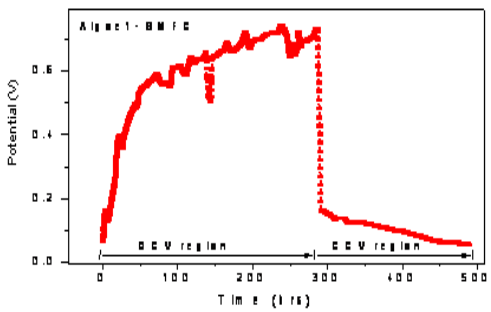
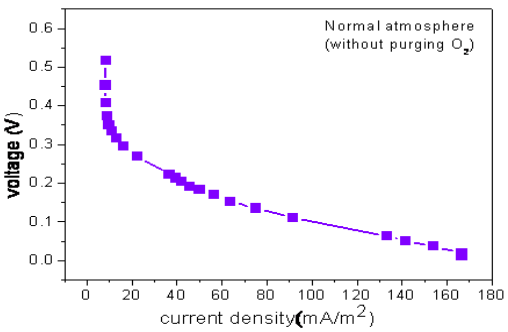


Fig 6: Polarization and power generation curves for SMFC operated with ordinary sea water



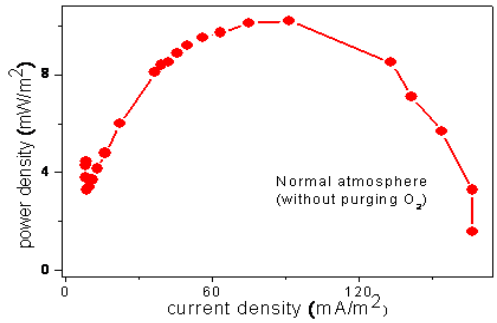
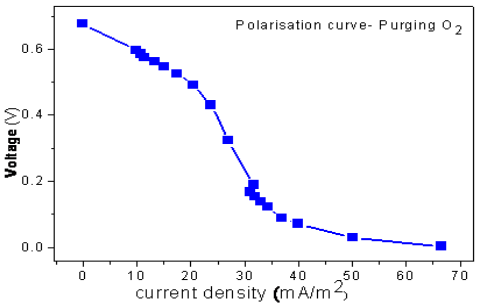


Fig 7: Polarization and power generation curves for SMFC operated with aerated sea water (air purging)



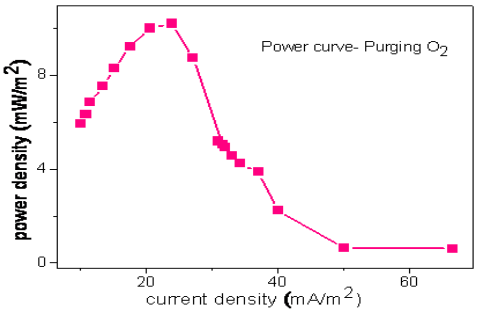
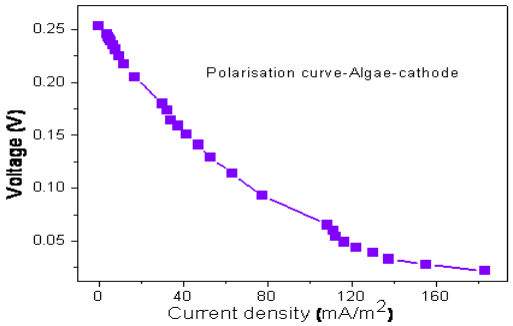


Fig 8: Polarization and power generation curves for SMFC operated with growing algae in sea water



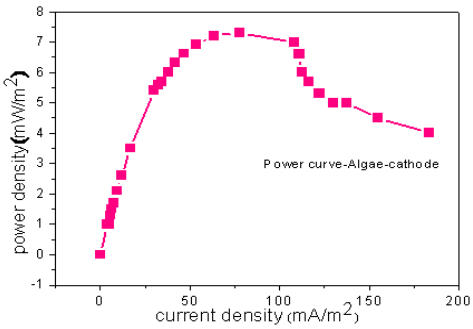
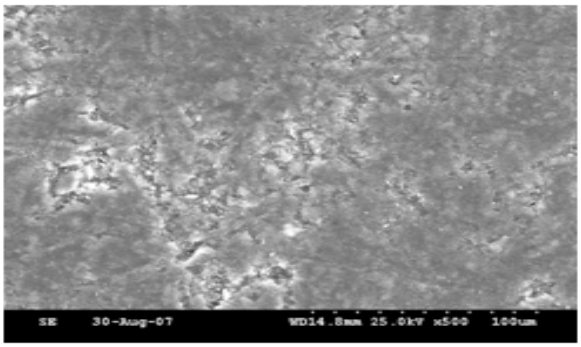


Fig 9: SEM image of Graphite anode and Biofilm formation at the end 95th day

(Graphite)



(Biofilm)

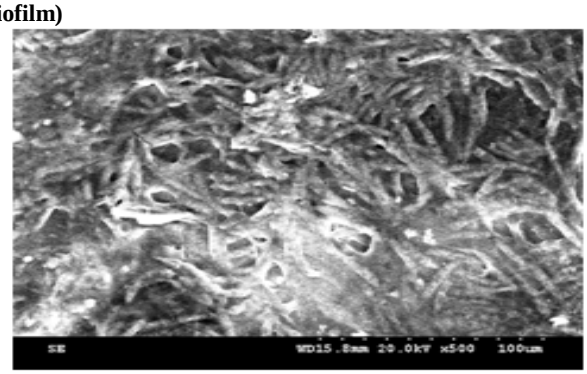


Fig 10: SDS PAGE image of Isolated bacterial strain collected from graphite anode (Lane 5= Marker protein, Lane 1 to 4 bacterial protein)

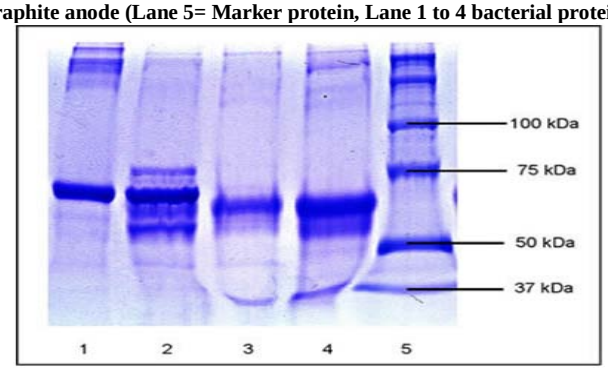


Plate 1: Laboratory setup of SMFC with real Sea water

Plate 2: Laboratory set up of SMFC with overlying water with purging oxygen

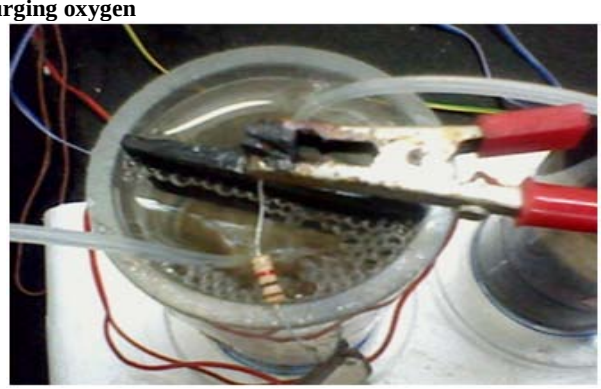
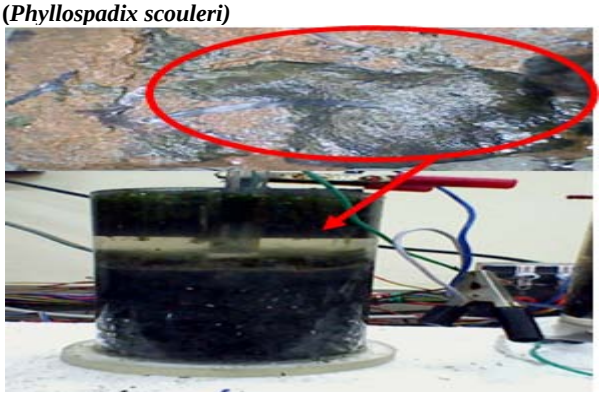


Plate 3: Laboratory set up of SMFC with surface Algae (Sea grass)

Plate 4: Laboratory set up of SMFC with surface Algae (*Phyllospadix scouleri)*



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