P-3085

MICROBIAL FUEL CELLS FOR POWER GENERATION AND WASTE WATER TREATMENT

PROJECT REPORT

Submitted by

A.U.AKNI

Register No: 0820203001

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in partial fulfillment for the award of the degree

Of

MASTER OF TECHNOLOGY

in

BIOTECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY, COIMBATORE-06.

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ANNA UNIVERSITY:COIMBATORE

BONAFIDE CERTIFICATE

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COIMBATORE-641 006

Department of Biotechnology

PROJECT WORK -PHASE II

MAY 2010

This is to certify that the project entitled **"MICROBIAL FUEL CELLS FOR POWER GENERATION AND WASTE WATER TREATMENT"** is the bonafide record of project work done by **A.U.AKNI Register No: 0820203001** of M.Tech during the year 2009-2010.

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CERTIFICATE

This is to certify that the dessertation entitled "**Microbial fuel cells for power generation and waste water treatment**" submitted to **Kumaraguru College of Technology, Coimbatore** in partial fulfillment for the award of the degree **M.Tech.** (Biotechnology) is a record of original work done by **Miss. A.U.Akni** (Reg. No: **0820203001**) at the Centre for Advanced Studies in Botany, University of Madras, Guindy campus, Chennai-25 from December 2009 to April 2010 under my guidance and that the dessertation has not formed a part of any previous work.

Date: 20.04.2010
Place: Chennai

Signature of the Guide

(P.T. Kalaichelvan)

DECLARATION

I affirm that the project work titled **Microbial fuel cells for power generation and waste water treatment** being submitted in partial fulfilment for the award of **M.Tech** is the original work carried out by me. It has not formed the part of any other project work submitted for award of any degree or diploma, either in this or any other University.

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I certify that the declaration made by the above candidate is true.

Signature of the Guide,

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A.U.AKNI

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ABSTRACT

Wastewater is an abundant biological resource and a potential source of clean energy. In a microbial fuel cell (MFC), bacteria oxidize substrates to produce free electrons, or electricity. The treatment of wastewater is one benefit of this process. Optimum design and condition for MFC operation are not yet fully defined. A novel low cost MFC was designed to enhance biofilm growth and substrate consumption. Several MFCs were chemically prepared to encourage electron deposition. Biofilm from the MFC producing the most power was used to seed new fuel cells; this process can be repeated to select the most efficient energy-producing microorganisms. Microbial fuel cells were designed and operated using waste activated sludge as a substrate and a source of microorganisms for the anodic chamber. Waste activated sludge provided a bacterial consortium predisposed to the solubilisation of particulate matter and utilization of substrates commonly found in wastewater. Dissolved oxygen and ferricynaide were used as the electron acceptor in the catholytes. Microbial fuel cell comparison was made while operating under identical condition using different electron acceptors. Comparisons were based on the electricity production observed during MFC operation. The anode and chamber connected by salt bridge to allow for action migration. Various soluble carbon sources, mediators etc were dosed to the Microbial fuel cells at measured intervals during operation via direct injection to the analyte. Microbial fuel cells were designed and operated utilizing various sludge in the anodic compartment and various oxidants in the cathodic compartment. In the cathodic compartment we used as an oxidant like potassium ferricyanide, potassium permanganate. Carbon rods from batteries were used as an electrode material. When fuel cells reached steady state we discharged them through resistor and evaluated the available power.

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Introduction

INTRODUCTION

Renewable energy is an increasing need in our society. Microbial fuel cell (MFC) technology represents a new form of renewable energy by generating electricity from waste. Microbial fuel cells (MFCs) are bio electro chemical systems that generate electricity by oxidation of organic or inorganic substrates catalyzed by microorganisms. Energy in any form plays the most important role in the world. We need energy, especially electrical energy in daily needs of life, such as operating toaster to steel plant. Energy output has become one of the countries progress indicating factors. We have been completely dependent on conventional energy sources such as coal and oil for quite a long time. These two non-replenish able sources of energy contribute to the major part of our energy consumption and we are slowly approaching a stage were all these fuels are fast becoming scarce due to the huge increase in the demand. Oil will not suddenly run out, but it is a finite resource. We must develop energy saving technologies that can stretch oil reserves.

1.1 FUEL CELLS

Fuel cells are a clean and simple way to convert chemical energy of fuels directly in to electricity. Specifically, they transform hydrogen and oxygen in to electric power, emitting water as their only waste product.

A fuel cell consists of two electrodes, an anode and cathode sandwiched around an electrolyte (An electrolyte is a substance, usually liquid, capable of conducting electricity by means of moving ions, charged atoms or molecules). The fuel usually hydrogen enters at the anode of the fuel cell while oxygen enters at the cathode. The hydrogen is split by a catalyst in to hydrogen ions and electrons. Both move towards the cathode, but by different paths. The electrons pass through an external circuit, where they constitute electricity, while the hydrogen ions pass through the electrolyte. When the electrons return to the cathode, they are reunited with the hydrogen and the oxygen to form a molecule of water.

1.2 ADVANTAGES OF FUEL CELLS

They are simple, produce only water as waste product, and extract electricity from fuel more effectively than combustion- boiler- generator systems. They can run on pure hydrogen usually derived from methane by combining methane with steam at high temperature or, in one recently developed design, on methane itself. Biomass, wind, solar power, or other renewable sources can supply energy to make hydrogen or other fuels for use in fuel cells, which could be installed in buildings (e.g., schools, hospitals, homes), in vehicles, or in small devices such as mobile phones or laptop computers. Fuel cells today are running on many different fuels, even gas from landfills and waste water treatment plants.

1.3 MICROBIAL FUEL CELLS

Microbial fuel cells (MFCs) are devices that exploit microorganisms to generate electric power from organic matters and potentially applicable to wastewater treatment and energy recovery from organic wastes. Recent technical developments of MFC processes are noteworthy, and the power output of MFC has been rapidly increasing in recent several years. Some workers consider that MFCs will be practically applied in the near future, but further studies, such as stability improvements and scale-up evaluations, are necessary for that.

A conventional MFC reactor is comprised of two chambers, the anode and cathode chambers. In the anode chamber, organic matter is oxidized to carbon dioxide by microorganisms under anaerobic conditions, reducing equivalents are discharged to the anode as electrons, and these electrons are transferred to the cathode. Protons are simultaneously generated in the anode chamber, passively transferred to the cathode chamber through a membrane and react with oxygen molecules on the cathode electrode to form water molecules. All these steps influence the total efficiency (e.g., power density, coulombic efficiency and organic-loading rate) of a MFC process, and each of these steps has been a subject for technical improvement. For example, the membrane has been optimized to

achieve efficient proton transfer and the cathode was modified to accelerate the proton-reducing reaction.

The principle of MFC lies on the oxidation of the fuel through microbial catabolism in the anaerobic anodic compartment of a fuel cell. The current is generated by diverting electrons from the microbe membrane respiratory chain to the anode. Electron transfer between a bacterium and an electrode is likely to occur through different mechanisms such as:

- Direct electron transfer
- Direct electron transfer through pili
- Mediated electron transfer through naturally exported or artificially added redox mediators
- Anodic oxidation of excreted metabolites (e.g. formate)

This technology can use bacterium already present in wastewater as catalysts to generating electricity while simultaneously treating wastewater (Lui *et al.*, 2004; Min and Logan, 2004). Although MFCs generate a lower amount of energy than hydrogen fuel cells, a combination of both electricity production and wastewater treatment would reduce the cost of treating primary effluent wastewater. Currently, most of the research performed on MFCs is concerned with increasing the power density of the system with respect to the peripheral anode surface area, while little research has been done on determining the size of a microbial fuel cell needed for a typical waste water treatment facility.

1.4 DESIGN STRUCTURES

A typical microbial fuel cell (MFC) consists of two separate chambers which can be inoculated with any type of liquid media. These chambers, an anaerobic anode chamber and

anaerobic cathode chamber, are generally separated by a Proton Exchange Membrane (PEM) such as Nafion (Oh and Logan, 2004).

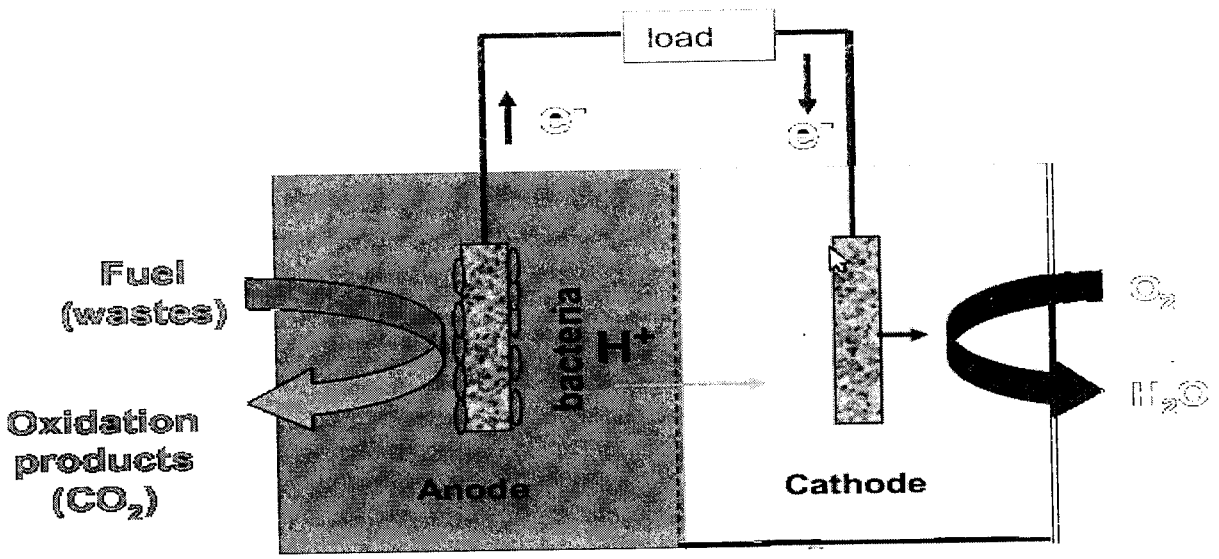


Fig 1.1: A Microbial fuel cell setup

1.5 TYPES OF MICROBIAL FUEL CELL

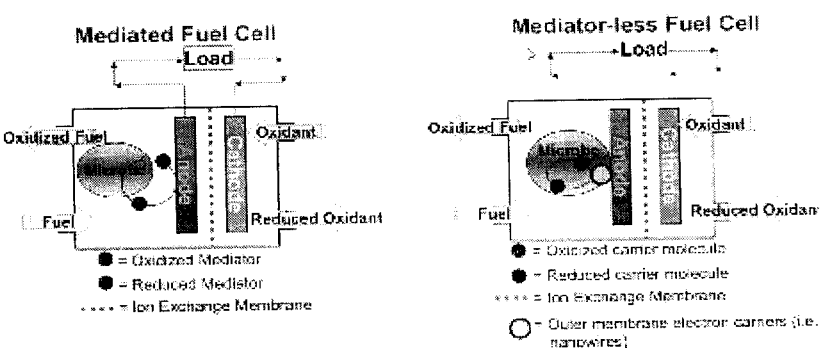


Fig 1.2. Types of microbial fuel cells

1.5.1 Mediator fuel cell

This type generates electricity from the addition of artificial electron shuttles (mediators) to accomplish electron transfer to the electrode. Most of the microbial cells are electrochemically inactive. The electron transfer from microbial cells to the electrode is facilitated by mediators such as thionine, methyl viologen, methyl blue, humic acid, nuteral red.

1.5.2 Mediator-less fuel cell

Mediator-less fuel cell does not require a mediator but uses electrochemically active bacteria to transfer electrons to the electrode (electrons are carried directly from the bacterial respiratory enzyme to the electrode).This type does not require these additions of exogenous chemicals and can be loosely defined as a mediator-less MFC (Bond and Lovely 2003, Chundhuri and Lovely 2003, Lui *et al.,* 2004). Mediator-less MFCs can be considered to have more commercial potential then MFCs that require mediators because the typical mediators are expensive and toxic to the microorganisms (Bond *et al.,* 2003). Among the electrochemically active bacteria are *Shewanella putrefaciens, Aeromonas hydrophila.* Some bacteria, which have pili on their external membrane, are able to transfer their electron production via these pili.

1.6 BIOLOGICAL MECHANISM

In normal microbial catabolism, a substrate such as a carbohydrate is initially oxidized anaerobically when its electrons are released by enzymatic reactions (Bennetto, 1990). The electrons are stored as intermediates (e.g., NADH, quinones) which become reduced and are then used to provide the living cell with energy (Bennetto,1990). The ending location for the electrons is molecular oxygen or dioxygen at the end of the respiratory chain (Bennetto,1990).

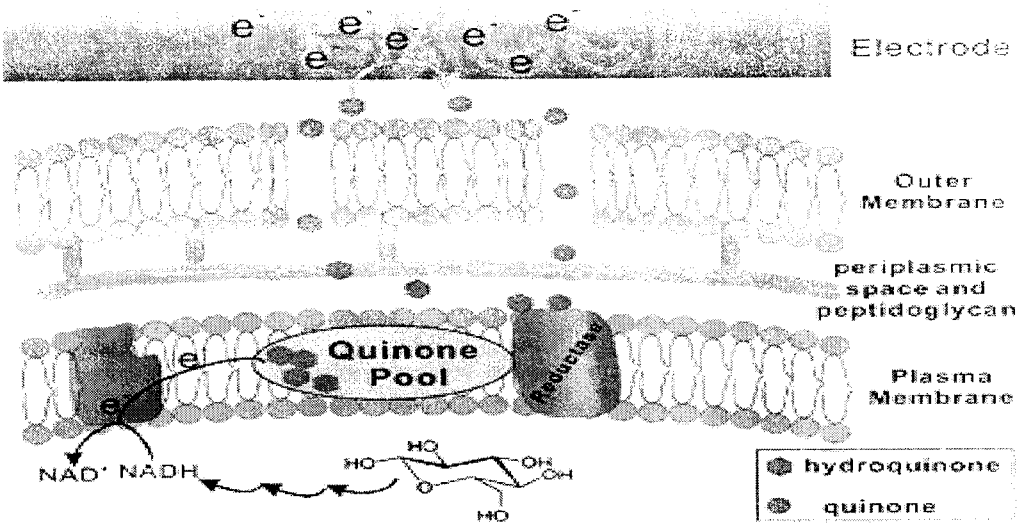


Fig 1.3: Glucose metabolism and the electron transport chain

A MFC uses bacteria to catalyze the conversion of organic matter into electricity by transferring electrons to a developed circuit (Bond *et al.,* 2002). Microorganisms can transfer electrons to the anode electrode in three ways:

- Exogenous mediators (ones external to the cell) such as potassium ferricyanide, thionine, or neutral red;
- Using mediators produced by the bacteria;
- By direct transfer of electrons from the respiratory enzymes (i.e., cytochromes) to the electrode (Bond et al. 2003, Min 2004).

These mediators can divert electrons from the respiratory chain by entering the outer cell membrane, becoming reduced, and then leaving in a reduced state to shuttle the electron to the electrode (Bennetto,1990). *Shewanella putrefaciens, Geobacter sulfurreducens, Geobacter metallireducens* and *Rhodoferax ferrireducens* have been shown to generate electricity in a mediator less MFC (Bond *et al.*,2003).

When these bacteria oxidize the organic matter present in the wastewater, the electron is shuttled to the electrode and the protons produced diffuse through the water the counter

electrode (cathode) giving this particular electrode a positive characteristic. Oxygen, the hydrogen protons, and the electron that is connected by a circuit from the anode to the cathode, are then catalytically combined with a platinum catalyst to form water at the cathode (Bond and Lovely, 2003, Bond *et al.*, 2002, Lui *et al.*, 2004).

1.7 Typical electrode reactions are shown below;

1.7.1 Anode reaction

The electron donors are oxidized in the anode compartment of a microbial fuel cell

$$nCH_2O + nH_2O \rightarrow nCO_2 + 4ne^- + 4nH^+$$

1.7.2 Cathode reaction

The electrons are transferred to the cathode compartment through the external circuit, because of the potential difference between the anode and cathode supplied with air. The protons are transferred to the cathode through the membrane. The electron and proton are consumed, reducing oxygen in the cathode compartment

$$nO_2 + 4ne^- + 4nH^+ \rightarrow 2nH_2O$$

The overall reaction is the breakdown of the substrate to carbon dioxide and water with a concomitant production of electricity as a by-product. Based on the electrode reaction pair above, an MFC bioreactor can generate electricity from the electron flow from the anode to cathode in the external circuit.

The bacteria depend on the anode for life. The bacteria at the anode breathe the anode, much like people breathe air, by transferring electrons to the anode. Because bacteria use the anode in their metabolism, they strategically position themselves on the anode surface to form a bacterial community called biofilm.

Bacteria in the biofilm produce a matrix of material so that they stick to the anode. The biofilm matrix is rich with material that can potentially transport electrons. The sticky biofilm matrix is made up of complex extracellular proteins, sugars, and bacterial cells. The matrix also has been shown to contain tiny conductive nanowires that may help facilitate electron conduction.

Bacteria have evolved to utilize almost any chemical as a food source. In the MFC, bacteria form a biofilm, a living community that is attached to the electrode by a sticky sugar and protein coated biofilm matrix. When grown without oxygen, the byproducts of bacterial metabolism of waste includes carbon dioxide, electrons and hydrogen ions. Electrons produced by the bacteria are shuttled on to the electrode by the biofilm matrix, creating a thriving ecosystem called the biofilm anode and generating electricity.

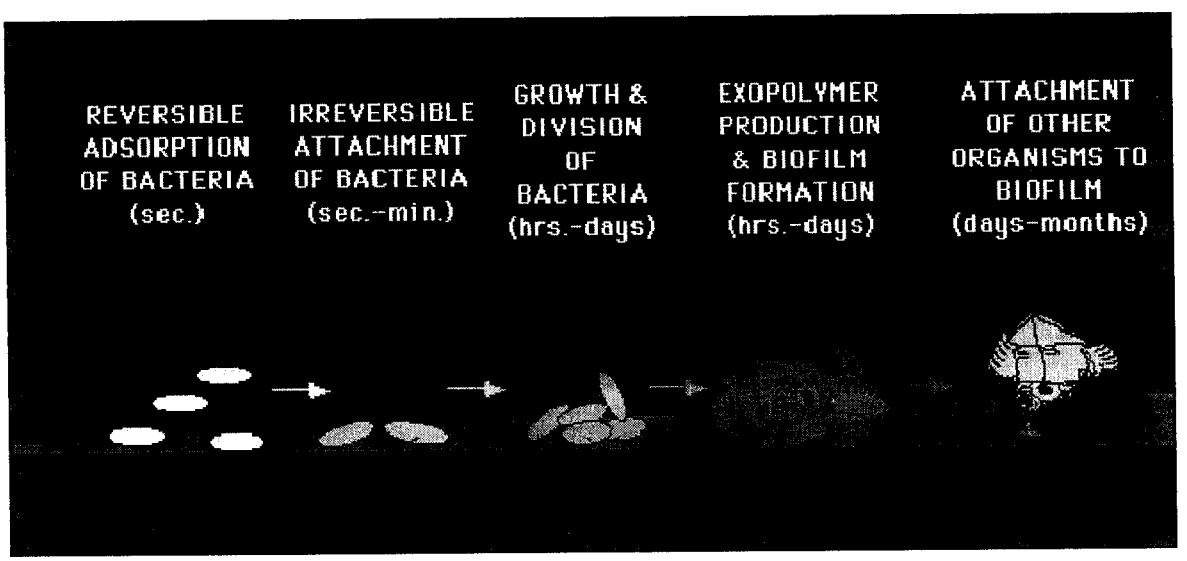


Fig. 1.4: Biofilm formation on the anode surface

Objective of the Study

2. OBJECTIVE

1. To use different waste water collected from different localities for the production of electricity using MFC.
2. To compare the Voltage produced by different waste water.
3. To change the catholyte (electron acceptors) and check their impact on the power generation.
4. Isolation and identification of *Staphylococcus aureus and Pseudomonas aeruginosa* from waste water.
5. To generate electricity and treat the wastewater with the two species that was isolated.

Review of Literature

3. REVIEW OF LITERATURE

Microbial fuel cell (MFC) is a device which converts chemical energy to electrical energy during substrate oxidation with the help of microorganisms (Allen and Bennetto, 1993; Byung Hong Kim *et al.*, 1999, Park and Zeikus, 2000, Bond and Lovely, 2003, Gil *et al.*, 2003, Liu *et al.*, 2004). Microbial fuel cell is made up of two compartments, anode and cathode, separated with proton cation exchange membrane. Microorganisms oxidize the substrate and produce electrons and protons in the anode chamber of MFC. Electrons collected on the anode are transported to cathode by external circuit and protons are transferred through the membrane internally. Thus, potential difference is produced between anode and cathode chamber due to dissimilar liquid solutions. Electrons and protons are consumed in the cathode compartment by utilizing oxygen from water. Most studies have used electrodes of solid graphite, graphite-felt, carbon cloth and platinum coated graphite cathode electrode.

There is an increasing need in the world today for alternative forms of energy production. This research utilized microbial fuel cell (MFC) technology to treat landfill leachate as well as produce energy. While the technology of MFCs is not new, recent developments have brought the technology to a more useful and practical level. MFCs are devices that operate similar to a battery but utilize anaerobic bacteria as the catalyst to oxidize organic and inorganic matter to generate electrical current (Logan *et al.*, 2006). They consist of conductive electrodes in an anode and cathode compartment. The electrodes facilitate the transfer of electrons from the anode over a resistance towards the cathode. The conductive electrodes are often composed of carbon or graphite as felt, cloth, paper, rods, or plates. Bacterial energy is converted into direct current (DC) that can then be stored in a battery or converted into alternating current (AC).

3.1 ROLE OF METAL REDUCING BACTERIA IN MFC

A dissimilatory metal- and sulfur-reducing microorganism was isolated from surface sediments of a hydrocarbon-contaminated ditch in Norman, Okla. The isolate, which was designated strain PCA, was an obligately anaerobic, nonfermentative, nonmotile, gram-negative rod. PCA grew in a defined medium with acetate as an electron donor and, ferric

oxyhydroxide, ferric citrate, elemental sulfur, Co (III)-EDTA, fumarate, or malate as the sole electron acceptor. PCA also coupled the oxidation of hydrogen to the reduction of Fe (III) but did not reduce Fe(III) with sulfur, glucose, lactate, fumarate, propionate, butyrate, isobutryate, isovalerate, succinate, yeast extract, phenol, benzoate, ethanol, propanol, or butanol as an electron donor. PCA did not reduce oxygen, Mn (IV), U (VI), nitrate, sulfate, sulfite, or thiosulfate with acetate as the electron donor. Cell suspensions of PCA exhibited dithionite-reduced minus air-oxidized difference spectra which were characteristic of c-type cytochromes. Phylogenetic analysis of the 168 rRNA sequence placed PCA in the delta subgroup of the proteobacteria. Its closest known relative is *Geobacterm etallireducens*. The ability to utilize either hydrogen or acetate as the sole electron donor for Fe (III) reduction makes strain PCA a unique addition to the relatively small group of respiratory metal-reducing microorganisms available in pure culture. A new species name, *Geobacter sulfurreducens*, is proposed (Frank Caccavo *et al.*, 1994).

3.2 MEDIATORS USED IN MFC

Most of the bacterial species used in MFC are inactive for transport of electron; hence for intervention, synthetic and natural compounds called redox mediators are required. Dye mediators such as neutral red, methylene blue, thionine, humic acid are used as a mediator (Park and Zeikus, 2000).

There are three ways by which microorganisms can transfer electrons to the anode electrode: using exogenous mediators, such as potassium ferric cyanide, thionine, or neutral red using mediators produced by the bacteria or by direct transfer of electrons from respiratory enzymes (i.e., cytochromes) to the electrode. Mediators provide a method of shuttling the electrons from inside of the bacterial cell to the electrode. There are several drawbacks to using exogenous mediators, such as their expense, short lifetime, and toxicity to the microorganisms. However, when the bacteria produce their own mediators, or they transfer electrons directly to the electrode, the system operates at a high, sustained level of activity. It is define as the system as a mediator-less MFC based on the observation that exogenous mediators do not need to be added. Several isolates, including *Shewanella putrefaciens*, *Geobacter sulfurreducens*, *Geobacter metallireducens* and *Rhodoferax ferrireducen*. have been shown to generate electricity in mediator-less MFC systems (Bond *et al.*, 2002).

A MFC consists of anode and cathode separated by a cation-specific membrane. Microbes in the anode oxidize fuel, and the resulting electrons and protons are transferred to the cathode through the circuit and the membrane, respectively. Electrons and protons are consumed in the cathode, reducing oxidant, usually oxygen. Since the microbial cells are electrochemically inactive due to the nonconductive cell surface structure, mediators are employed to facilitate electron transfer from the microbial cells to the anode in MFCs (Catal. T. *et al.*, 2008).

3.3 MEDIATOR – LESS MICROBIAL FUEL CELL

A mediator-less microbial fuel cell was optimized in terms of various operating conditions. Current generation was dependent on several factors such as pH, resistance; electrolyte used, and dissolved oxygen concentration in the cathode compartment. The highest current was generated at pH 7. Under the operating conditions, the resistance was the rate-determining factor. With the lower resistance, proton transfer and dissolved oxygen (DO) supply limited the cathode reaction. A high strength buffer reduced the proton limitation to some extent. The fact that oxygen limitation was observed at high DO concentration is believed to be due to the poor oxygen reducing activity of the electrode used, graphite. The current showed linear relationship with the fuel added at low concentration, and the electronic charge was well correlated with substrate concentration. The microbial fuel cell might be used as a biochemical oxygen demand (BOD) sensor (Geun-Cheol Gil *et al.*, 2003).

3.4 MEMBRANE-LESS MICROBIAL FUEL CELL

A membrane-less microbial fuel cell (ML-MFC) was used to enrich a microbial consortium oxidizing electron donors with concomitant current generation. Forced aeration to the cathode compartment generated higher current. Use of a cathode with a higher affinity for oxygen could improve the current yield. Additions of NaCl or HCl increased the current generation. Aerobic microbes turned out to be the predominant oxygen consumer at the cathode. Based on these findings suggestions are made for a ML-MFC configuration with better performance. (Jae Kyung Jang *et al.*, 2004).

A membrane-less microbial fuel cell (ML-MFC) with the internal resistance of 3.9M was used to enrich a microbial consortium oxidizing electron donors with concomitant current generation. Within 4 weeks the system generated a stable current of 2 mA. The current yield was less than 10%. Forced aeration to the cathode compartment generated higher current, but the yield was similar. Use of a cathode with a higher affinity for oxygen could improve the current yield. Additions of NaCl or HCl increased the current generation further with the current yield of 15%. Aerobic microbes turned out to be the predominant oxygen consumer at the cathode. Based on these findings suggestions are made for a ML-MFC configuration with better performance (Jae Kyung Jang *et al.*, 2003).

3.5 MFC USING PROTON EXCHANGE MEMBRANE

In a microbial fuel cell (MFC), power can be generated from the oxidation of organic matter by bacteria at the anode, with reduction of oxygen at the cathode. Proton exchange membranes used in MFCs are permeable to oxygen, resulting in the diffusion of oxygen into the anode chamber. This could either lower power generation by obligate anaerobes or result in the loss in electron donor from aerobic respiration by facultative or other aerobic bacteria. In order to maintain anaerobic conditions in conventional anaerobic laboratory cultures, chemical oxygen scavengers such as cysteine are commonly used. It is shown here that cysteine can serve as a substrate for electricity generation by bacteria in a MFC. A two-chamber MFC containing a proton exchange membrane was inoculated with anaerobic marine sediment. Over a period of a few weeks, electricity generation gradually increased to a maximum power density of 19mW/m^2 (700 or 1000O resistor; 385 mg/L of cysteine). Power output increased to 39mW/m^2 when cysteine concentrations were increased up to 770 mg/L. The use of a more active cathode with Pt- or Pt–Ru, increased the maximum power from 19 to 33mW/m^2 demonstrating that cathode efficiency limited power generation. Power was always immediately generated upon addition of fresh medium, but initial power levels consistently increased by Ca. 30% during the first 24 h. Electron recovery as electricity was 14% based on complete cysteine oxidation, with an additional 14% (28% total) potentially lost to oxygen diffusion through the proton exchange membrane. 16S rRNA based analysis of the biofilm on the anode of the MFC indicated that the predominant organisms were *Shewanella spp.* closely related to *Shewanella affinis* (37% of 16S rRNA gene sequences recovered in clone libraries). (Bruce E. Logan *et al.*, 2005).

3.6 APPLICATIONS

The discovery of microorganisms known as electricigens had cleared that the highly efficient and sustainable microbial fuel cells are used in a diversity of applications.

3.6.1 Energy autonomy in robots through microbial fuel cells

A robot powered by live microorganisms can utilize as fuel a wide range of organic substrates of the types found in agriculture or food wastes. This implies that can be designed to operate in a range of habitats where they can exploit various forms of energy sources and hence illustrate a different (perhaps higher) level of autonomy. Microbial Fuel Cell (MFC) technology offers the potential of exploiting microbial metabolism to produce electrical energy. This is a good way forward, as the robot will incorporate in its behavioural repertoire actions that involve search and get hold of food and also remain inactive until energy is sufficient to do the next task. (Ioannis Ieropoulos *et al.*, 2003).

3.6.2 Transport and energy generation

At present the world's largest source of power is derived from the use of fossil fuels and especially petroleum. However the burning of hydrocarbons cannot continue indefinitely because of environmental problems and also the simple fact that we have a finite supply of these fuels. The utilization of biofuel cells with carbohydrates as a power source would, if they could be developed, help to mitigate at least some of these problems. It has been calculated that a litre of a concentrated carbohydrate solution could power a car for 25-30 km. it follows that if a car were to be fitted with a 50 L tank, the car could travel over 1000 km without refuelling. Not only would this offer environmentally benefits, it would also remove the risk associated with transport of large amounts of volatile, flammable fuels in addition to the risk of fire following a road traffic accident (Shukla *et al.*, 1999).

3.6.3 Implantable power sources

Since biofuel cells can potentially be run in living systems, taking the oxygen and fuel required for their operation can conceivably be taken from their immediate environment and this offers great potential as power sources within a range of possible implantable medical devices. For example, a biosensor for glucose has been developed utilizing a glucose oxidase based anode and cytochrome -C cathode to generate electrical current (Katz *et al.*, 2001).

3.6.4 Waste water treatment:

Numerous fuel cells have been shown to generate power by oxidation of compounds found in waste water streams. Two useful purposes can be realized by this procedure; (a) for the removal of organic compounds from the waste stream and (b) for the generation of electrical power. A recent review on the subject (Logan, 2005) calculates that the waste water from the town of 150,000 people could potentially be used to generate up to 2.3 MW of power (assuming 100% efficiency), although a power of 0.5 MW might be more realistic. It should be mentioned in this context that up to 80% of the chemical oxygen demand of waste water can be removed by treatment in a microbial fuel cell and it is possible that the electricity generated in this manner could be used on site to power further treatment of waste water. An economic study with in the review (Logan, 2005) shows the potential for this application, although this is highly dependent on local power cost (Gil *et al.*, 2003, Rabaey *et al.*, 2004, Liu and Logan ,2004).

3.6.5 Metal reduction

MFCs are used in the treatment of fresh water contaminated with metals like uranium, sulphur, iron etc., Metal reducing bacteria such as Desulfuromonas acetoxidants is involved in the reduction of sulphur and iron contaminated water.

A very recent study utilized some of the accepted electron mediators from earlier studies, to examine the effects of mediators have on electricity production and fermentation within the anolyte (Sund *et al.*, 2007). The effects of electrode materials have been tested by

a few research groups, focusing on the use or addition of metals such as manganese, copper and gold (Park & Zeikus, 2002; Crittenden, Sund, & Sumner, 2006; Kargi & Eker, 2007).

3.6.6 Bioremediation

Mechanism of electron transfer of *Geobacter* contributes to bioremediation of ground water contaminated with organic and metal contaminants. The ability to produce fine, long conductive filaments has application in the development of nanoelectric devices, sensors and microbially based fuel cells. *Geobacter* species play an important role in bioremediation of ground water contaminated with petroleum and landfill leachate. Soluble organic contaminants are oxidized to carbon dioxide with the reduction of iron oxides that are abundant in most subsurface environments.

Materials and Methods

4. MATERIALS AND METHODS

4.1 COLLECTION OF SAMPLE

The wastewater used in this study was collected from in and around the locations of Chennai, TamilNadu.

4.2 CLEANING SOLUTION

The glass wares used were first soaked in chromic acid cleaning solution (10% potassium dichromate solution in 25% concentrated sulphuric acid) for three hours and then washed thoroughly in tap water. Then washed using commercial detergent, tap water, finally rinsed in distilled water and oven dried at 80 ^{0}C.

Potassium dichromate	- 60 g
Conc. H_2SO_4	- 60 ml
Distilled water	- 1000 ml

Potassium dichromate was dissolved was dissolved in warm water, cooled and sulphuric acid was added slowly. It was mixed thoroughly and used for cleaning of glasswares.

4.3 STERILIZATION

Dried glasswares and media were sterilized in autoclave at 121 ^{0}C, 15psi for 20 minutes.

4.4 CHEMICALS

Analytical grade chemicals such as potassium ferricynide, potassium permanganate, potassium hydroxide, sodium chloride, potassium nitrate and other were purchased from Hi- Media. Loba Fisher chemicals and Sisco research laboratories. Mumbai.

4.5 MEDIA PREPARATION

4.5.1 Nutrient agar:

It acts as a best substrate for the bacterial growth.

Peptone	-	5 g
Beef extract	-	3 g
NaCl	-	5 g
Agar	-	20 g
Dis.H_2O	-	1000 ml

The medium was prepared and sterilized in the autoclave at 15psi (121°C) for 15 minute

4.5.2 Nutrient broth:

The same composition as mentioned above without adding agar.

The broth for the respective media is prepared without adding agar and they are sterilized in autoclave (121°C for 15 mins). A loop full of colony which is isolated from the streaking techniques are inoculated in the broth and kept in shaker for overnight at 36 °C. Presence of turbidity in the media shows the presence of the microorganisms. They can also be preserved in effendorf tube for future use.

4.5.3 Mannitol salt agar (MSA) media

This is specific medium for *staphylococcus aureus*

MSA agar -35g

Distilled water -1000ml

The medium was prepared and sterilized in the autoclave at 15psi (121°C) for 15 minute

4.6 ISOLATION OF MICROORGANISMS FROM WASTE WATER

In this method 1 ml of the waste water was mixed with 100ml of sterilized distilled water. Dilutions were made usually in multiples of 10 in the range of 10^{-1} to 10^{-7}

The samples were prepared by transferring 1ml of the soil solution to 9 ml of the second dilution and so on. Once diluted, specified volume (1 ml) of diluted sample from various dilutions was added to sterile petriplates to which 20 ml of sterilized nutrient agar medium was added. In order to arrest the fungal growth, anti-fungal agent Gresoflavin was added to the nutrient agar media. The plates were then allowed to solidify and incubated in an inverted position at 30 ^{0}C for a period of 24-48 hours. The organisms were then identified and observed by their morphological and biochemical characteristic features.

4.6.1 SPREAD PLATE TECHNIQUE

Principle:

Microorganisms are ubiquitous in nature, so it is necessary to isolate and culture pure culture of organisms in order to study the properties of a particular organism. Pure culture represents the population of organism of a single species in the absence of living cell of any other species.

Method:

All the industrial waste water is cultured in Nutrient Agar Media. The media is poured in the plates and allowed to satisfy. For the isolation of microorganisms, the samples are first serial diluted and 5^{th}, 6^{th}, and 7^{th} diluted samples are plated for isolation of bacteria in NA medium. Spread plate is done for these dilutions and incubated at 36 °C for 18-24 hrs.

4.6.2 SUB-CULTURE (STREAK PLATE TECHNIQUE)

Principle:

A single organism, physically separated from others on the surface of medium, will multiply and give rise to a localized colony of descendants

Method:

The colonies are selected in Nutrient Agar Media. The media is poured in the plates and allowed till it gets solidified. Inoculation loop is heated red in flame and allowed to cool for 30 seconds. Touch the culture with the loop and take the culture. Plate it on agar plate and streak it on the plates, again sterilize the loop and after cooling take the culture from one end to another and complete the streaking. These plates are inoculated at 36 °C for 18-24 hrs. For slants, the media are poured in the test tubes and kept them in slanting position with plugged end over the glass rod and allowed till the media gets solidified. Culture is taken in the sterilized loop and then streaked. They are incubated at 36 °C for 18-24 hrs. These slants are used for the bio-chemical test and identification of the microorganisms.

4.7 BIOCHEMICAL TEST

4.7.1 METHYL-RED AND VOGES-PROSKAUER TESTS

The methyl red (MR) and the Voges-Proskauer tests were employed to detect the production of ethanol and acetoin (nonacidic end products) from the fermentation of glucose.

Preparation of MR-VP Broth:

MR-VP broth was prepared by dissolving 7.0 grams of peptone, 5.0 grams of glucose and 5.0 grams of potassium phosphate in 1000.0 ml of distilled water and was autoclaved at 15psi for 15 minutes.

Procedure:

MR test was performed by inoculating a loopful of culture in the MRVP broth and keeping one uninoculated tube of broth as control. The tubes were then incubated at 35 ^{0}c for 48 hours. Then 5 drops of methyl red indicator was added to the tubes and the colour change was observed. Cherry red colour indicates the positive result.

VP test was performed by adding 12 drops of 40% KOH(VP-reagent I) and 2-3 drops of 45% α-naphthol(VP-reagent II) and the tubes were shaken gently for few minutes, allowed to stand for 15-30 minutes and the colour change was observed. Pink colour indicates the positive result.

4.7.2. CITRATE UTILIZATION TEST

Principle:

Citrate utilization test indicates the breakdown of citrate to oxaloacetic acid and acetic acid in the presence of the enzyme citrase.

Procedure:

The Simmon's citrate agar medium was prepared, sterilized and distributed into sterile test tubes. The culture was then inoculated on the surface of Simmon's citrate agar and incubated at 37 ^{0}C for 24-48 hours. The colour change from green to deep Prussian blue indicates a positive result.

4.7.3. CATALASE TEST

Principle:

Catalase test was performed to indicate the degradation of hydrogen peroxide by the synthesis of Catalase enzyme.

Procedure:

The trypticase soy agar medium was prepared, sterilized and poured into the culture tubes. The culture was then inoculated in the medium and one uninoculated agar slant was kept as control. The culture tubes were incubated at 35 ^{0}C for 24-48 hours. Then 3-4 drops of hydrogen peroxide was allowed to flow over the growth of each culture slant. Production of bubbles of oxygen after the addition of hydrogen peroxide indicates positive response.

4.8 IMMOBILIZATION OF THE ISOLATED ORGANISMS

In order to increase the survivality and longevity, the organisms were immobilized in sodium alginate beads.

Preparation of reagents:

3% sodium alginate solution was prepared by dissolving 30 grams of sodium alginate in 1000 ml of distilled water and kept in water bath until it gets completely dissolved.

0.2 M calcium chloride solution was made by dissolving 110.99 grams of calcium chloride in 1000 ml of distilled water.

Procedure:

5 ml of liquid culture was mixed with 100 ml of 3% Sodium alginate solution. This polymer solution was dropped from a height of approximately 20 cm into an excess amount of stirred 0.2 M $CaCl_2$ solution with a syringe and needle at room temperature. The beads formed were then collected, dried using filter paper and stored at 30 °C for further usage.

4.8.1 MUTATED BEADS

The immobilized beads were kept under UV-light for 10 minutes.

4.9 MICROBIAL FUEL CELL

The microbial fuel cell was constructed using salt bridges. The construction based on two chamber fuel model which has been connected by salt bridge.

4.9.1 ELECTRODES

S.NO	ELECTRODE TYPE	SIZE	USES
1	Graphite sheet	0.5mm (Thick) x 5 cm (length) x 1cm (Breath)	Sewage waste, Sea water
2	Graphite rod	0.3cm (Diameter) x 5 cm (length)	Sewage waste, Seawater, starch waste, fish waste, cow urine
3	Galvanised Zinc mesh	0.5mm (Thick) x 5 cm (length) x 1cm (Breath)	Sewage waste, Sea water
4	Steel mesh	0.5mm (Thick) x 5 cm (length) x 1cm (Breath)	Sewage waste, Sea water

The electrode in the anodic compartments was made of graphite sheets, graphite rods, steel mesh and Galvanised mesh in some experiments. Since current production is proportional to the surface area of the electrode, we used wavy graphite sheets for most of the fuel cell experiments.

Graphite sheets was used for it chemical inertness, high temperature strength, high surface area to volume ratio, low resistance and for its electrical conductivity. The graphite sheets were connected to copper wires by direct insertion of the wires into the graphite sheet. The new electrodes were soaked in 100% ethanol for 30 minutes and in 1M HCL for 1 hour. After each use the electrodes were washed in 1.0M HCL followed by 1. M NaOH, each for 1 hour to remove possible metal and inorganic contamination, and stored in distilled water before use.

4.9.2 SALT BRIDGE

A salt bridge was formed using plastic tube as described by (Geiser *et al* 2002). The ends of the salt bridge tube were placed into the top of the anodic and cathodic chamber of the microbial fuel cell. The filling solution for the salt bridge consisted of 2 g/100ml of agar and 1M KCL. The filling solution and syringe apparatus were autoclaved at 121^0C and 1 atm before filling the plastic tube. The filling solution was then added to the plastic tube using the syringe apparatus. The salt bridge was used for selective transport of protons or cations the bridge

4.9.3 ANODIC COMPARTMENTS

We used different type of cells. We collected sludge's from different places. Waste used for microbial fuel cells are

- Sewage waste
- Cow urine

- Reverse osmosis water
- Sea water
- Fish waste
- Beverage waste

4.9.4 CATHODIC COMPARTMENTS

0.1M potassium ferricynide or 0.1 M copper sulphate or 0.1M potassium permanganate was used in the cathodic compartment of the fuel cell. They are good oxidant which they accepts the electrons and get reduced.

- Potassium ferricynide: 0.1M potassium ferricynide was prepared adding 32.925g in 1000ml of distilled water.
- Potassium permanganate: 0.1M potassium permanganate was prepared adding 15.9g in 1000ml of distilled water.
- Copper sulphate: 0.1M Copper sulphate was prepared adding 24.9g in 1000ml of distilled water.

4.10 MICROBIAL FUEL CELLS OPERATION

The microbial fuel cell was operated by following the protocol:

1. Prepared the salt bridge using KCl in a flexible plastic tube.
2. Assembled the bottles with salt bridge and electrodes.
3. The microbial fuel cell was sterilized after complete assembly. The assembled set up was wiped with spirit and kept under UV light for half an hour in order to sterilize the apparatus.
4. The samples were poured in the anodic compartment carefully and closed tightly the provisions were sealed tightly the provisions were sealed tightly using sealing materials.
5. In cathodic compartment the potassium ferricynide or potassium permanganate or copper sulphate were poured and closed tightly. These steps have to be carried out in laminar air flow chamber.
6. Operated the entire fuel cell once a constant anodic potential was

established and the first current measurement was taken as a control, during the entire experiment, potential and current measurement were taken.

4.11 PHYSICO-CHEMICAL ANALYSIS OF THE EFFLUENT

The physico-chemical parameters viz., pH, BOD and COD were analysed by the following methods.

4.11.1 MEASUREMENT OF pH

The pH was determined by taking about 50 ml of effluent in a 100 ml cleaned beaker and immersing the calomel electrode of the pH meter and the pH indicated on the dial was noted.

4.11.2 MEASUREMENT OF BIOCHEMICAL OXYGEN DEMAND

The BOD is generally measured by incubating the sample at 20^0C for five days in the dark under aerobic conditions.

Preparation of reagents

1N Sodium hydroxide was made ready by dissolving 4grams of NaOH in 100ml of distilled water.

Phosphate buffer solution was prepared by dissolving 8.5 grams of potassium dihydrogen phosphate, 21.75 grams of dipotassium hydrogen phosphate, 32.4 grams of disodium hydrogen phosphate heptahydrate and 1.7 grams of ammonium chloride in 1000 ml of distilled water and the pH was adjusted to 7.2

Magnesium sulphate solution was prepared by dissolving 25 grams of magnesium sulphate in 1000ml distilled water.

Ferric chloride solution was prepared by dissolving 0.125grams of ferric chloride in 1000ml of distilled water.

Calcium chloride solution was made ready by 27.5 grams anhydrous calcium chloride in 1000ml of distilled water.

Procedure

50ml of tannery effluent were measured directly into sterilized clean bottles of known capacities and the bottles were filled with sufficient diluted water to permit insertion of the stopper without leaving air bubbles. Another bottle with diluted water alone was kept as blank. Then the blank and the diluted sample were incubated for 5 days in the dark at 20^0C and after five days the bottles were kept out from the incubator. Then 2ml manganese sulphate solution, followed by 2ml alkali iodine-azide reagent was carefully added and the contents were mixed by inverting the bottles for 15 minutes

After 2 minutes of precipitate formation, 2ml concentrated sulphuric acid was added and was mixed gently. Then the contents were titrated with starch indicator added to it and the end point was the appearance of blue colour.

Calculation

The BOD (mg/lit) of the tannery effluent was found by applying the formula.

$$\text{BOD of sample (mg/lit)} = \frac{300 \times (B-S)}{A}$$

B = ml of Thio consumed for sample

S = ml of Thio consumed by blank.

A = Volume of sample (ml)

4.11.3 DETERMINATION OF CHEMICAL OXYGEN DEMAND

The amount of organic matter in tannery effluent was estimated by their oxidability by chemical oxidant such as potassium permanganate or potassium dichromate.

Preparation of reagents

0.1N Potassium dichromate solution was made ready by dissolving 3.676 grams of $K_2Cr_2O_7$ in 1000 ml of distilled water.

0.1M Sodium thiosulphate solution was prepared by adding 15.811 grams of sodium thiosulphate in 2000ml of distilled water.

Sulphuric acid solution was obtained by mixing 10.8 ml of concentrated H_2SO_4 in 100 ml of distilled water.10% potassium iodide solution and 1% starch solution were prepared.

Procedure

50 ml of the effluent was taken in three cleaned 100ml Erlenmeyer flasks. Another three 100ml Erlenmeyer flasks with distilled water was read as blank standards. 5 ml of $K_2Cr_2O_7$ solution was added to all the flasks and the flasks were kept in the water bath at 100^0C for one hour. The samples were then allowed to cool for 10 minutes and to the cooled samples 5 ml of Potassium iodide and 10 ml of H_2SO_4 were added. The contents of each flask were titrated with 0.1M sodium thiosulphate solution until the appearance of pale yellow colour. Then 1 ml of starch solution was added and the contents of the flasks turned blue. Finally the contents were titrated with 0.1M Sodium thiosulphate solution till the complete disappearance of the blue colour.

Calculation

The COD (mg/lit) of the tannery effluent was found by applying the formula.

$$\text{COD of sample (mg/lit)} = \frac{8 \times C \times (B-A)}{S}$$

C = Concentration of Titrant (m mol/lit)

A = Volume of Titrant used for blank (ml)

B = Volume of Titrant used for sample (ml)

S = Volume of effluent sample taken (ml).

4.12 SCANNING ELECTRON MICROSCOPE

For scanning electron microscope (SEM) analysis, parts of the graphite were removed from the anode chambers, rinsed with a sterile medium, and immediately fixed using an anaerobic solution of 3% glutaraldehyde for 3 hrs. Sample were the subjected to a serial dehydration protocol using increasing concentrations of ethanol (10%, 40%, 70%, 100%; 30 minutes for each stage) and dried completely at room temperature. The desiccated samples were then analyzed using scanning electron microscope (HITACHI, 5X TO 300000 X) with gold coating.

Results and Discussion

5. RESULTS AND DISCUSSION

5.1 RESULTS FOR BIO-CHEMICAL TEST

The microorganisms present in various samples are identified from the bio-chemical tests performed.

5.1.1 Catalase test:

when few drops of hydrogen peroxide was added directly to the colonies, brisk effervescence followed by bubbling or foaming takes place in the slides (Fig 5). This shows the presence of *Pseudomonas aeruginosa* and *staphylococcus* in the samples.

Fig 5.1: Catalase test (Slide)

5.1.2 Simmon's Citrate Broth:

After incubation of the samples, colour from green to blue takes place in the tubes (Fig4.2). This shows the presence of *Pseudomonas aeruginosa* and *staphylococcus* in the samples.

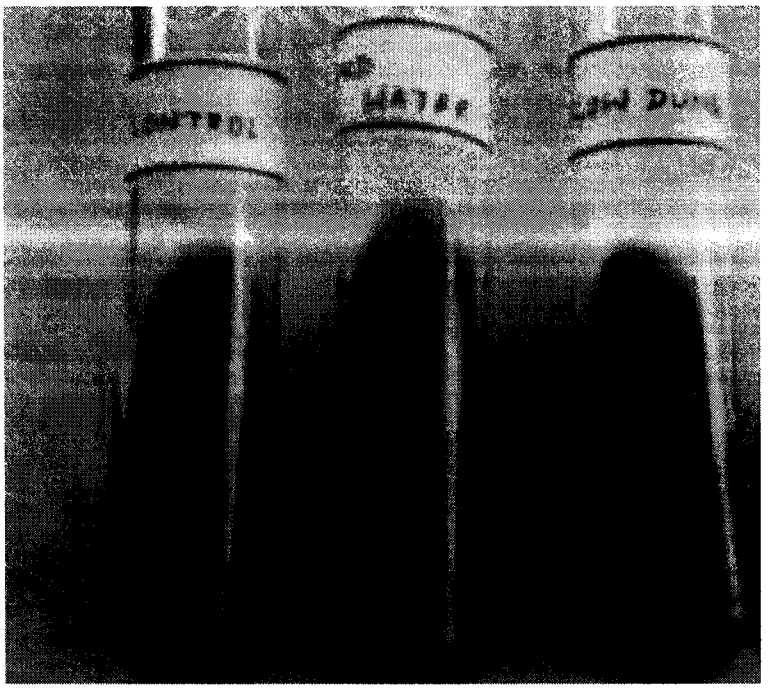


Fig 5.2: Simmon's Citrate Broth Test

5.1.3 Methyl Red Test:

The samples which are kept for incubation for 48 hrs showed some sewage waste changes from yellow to red (Fig4.3). This shows the presence of *Pseudomonas aeruginosa* and *staphylococcus* in the sample.

Fig 5.3: Methyl Red Test

5.1.4 Carbohydrate fermentation test

(a) Lactose broth test

The change in pH was indicated by the colour change of the broth from red to yellow. Rise in the height of Durham's tube showed the presence of gas formation (Fig-4.4). This confirmed the presence of *Pseudomonas* in the samples.

(b) Sucrose broth test

The change in pH was indicated by the colour change of the broth from red to yellow. Rise in the height of Durham's tube showed the presence of gas formation (Fig-4.5). This confirmed the presence of *Pseudomonas* in the samples.

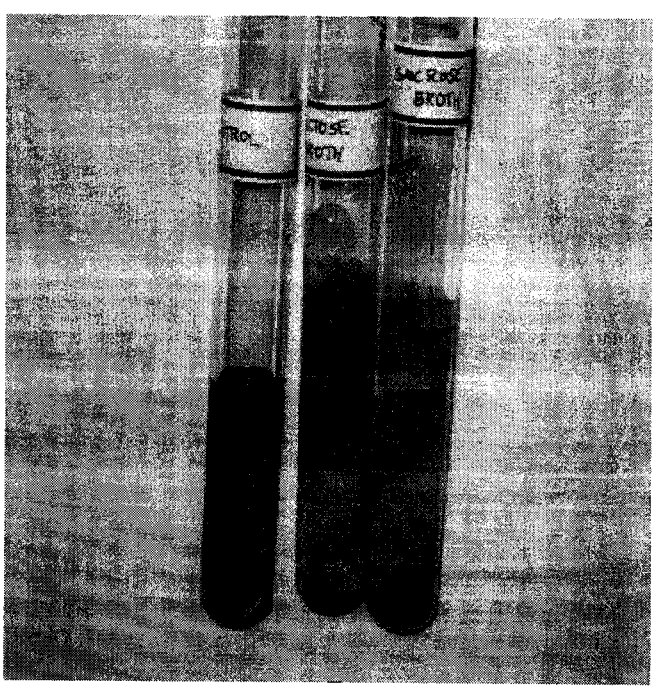


Fig 5.4: Carbohydrate fermentation test

5.2 CONFIRMATORY TEST

5.2.1 Pseudomonas

Nutrient Agar

Colour of the medium to blue indicated the presence of *Pseudomonas* in the samples. (Fig-4.6)

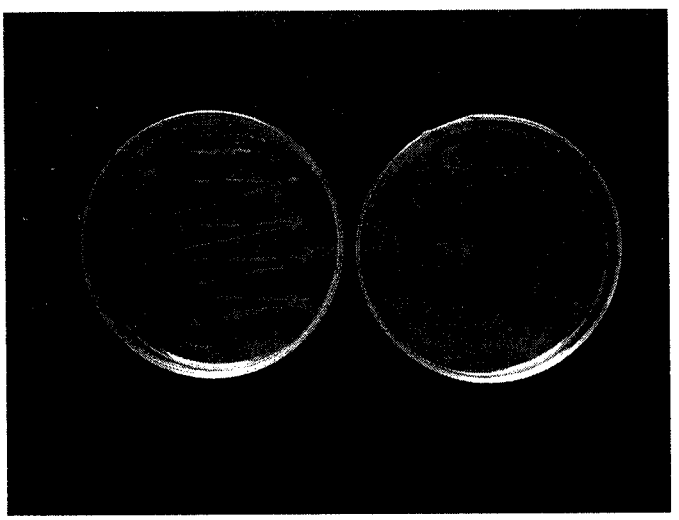


Fig 5.6:*Pseudomonas* in Nutrient Agar

Citrimide Agar Base

The plate turned yellow confirming the presence of *Pseudomonas* in the samples. (Fig-4.7)

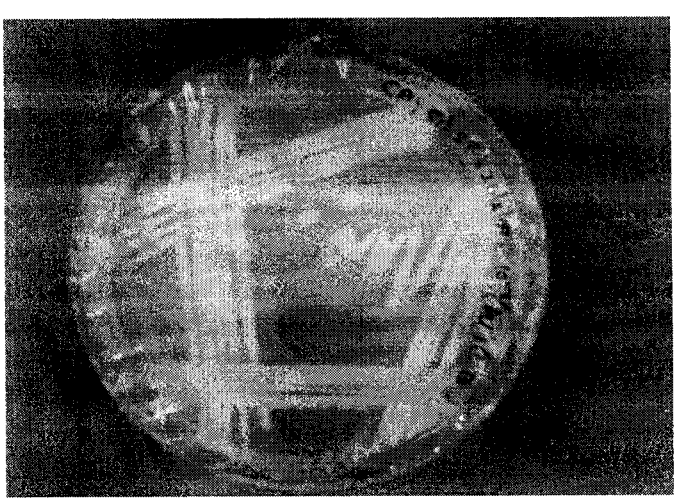


Fig-5.7:- *Pseudomonas* in Citrimide Agar Base

EMB Agar

Purple colour colonies in the plate confirmed the presence of *Pseudomonas* in the samples. (Fig-4.8)

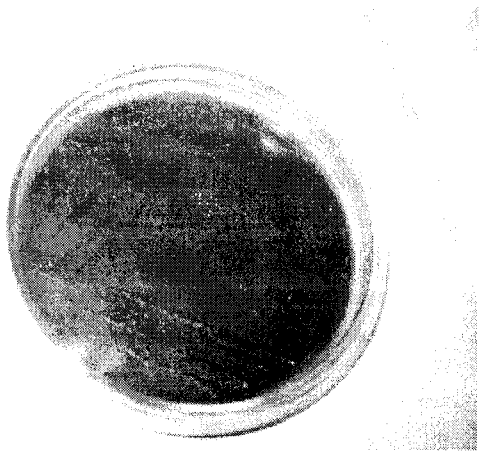


Fig 5.8 : *Pseudomonas* in EMB Agar

5.2.2 *Staphylococcus aureus*

MSA medium

Red colour of medium turn to yellow colour medium and organisms in the plate confirmed the presence of *Staphylococcus aureus* in the samples. (Fig-4.9)

Fig : *Staphylococcus aureus* in Mannitol Salt Agar

5.3 IMMOBILIZED CELLS

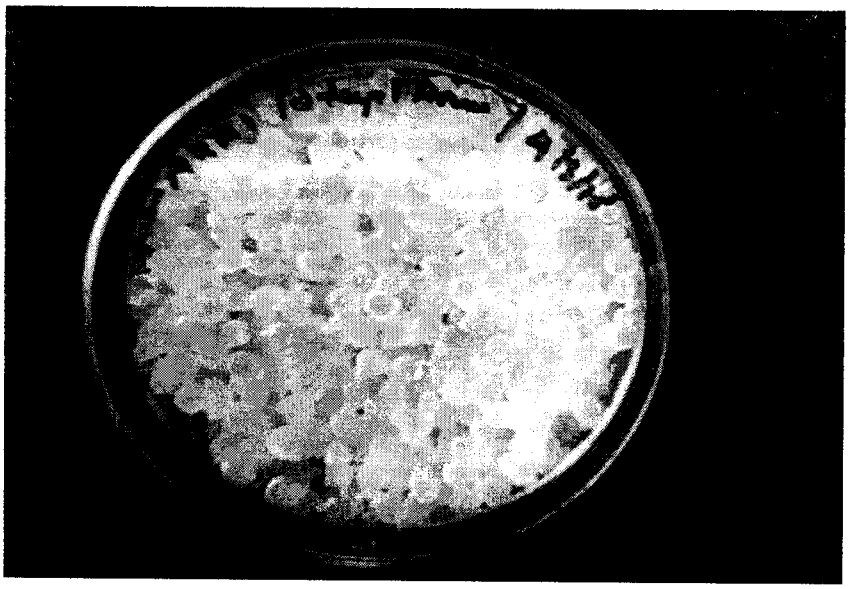


Fig 5.9: Immobilized beads of *Staphylococcus* using Sodium Alginate and calcium chloride

Fig 5.10: Immobilized beads of *Pseudomonas* using Sodium Alginate and calcium chloride

5.4 MFC CONSTRUCTION:

We have constructed 7 different Microbial fuel cells with samples from various industrial sources:

- Sewage waste
- Sea water
- Cow urine and sea water
- Reverse osmosis water
- Fish waste
- Beverage waste

5.4.1 MICROBIAL FUEL CELLS 1 (MFC-1)

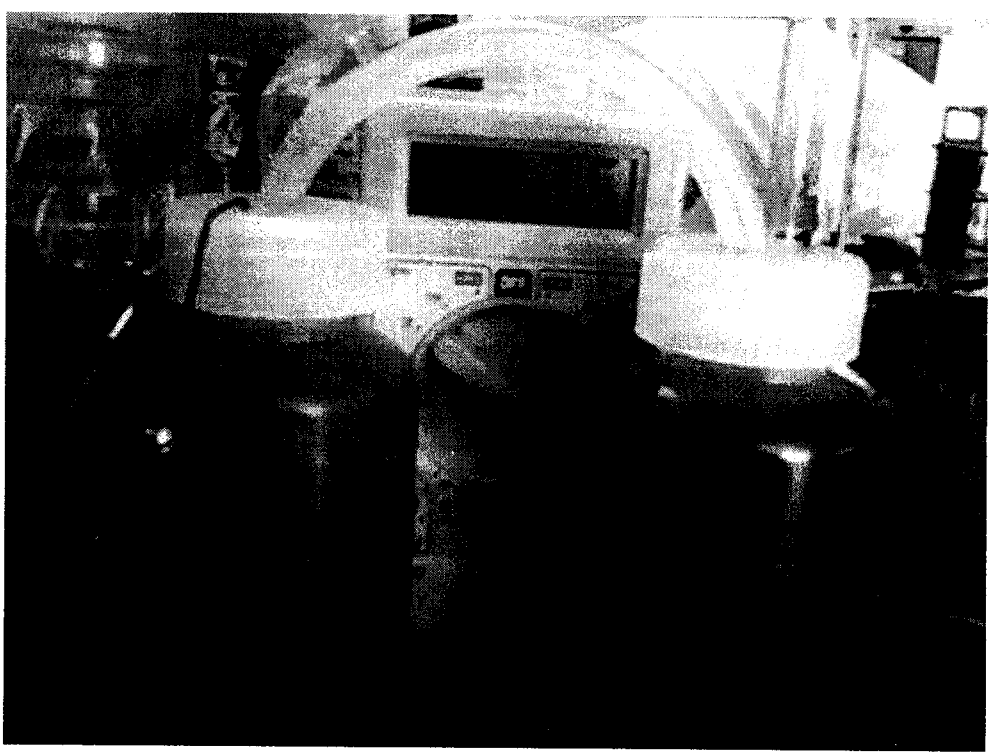


Fig 5.11: Double chamber system for MFCs

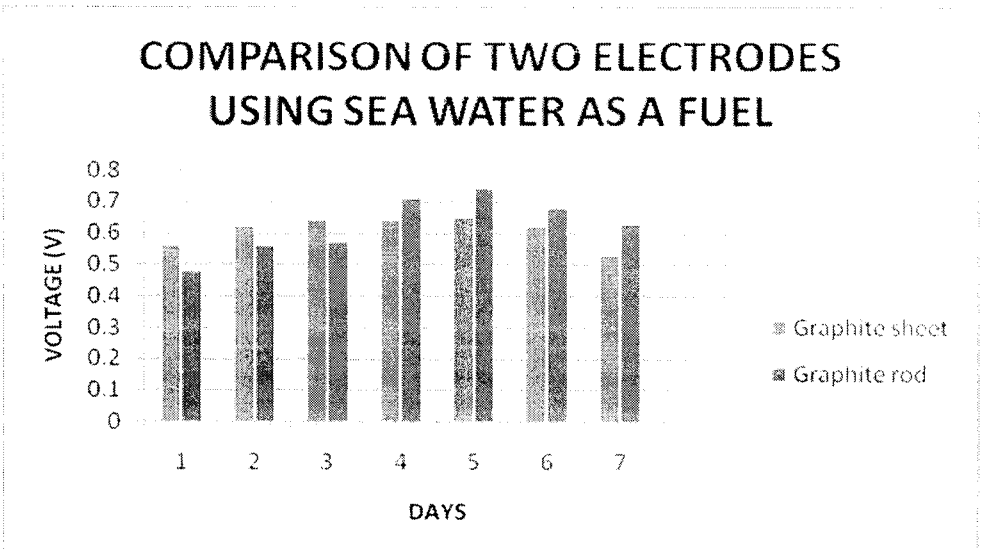
Cathode compartment - Potassium permanganate ($KMnO_4$)

Maximum electricity production in the single set-up was 1.35V.

TABLE 5.1: Comparisons of two electrodes using sea water as a fuel

	VOLTAGE(V)	
Days	**Graphite sheet**	**Graphite rod**
1	0.56	0.48
2	0.62	0.56
3	0.64	0.57
4	0.64	0.71
5	**0.65**	**0.74**
6	0.62	0.68
7	0.53	0.63

Fig 5.12: Comparisons of two electrodes using sea water as a fuel



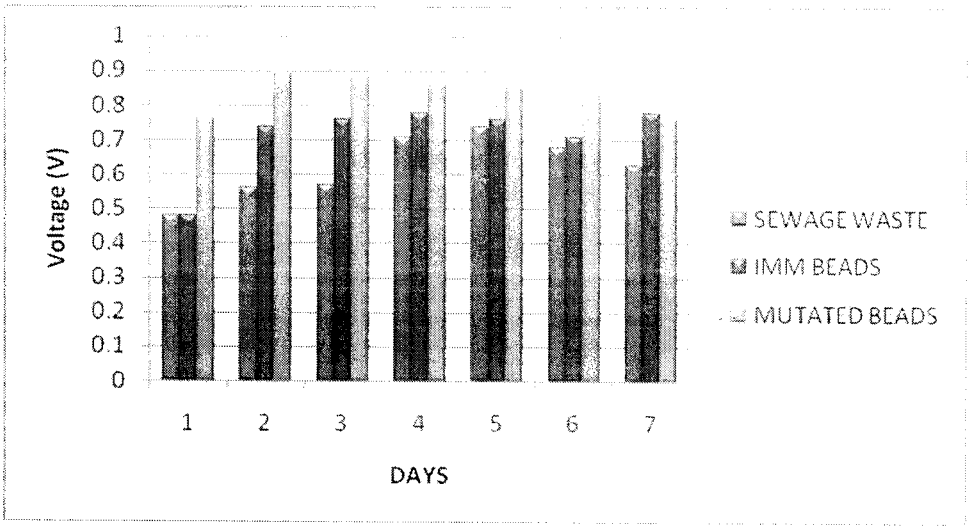
The microbial fuel cell (MFC-1 A&B) was constructed using the sea water from Eliots's beach, Chennai in the anode. Two set ups were done for the same fuel. The electrodes used were graphite rod and graphite sheet. The chambers are connected together by the salt bridge made out of KCl salt and agar. The oxidizing agent used in cathode here was Potassium permagnate ($KMnO_4$). The voltage is measured in both the set up and was found to be **0.65V, 0.74V** respectively. Here the potential voltage generation using graphite rod showed best result.

5.4.2 MICROBIAL FUEL CELLS 2 (MFC-2)

TABLE 5.2: Comparison between sea water, immobilizes bead and mutated beads of *Staphylococcus aureus*

	Voltage (V)		
Days	**Sea Water**	**Immobilized Beads**	**Mutated Beads**
1	0.48	0.48	0.76
2	0.56	0.74	0.89
3	0.57	0.76	**0.88**
4	0.71	**0.78**	0.86
5	**0.74**	0.76	0.85
6	0.68	0.75	0.83
7	0.63	0.74	0.76

Fig 5.13: Comparison between sea water, immobilizes bead and mutated beads of *Staphylococcus aureus*



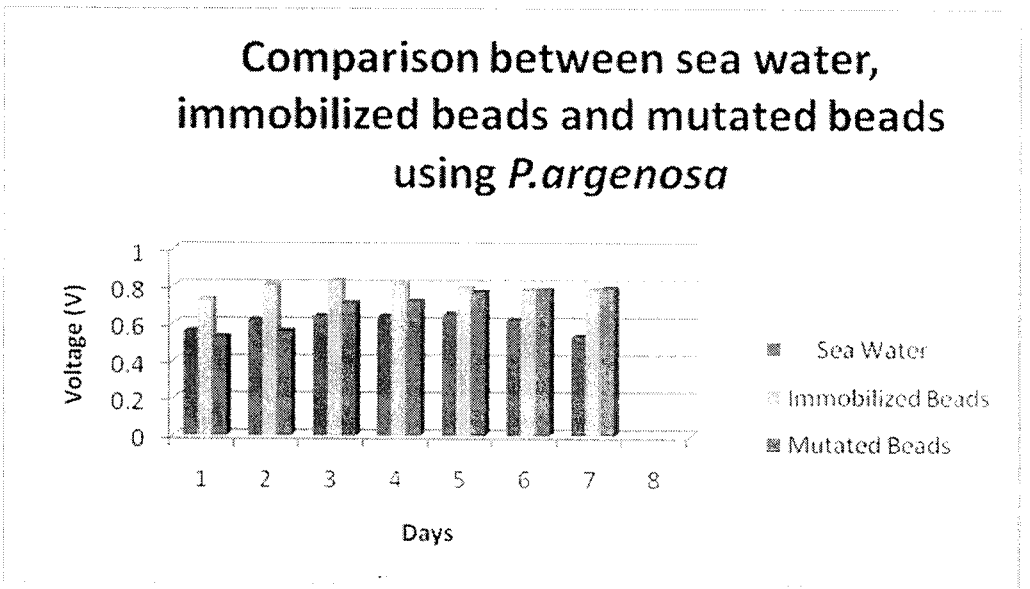
The microbial fuel cell (MFC-2 A,B&C) was constructed using the sea water from Eliots's beach, Chennai in the anode. Three set ups were done for the same fuel. The electrodes used were graphite rod. The chambers are connected together by the salt bridge made out of KCl salt and agar. The oxidizing agent used in cathode here was Potassium permagnate ($KMnO_4$). The voltage is measured in both the set up and was found to be **0.74V,0.78V,0.88V** respectively. Here the potential voltage generation using mutated beads showed best result.

5.4.3 MICROBIAL FUEL CELLS 3 (MFC-3)

TABLE 5.3: Comparison between sea water, immobilized beads and mutated beads using *Psedomonas aueroginosa*

		VOLTAGE (V)	
Days	**Sea Water**	**Immobilized Beads**	**Mutated Beads**
1	0.56	0.73	0.53
2	0.62	0.81	0.56
3	0.64	**0.83**	0.71
4	0.64	0.81	0.72
5	**0.65**	0.79	0.77
6	0.62	0.78	0.78
7	0.53	0.78	**0.79**

Fig 5.14: Comparison between sea water, immobilized beads and mutated beads using *Psedomonas aueroginosa*



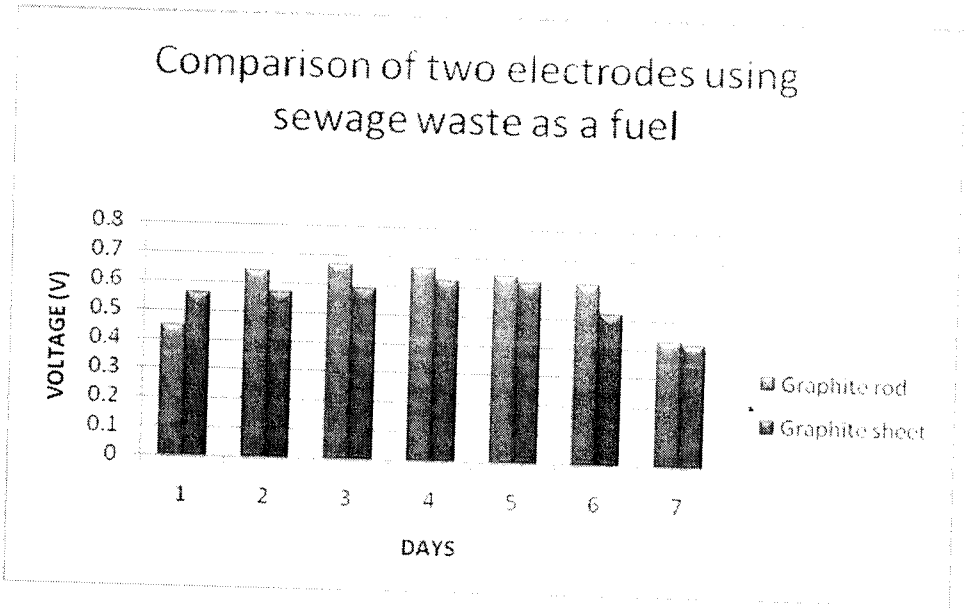
MFC-3(A,B&C) was constructed using the Immobilized cell of *Pseudomonas aueroginosa* (wild and mutated beads) in anode compartment. The voltage was measured in these set ups and is found to be **0.74V,0.82V and 0.78V** respectively.

5.4.4 MICROBIAL FUEL CELLS 4 (MFC-4)

TABLE 5.4: Comparison of two electrodes using sewage waste as a fuel

	VOLTAGE (V)	
Days	**Graphite rod**	**Graphite sheet**
1	0.45	0.56
2	0.64	0.57
3	**0.67**	0.59
4	0.66	**0.62**
5	0.64	0.62
6	0.62	0.52
7	0.43	0.42

Fig 5.15: Comparison of two electrodes using sewage waste as a fuel



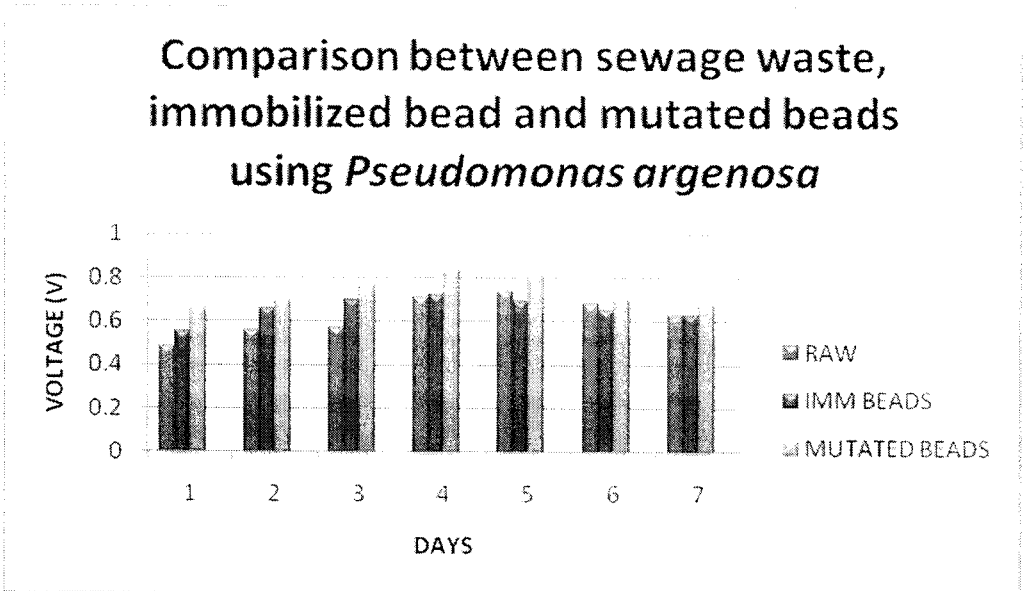
The MFC-4(a& b) was constructed using the sewage waste water collected from Koyembed, Chennai. Two set ups were done for the same fuel. The electrodes used graphite rod and graphite sheet. The chambers are connected together by the salt bridge made out of KCl salt and agar. The oxidizing agent used here is Potassium permanganate ($Kmno_4$). The voltage is measured in both the set up and is found to be **0.67V, 0.62V** respectively. Here the potential voltage generation in graphite rod.

5.4.5 MICROBIAL FUEL CELLS 5 (MFC-5)

TABLE 5.5: Comparison between sewage waste, immobilized bead and mutated beads using *Pseudomonas aueroginosa*

		VOLTAGE (V)	
Days	**Raw waste**	**Immobilized Beads**	**Mutated Beads**
1	0.48	0.55	0.66
2	0.56	0.66	0.69
3	0.57	0.7	0.77
4	0.71	**0.72**	**0.83**
5	**0.74**	0.69	0.81
6	0.68	0.65	0.7
7	0.63	0.63	0.68

Fig 5.15: Comparison between sewage waste, immobilized bead and mutated beads using *Pseudomonas aueroginosa*



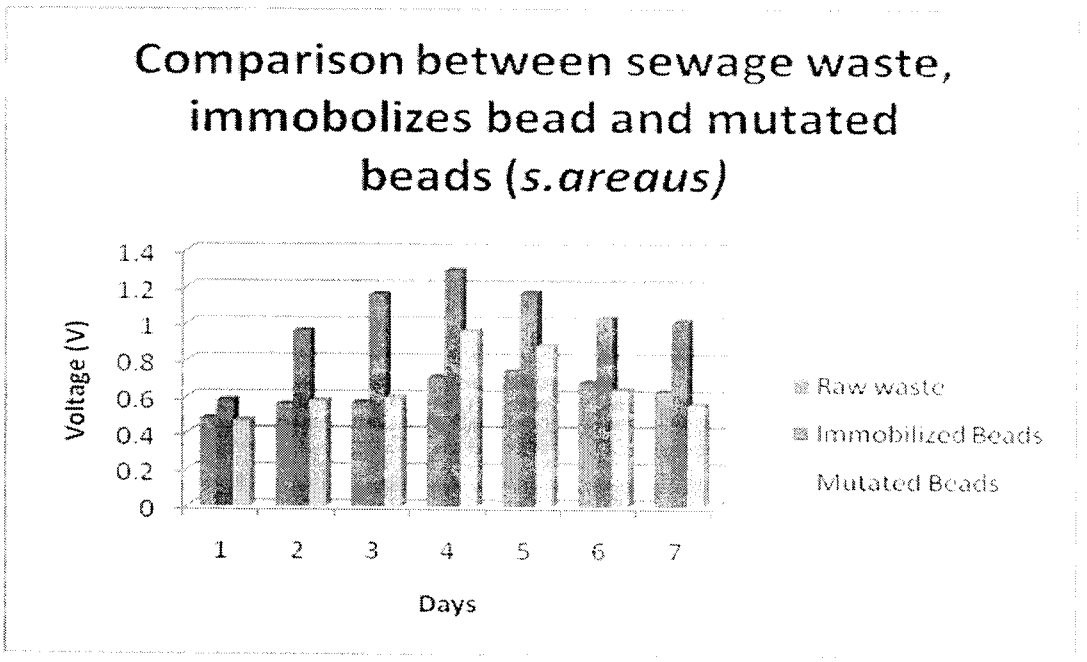
MFC 5 (A,B&C) was developed using Aerobic Sludge from Waste water treatment plant in Koyambedu as fuel.The Immobilized cell using *Psedomonas Aueroginosa* and mutated beads are used in anode compartment. The voltage is measured in these set up and is found to be **0.74 V, 0.72 V, and 0.83V** respectively.

5.4.6 MICROBIAL FUEL CELLS 6 (MFC-6)

TABLE 5.6: Comparison between sewage waste, immobilizes bead and mutated beads (*s.aureus)*

		VOLTAGE (V)	
DAYS	**Raw waste**	**Immobilized Beads**	**Mutated Beads**
1	0.48	0.58	0.47
2	0.56	0.96	0.58
3	0.57	1.16	0.6
4	0.71	**1.29**	**0.96**
5	**0.74**	1.17	0.88
6	0.68	1.03	0.64
7	0.63	1.01	0.56

Fig 5.16: Comparison between sewage waste, immobilizes bead and mutated beads *(s.aureus)*



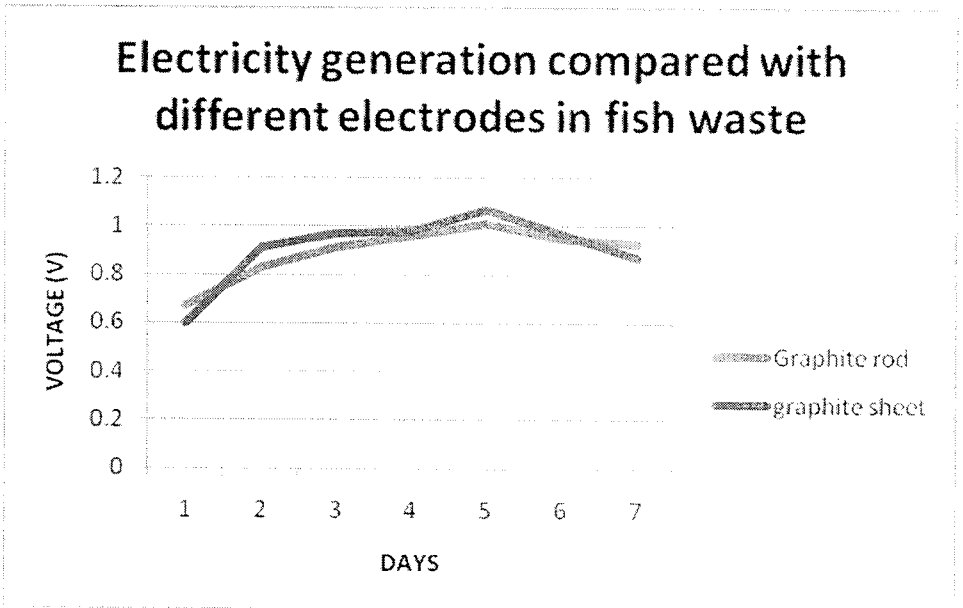
MFC-6(A,B&C) was constructed using the Immobilized cell of *Staphylococcus aureus* (wild and mutated beads) in anode compartment and cathode with Potassium permanganate. The voltage is measured in these set up and is found to be **0.74 V, 1.29 V, and 0.96V** respectively.

5.4.7 MICROBIAL FUEL CELLS 7 (MFC-7)

TABLE 5.7: Electricity generation compared with different electrodes in fish waste

	VOLTAGE (V)	
DAYS	**Graphite sheet**	**Graphite rod**
1	0.67	0.60
2	0.83	0.91
3	0.91	0.97
4	0.96	0.98
5	**1.01**	**1.07**
6	0.95	0.97
7	0.93	0.87

Fig 5.17: Electricity generation compared with different electrodes in fish waste



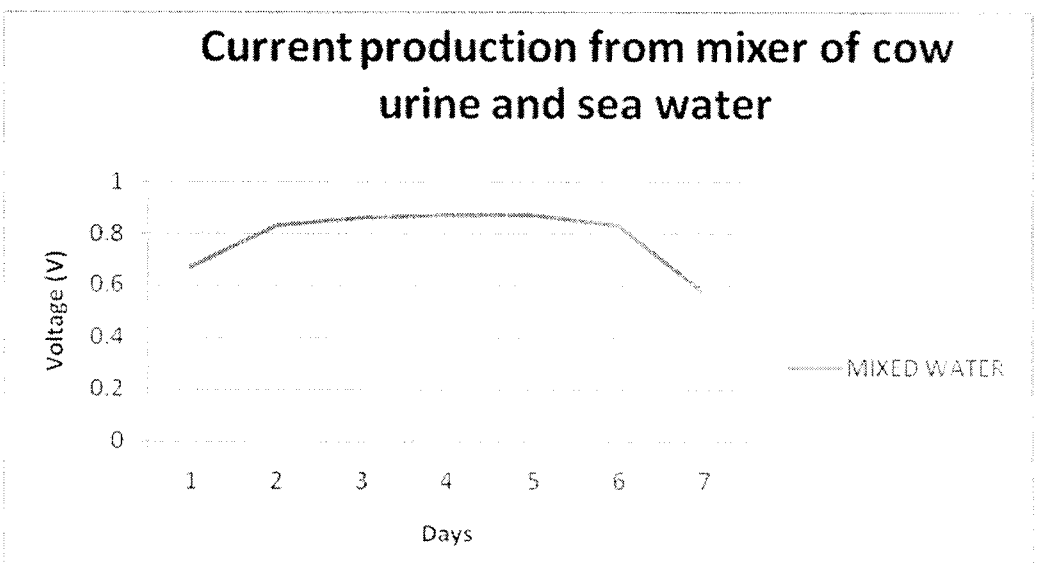
The MFC-7(a& b) was constructed using the fish waste water collected from Fish market ,Chennai . Two set ups were done for the same fuel. The electrodes used graphite rod and graphite sheet. The chambers are connected together by the salt bridge made out of KCl salt and agar. The oxidizing agent used here is Potassium permagnate ($Kmno_4$). The voltage is measured in both the set up and is found to be **1.01 V, 1.07 V** respectively. In double chamber microbial fuel cells produces high voltage within few day. Here the potential voltage generation in graphite rod.

5.4.8 MICROBIAL FUEL CELLS 8 (MFC-8)

TABLE 5.8: Current production from mixer of cow urine and sea water

	Voltage (V)
DAYS	MIXED WATER
1	0.67
2	0.83
3	**0.86**
4	0.87
5	0.87
6	0.83

Fig 5.18: Current production from mixer of cow urine and sea water



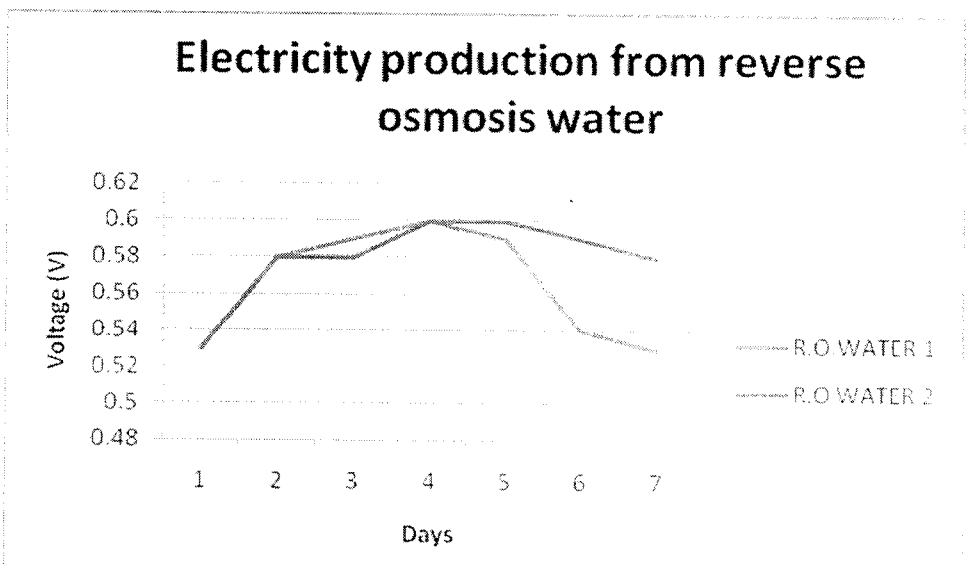
The MFC 8 was constructed using the equal amount of cow urine and sea water. The electrodes used graphite rod and graphite sheet. The mixer of cow urine and sea water shows maximum voltage was **0.85V.**

5.4.9 MICROBIAL FUEL CELLS 9 (MFC-9)

TABLE 5.9: Electricity production from reverse osmosis water

	VOLTAGE (V)	
DAYS	**R.O.WATER1**	**R.O WATER2**
1	0.53	0.53
2	0.58	0.58
3	0.59	0.58
4	**0.60**	**0.60**
5	0.59	0.60
6	0.54	0.59
7	0.53	0.58

Fig 5.19: Electricity production from reverse osmosis water



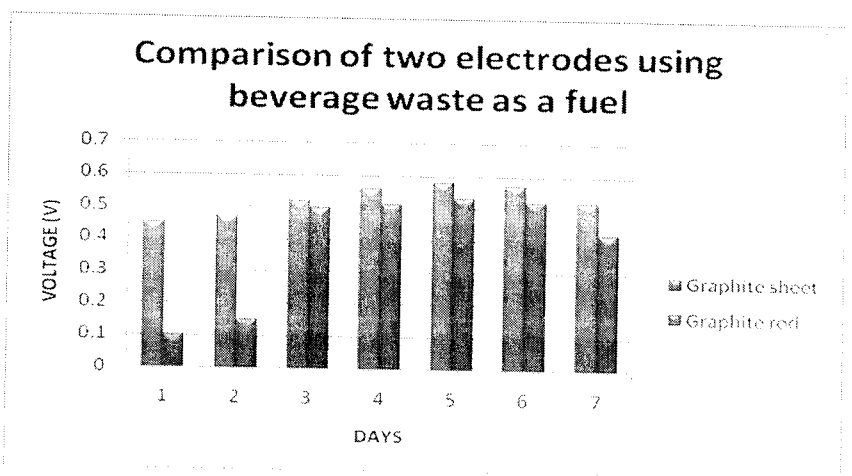
Reverse osmosis water was collected from different place and it's used for anode compartment and electricity is measured. Comprised to R.O water 1. R.O water 2 shows high voltage.

5.4.10 MICROBIAL FUEL CELLS 10 (MFC-10)

TABLE 5.10: Comparison of two electrodes using beverage waste as a fuel

VOLTAGE (V)		
Days	**Graphite sheet**	**Graphite rod**
1	0.45	0.1
2	0.47	0.15
3	0.52	0.5
4	0.56	0.51
5	**0.58**	**0.53**
6	0.57	0.52
7	0.52	0.42

Fig 5.20: Comparison of two electrodes using beverage waste as a fuel



The microbial fuel cell MFC-10 (A&B) was constructed using the beverage in the anode. Two set ups were done for the same fuel. The electrodes used were graphite rod and graphite sheet. The chambers are connected together by the salt bridge made out of KCl salt and agar. The oxidizing agent used in cathode here was Potassium permagnate ($KMnO_4$). The

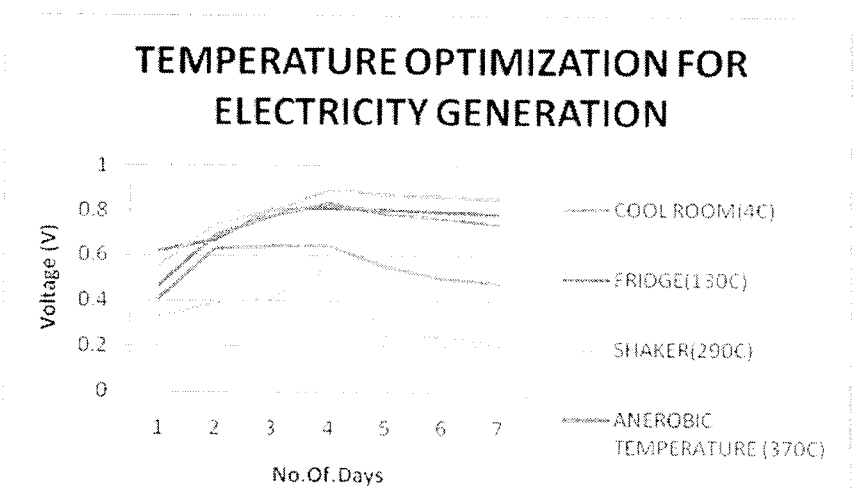
voltage is measured in both the set up and was found to be **0.58V, 0.53V** respectively. Here the potential voltage generation using graphite sheet showed best result.

5.4.11 MICROBIAL FUEL CELLS 11 (MFC-11)

TABLE 5.11: Temperature optimization for electricity generation

VOLTAGE (V)					
DAY	**Cold room (4^0C)**	**Fridge (13^0C)**	**Shaker (29^0C)**	**Anaerobic temperature (37^0C)**	**Lab temperature (24^0C)**
1	0.41	0.62	0.33	0.47	0.56
2	0.63	0.67	0.39	0.69	0.74
3	**0.64**	0.8	0.41	0.77	0.8
4	0.64	**0.81**	**0.55**	**0.83**	**0.89**
5	0.55	0.8	0.25	0.78	0.87
6	0.5	0.79	0.24	0.76	0.86
7	0.48	0.78	0.21	0.74	0.85

Fig 5.21: Temperature optimization for electricity generation



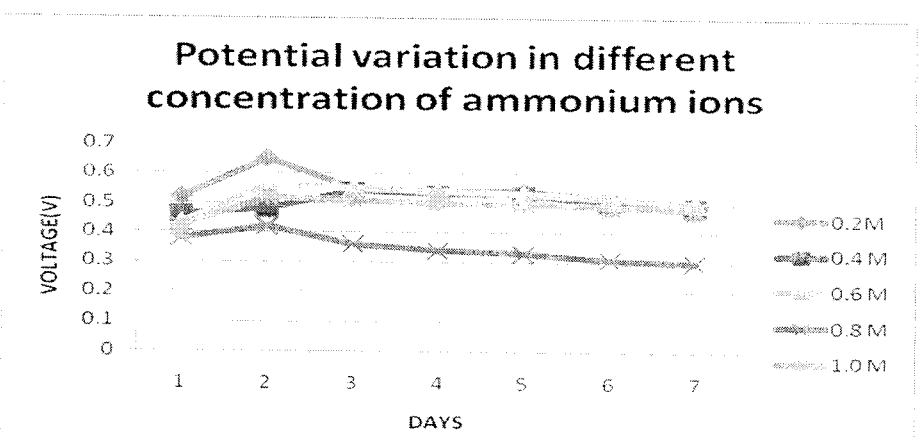
Electricity optimization was done under different temperatures- 4^0C in cold room. 13^0C under fridge, 29^0C in shaker, 37^0C under anaerobic temperature, 24^0C lab temperature. The optimum electricity shows only in room temperature.

5.4.12 MICROBIAL FUEL CELLS 12 (MFC-12)

TABLE 5.12: Potential variation in different concentration of ammonium ions

VOLTAGE (V)					
	AMMONIUN IONS				
DAYS	**0.2M**	**0.4 M**	**0.6 M**	**0.8 M**	**1.0 M**
1	0.52	0.46	0.43	0.38	0.42
2	**0.65**	0.48	0.56	**0.42**	**0.52**
3	0.55	0.54	0.55	0.36	0.51
4	0.54	**0.53**	**0.54**	0.34	0.50
5	0.54	0.53	0.53	0.33	0.50
6	0.52	0.5	0.52	0.31	0.49
7	0.50	0.49	0.5	0.30	0.48

Fig 5.22: Potential variation in different concentration of ammonium ions



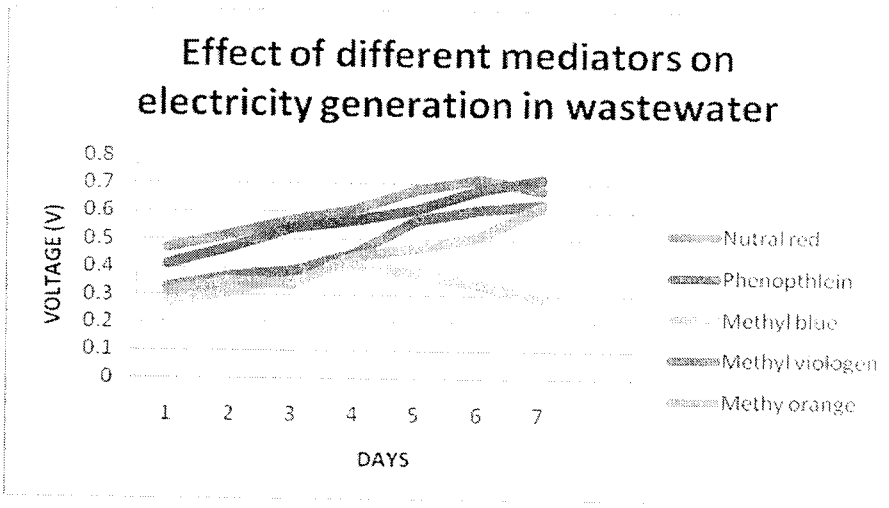
In anode compartment different concentration of ammonium ions were added to find their effect on the potential difference in voltage production. Here the minimum concentration of ammonium ions only gives the better result.

5.4.13 MICROBIAL FUEL CELLS 13 (MFC-13)

TABLE 5.13: Effect of different mediators on electricity generation in wastewater

VOLTAGE (V)					
	MEDIATORS				
Days	**Neutral red**	**Phenolphthalein**	**Methyl blue**	**Methyl viologen**	**methyl orange**
1	0.47	0.41	0.27	0.33	0.31
2	0.52	0.47	0.31	0.37	0.35
3	0.57	0.54	**0.37**	0.39	0.34
4	0.61	0.57	0.41	0.45	0.44
5	0.68	0.61	0.38	0.57	0.47
6	**0.78**	0.68	0.34	0.61	0.51
7	0.68	**0.72**	0.29	**0.63**	**0.62**

Fig 5.23: Effect of different mediators on electricity generation in wastewater



For this set ups MFC-13, the electrodes used was graphite rod in both anode and cathode chambers. The oxidizing agent is potassium permagnate. The mediators play important role in the current production. They help the microorganisms to get attached to electrode and so they release the electrons and protons. Though these mediators help in the current production they are toxic to the microorganisms. Hence , they were added only trace amount. In this set up added mediators are Nutral red, Phenolphthalein, Methyl blue, methyl viologen, methyl orange respectively. Nutral red gives high voltage.

5.5 MICROBIAL FUEL CELLS 14 (MFC-14)

Single chamber system

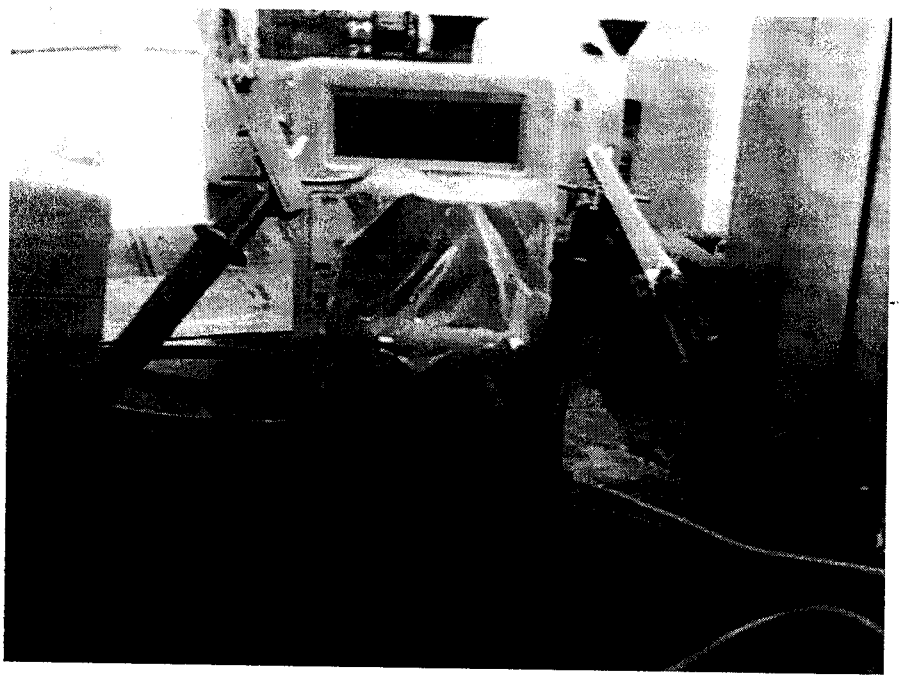
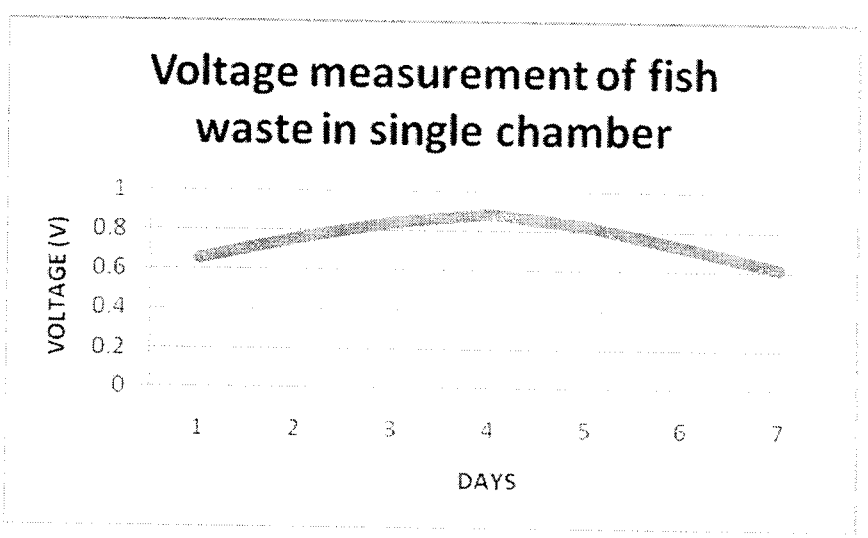


Fig 5.24: Single chamber system

TABLE 5.15: Voltage measurement of fish waste in single chamber

	Voltage (V)
Days	Fish waste
1	0.66
2	0.76
3	0.84
4	**0.89**
5	0.83
6	0.73

Fig 5.25: Voltage measurement of fish waste in single chamber



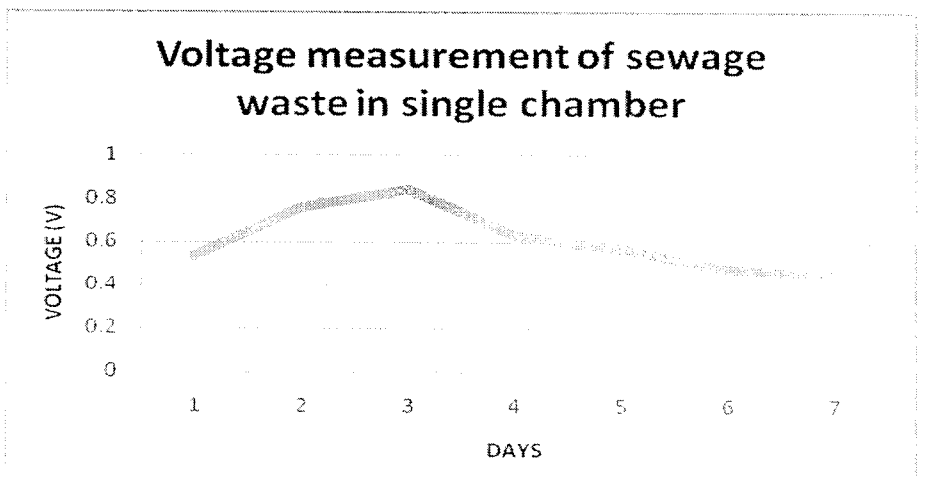
The MFC 13, the electrodes used was graphite rod and zinc was used in single chamber. Here the graphite rod act as anode and zinc electode acts as cathode. The fish waste was used in the single chamber. The single chamber fish waste shows maximum voltage was 0.89V.

5.5.1 MICROBIAL FUEL CELLS 15 (MFC-15)

TABLE 5.16: Voltage measurement of sewage waste in single chamber

	Voltage (V)
Days	**Sewage waste**
1	0.54
2	0.76
3	**0.84**
4	0.63
5	0.57
6	0.48

Fig 5.26: Voltage measurement of sewage waste in single chamber



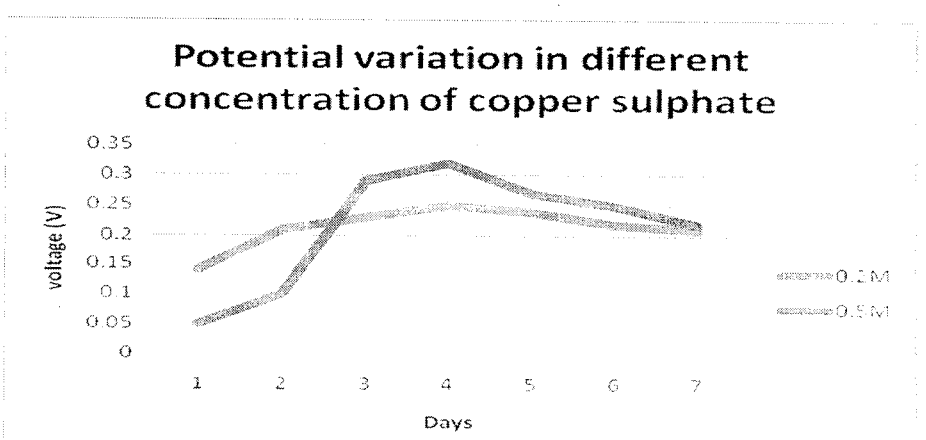
In the single chamber system using electrodes are Galvanised Zinc mesh and Graphite sheet these two electrodes used in the single system. Compared to the double chamber system its give good result.

5.5.2 MICROBIAL FUEL CELLS 16 (MFC-16)

TABLE 5.17: Potential variation in different concentration of copper sulphate

	VOLTAGE (V)	
Days	**0.2M**	**0.5M**
1	0.14	0.05
2	0.21	0.10
3	0.23	0.29
4	**0.25**	**0.32**
5	0.24	0.27
6	0.22	0.25
7	0.21	0.22

Fig 5.27: Potential variation in different concentration of copper sulphate



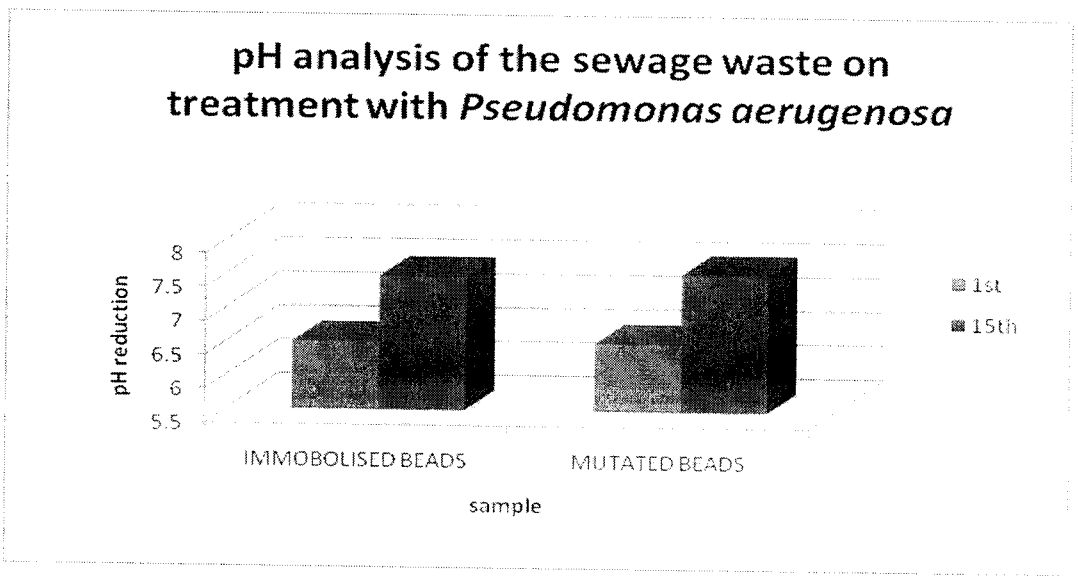
In this set up cathode compartment solution was used as copper sulphate ($CuSO_4.5H_2O$) in different concentration like 0.2M and 0.5M respectively. Here 0.2M concentration of copper sulphate shows the better result

5.6 TREATMENT OF WASTE WATER

TABLE 5.18: pH analysis of the sewage waste on treatment with *pseudomonas aerugenosa*

pH Reduction		
Days	Immobilized Beads	Mutated Beads
1	6.5	6.5
15	7.4	7.5

Fig 5.28: pH analysis of the sewage waste on treatment with *pseudomonas aerugenosa*

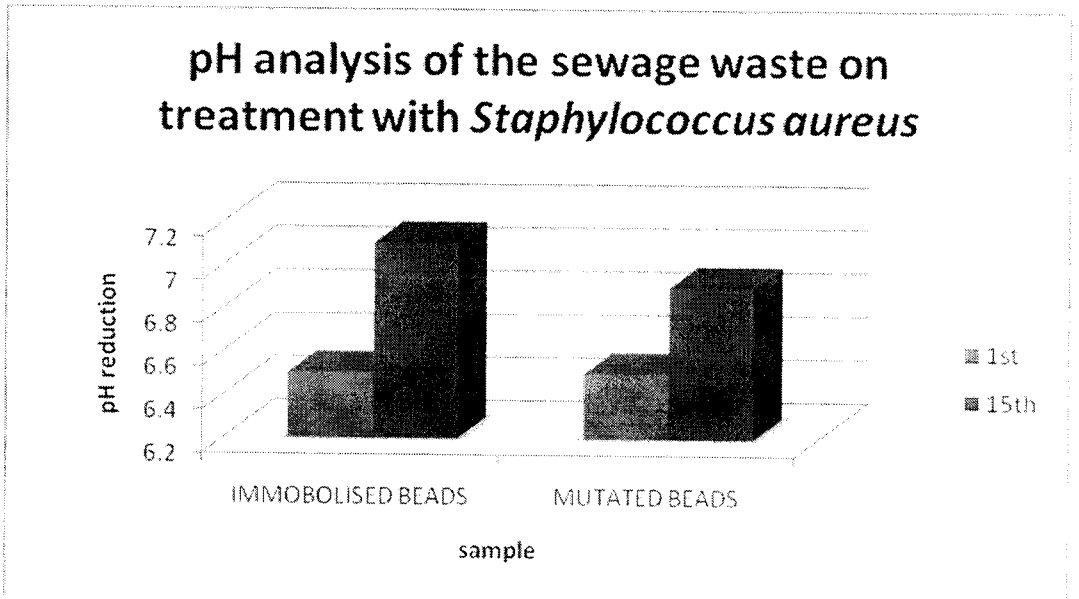


The above graphs-4.18 shows the variation in the pH of the wastewater sample which was treated with the immobilized cells (mutated and wild) of ***Pseudomonas aerugenosa.*** The pH changes from acid to neutral at the end of 15th day. The variation was more profound in mutated beads.

TABLE 5.19: pH analysis of the sewage waste on treatment with *Staphylococcus aureus*

pH Reduction		
Days	Immobilized Beads	Mutated Beads
1	6.5	6.5
15	7.1	6.9

Fig 5.29: pH analysis of the sewage waste on treatment with *Staphylococcus aureus*



The above graphs-4.19 shows the variation in the pH of the wastewater sample which was treated with the immobilized cells (mutated and wild) of ***Staphylococcus aureus.*** The pH changes from acid to neutral at the end of 15^{th} day. The variation was more profound in mutated beads.

5.6.3 Treated sewage waste with *Staphylococcus aureus* and *Pseudomonas aeruginosa* with immobilised beads after 5 days

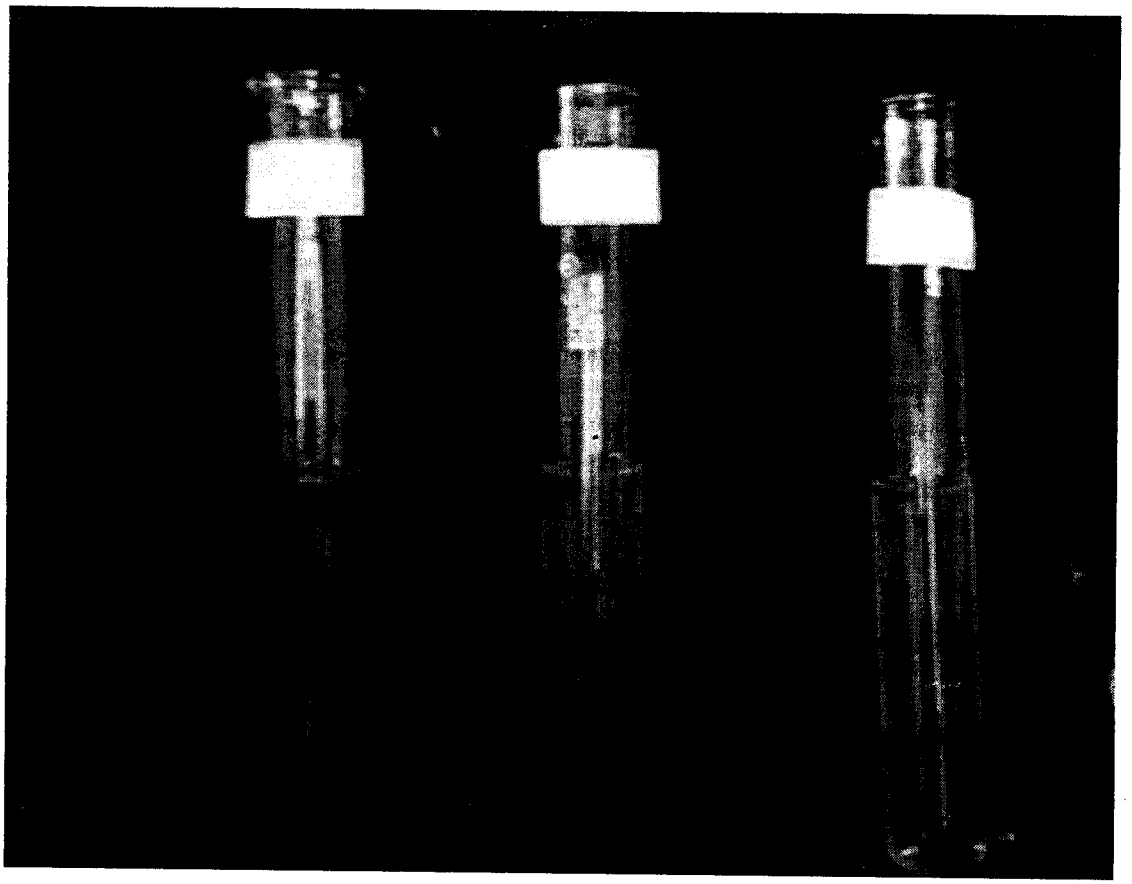
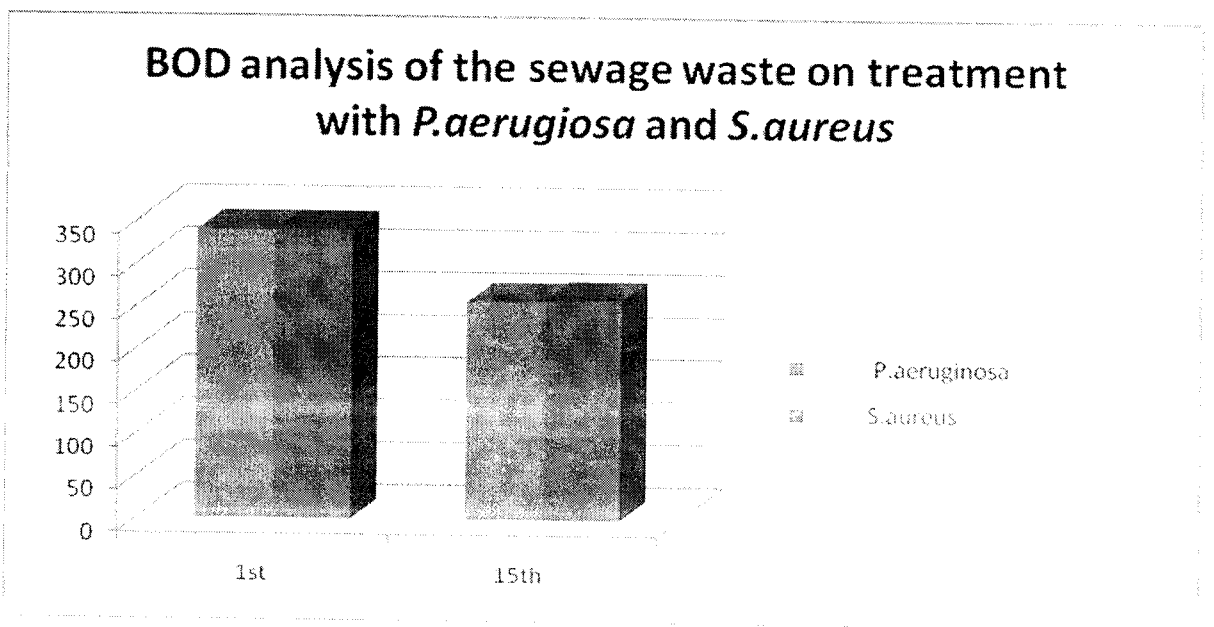


Fig 5.30: BOD analysis of the sewage waste on treatment with *Psedomonas aerugiosa* and *Staphylococcus aureus*

TABLE 5.20: BOD analysis of the sewage waste on treatment with Psedomonas aerugiosa and *Staphylococcus aureus*

BOD (mg/lit)		
Days	***P. aeruginosa***	***S. aureus***
1	339	339
5	257	259

Fig 5.31: BOD analysis of the sewage waste on treatment with *Psedomonas aerugiosa* and *Staphylococcus aureus*

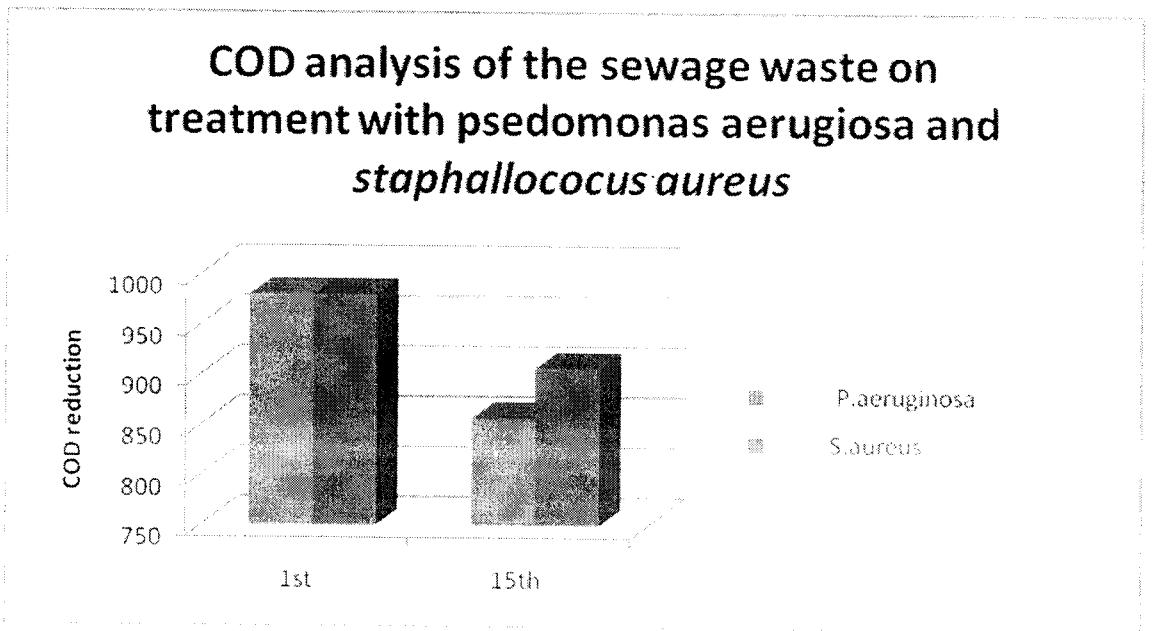


The initial BOD of the raw effluent was found to be 339 mg/lit. The ***Pseudomonas aeruginosa*** **and** ***Staphylococcus aureus*** treated effluent has the BOD of 247 mg/lit, 259mg/lit on 5^{th} respectively.

TABLE 5.21: COD analysis of the sewage waste on treatment with *Psedomonas aerugiosa* and *Staphylococcus aureus*

COD (mg/lit)		
Days	***P. aeruginosa***	***S. aureus***
1	980	980
5	857	907

Fig 5.32: COD analysis of the sewage waste on treatment with *Psedomonas aerugiosa* and *Staphylococcus aureus*



The initial COD of the raw effluent was found to be 980 mg/lit. The ***Pseudomonas aeruginosa and Staphylococcus aureus*** treated effluent has the BOD of 857 mg/lit, 907mg/lit respectively.

5.7 SEM Analysis of electrode:

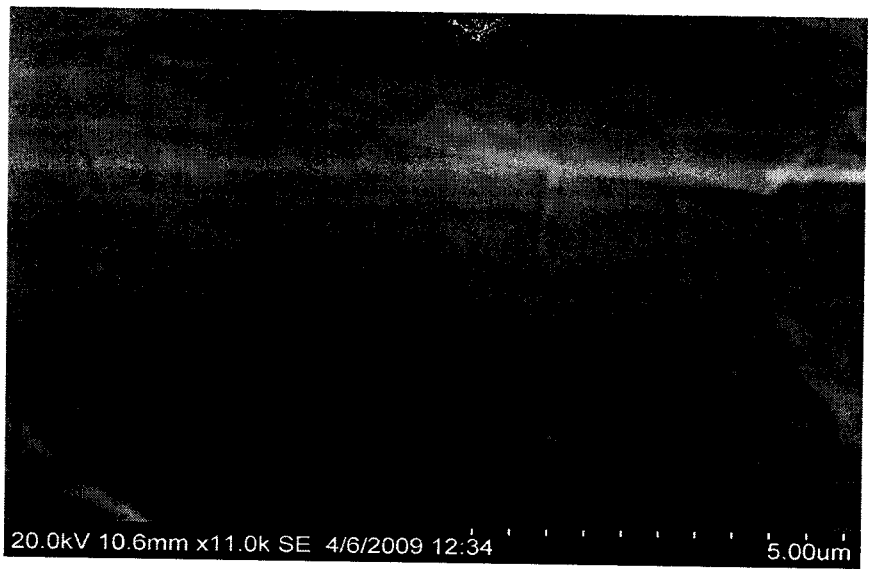


Fig 5.33: **SEM analysis of the untreated electrode**

The fig18 shows the cathode electrode without formation of any biofilm.

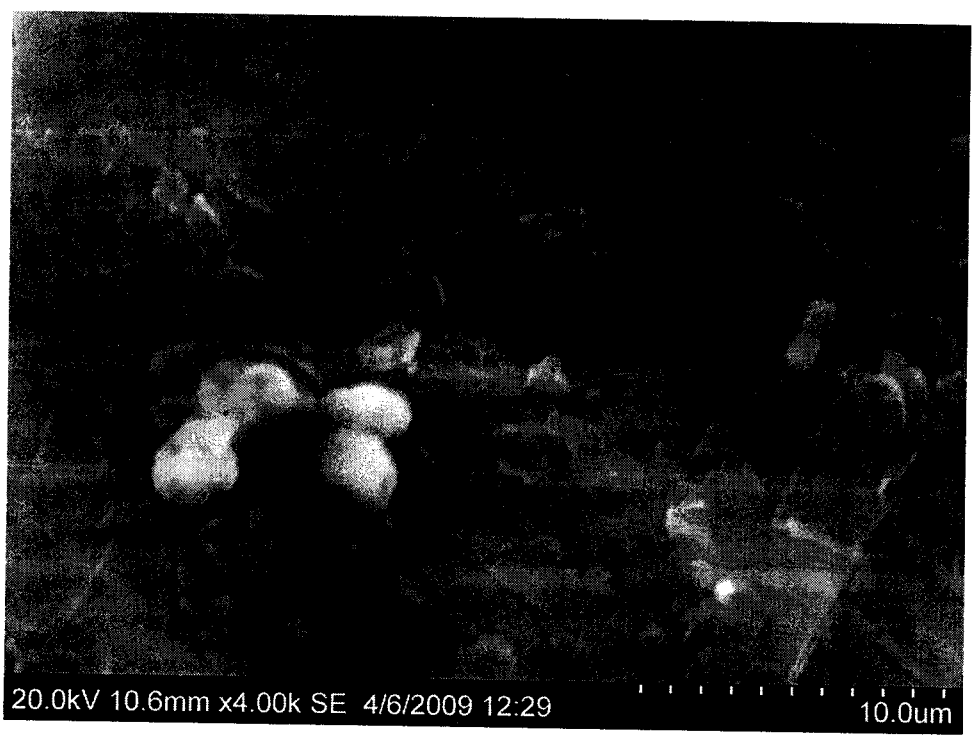


Fig 5.34: **SEM analysis of biofilm formation**

The SEM photo above shows the biofilm formed on the anode electrode by the microbes present in the wastewater.

Microbial Fuel Cells (MFC) have great potential for use in waste water treatment since; organic contaminants are converted into electricity with a reduction in amount of sludge produced (Rabaey *et al.*, 2004). It has been shown that a mediator -- less MFC can be constructed using electrochemically active microbial enrichment (ECM) culture where the waste water is utilized as a fuel (substrate), thus reducing the sludge production (Byung Kong Kim *et al.*, 1999). Metabolically active microorganisms can be recovered from a diversity of deep subsurface environments and have the potential to affect the rate of toxic organic and inorganic contaminants in groundwater. (Derek R Lovey *et al.*, 1995).

Pseudomonas aeruginosa is a soil-dwelling, Gram-negative, aerobic, rod-shaped bacterium belongs to the class gamma- Proteobacteria. *Pseudomonas aeruginosa* secretes variety of pigments, such as pyocyanin (blue-green), fluorescein (yellow-green and fluorescent, now also known as pyoverdin), and pyorubin (red-brown).

In the present study, four different fuels (mixed culture) were compared with universally accepted catholyte, Ferricyanide and also with permanganate and dichromate. This study is in complete accordance with findings of Shijie You *et al.*, 2006.

Our data shows the maximum voltage production under the optimum conditions (mediator-less, batch mode) at 5 days. The maximum voltage production was 1.28V. According to B. H. Kim and his colleuges maximum voltage was reached after 5-7 days.

5.1 Current Production:

The six different set ups had shown that $KMnO_4$ produces higher voltage with sewage waste of about 1.29V in batch mode. The sewage waste is the consortia of microbes which has *Staphylococcus aureus, Pseudomonas aeruginosa* (electrochemically active microbe. The maximum volts produced using sewage waste series was 3.37V. In all these set ups the initial readings was low as it is due to time taken by the biofilm formation on the graphite sheet and graphite rod.

In MFC-13 addition of mediator, methylene blue produces higher voltage than neutral red. But in MFC-1a double salt bridge produced higher OCV than single salt bridge which shows that higher voltage can be produced by increasing salt bridges rather than for the addition of mediators which are actually toxic to the microbes. According to Booki Min *et al.*, 2005, in both PEM and salt bridge the oxygen diffusion from the cathode chamber into the anode chamber was observed which was a factor to be considered in power generation.

Three different catholyte were used from which $KMnO_4$ showed higher result than ferricyanide and copper sulphate. Permanganate has been used as an environment-friendly oxidant in industries for many years. Its high redox potential offers the possibility of its application in a fuel cell system to establish a high potential difference between the anode and the cathode.

The mechanism of reduction reaction of $KMnO_4$ was as follows:

$$MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O$$

$$MnO_4^- + 2H_2O + 3e^- \rightarrow MnO_2 + 4OH^-$$

In acidic and alkaline condition it releases 3 e- (Shijie You *et al.*, 2006) whereas ferricyanide releases only 2 e-. Thus $KMnO_4$ produces higher voltage.

5.2Characteristics of the anode Surface:

The SEM analysis of sewage waste shows biofilm formation on the electrode in anode. The result showed a clear picture on the development of pili at 60,956x (500nm) and huge biofilm formation on the ESEM of sewage waste. This shows it may be due to the presence of *Pseudomonas aeruginosa* in the sample (sewage waste) was found to be electrochemically active bacteria as it produced comparatively higher voltage than other biological fuel cells.

Conclusion

6. Conclusion

In the present study, the organic wastes were collected and were analyzed. The identification of bacteria was done by the morphological features and culture characteristics.

The isolate were identified as a strain of *Staphylococcus aureus, Pseudomonas aeruginosa* , from the sample (sewage waste) and had electricity production which is detected by screening method.

Production was done for the different samples with three different catholytes. From this study, the sewage waste (mixed culture) had better electricity production with KMnO4 at pH 7.5, while the others had comparatively low electricity production. Similarly the use of Double Salt Bridge produces more voltage than single salt bridge.

Based on information obtained from this study, it is reasonable to conclude that permanganate is an effective electron acceptor. It is able to enhance the maximum power density of a MFC. Moreover, it is worth pointing out that this permanganate method has no need for a catalyst, which makes this process simple and economical.

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