Enrichment of Electrogenic Bacteria from Activated Sludge and Soil Samples in Dual Chamber Microbial Fuel Cells

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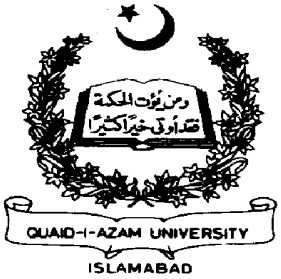
A thesis submitted in partial fulfillment of the requirements for the

Degree of

Master of Philosophy

In

Microbiology



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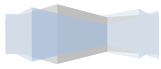
بسم الله الرحمن الرحيم
In the Name of Allah,
the Beneficent, the Merciful.

DEDICATED TO

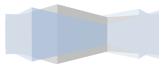
FAMILY



DECLARATION

The material and information contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

ZARGONA ZAFAR



CERTIFICATE

This thesis submitted by Zargona Zafar, is accepted in its present form by the Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan; as satisfying the thesis requirements for the degree of Master of Philosophy in Microbiology.

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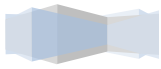
Chairperson: _____

(Dr. Fariha Hassan)

Dated: _____



ACKNOWLEDGMENT

All praises to **Almighty Allah,** The Light of Heavens and Earths, The One Who put good thoughts in ones mind, turn them into determinations and then makes the way towards their fulfillments showering all His Blessings throughout the journey.

Best of the praises and Peace be upon all the Sacred Messengers and especially for the Last of them **Hazrat Muhammad (SAWW)** who are the minarets of knowledge for all the mankind.

I am very thankful to my Supervisor ***Dr.Naeem Ali*** for giving me the opportunity to study MFCs. It has been a challenge that I have enjoyed grappling with and your guidance and comments have been invaluable. You were instrumental in shaping my understanding of this technology and I have a debt of gratitude that I hope you will recall one day. And the very hounarable Chairperson Department of Microbilogy ***Dr. Fariha Hassan*** and all the Faculty Members for supporting me with valueable suggestions for providing access to the facilities that ensured the successful completion of this work.

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At the end I would also like to thanks my parents, my brothers and sweet sister for their love and blessings.

ZARGONA ZAFAR

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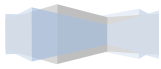

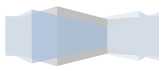

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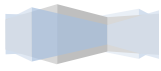
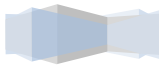
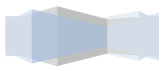


LIST OF ABBREVIATIONS

ASC	Anode surface coverage
ATP	**Adenosine triphosphate**
A	**Surface Area**
BES	**Bio-electrochemical system**
COD	**Chemical oxygen demand**
CSLM	**Confocal scanning laser microscopy**
CFU	**Colony Forming Unit**
DGGE	**Denaturant gel electrophoresis**
DET	**Direct Electron Transfer**
EET	**Extracellular electron transfer**
HRT	**Hydraulic retention time**
I	**Current**
L	**Length**
LMFC	**Large microbial fuel cell**
MFC	**Microbial fuel cell**
NADH	**Nicotine amide adenine nucleotide**
P_D	**Power density**
P	**Power**
PEM	**Proton Exchange Membranr**
R_{int}	**Internal Resistance**
R	**Resistance**
SEM	**Scanning Electron Microscopy**

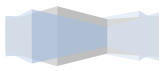


TSI	**Triple Sugar Iron**
V	**Voltage**
W	**Width**



LIST OF SYMBOLS

A	Ampheres
~	**Approximately**
COD/L	**Chemical oxygen demand per liter**
COD/L/D	**Chemical oxygen demand per day**
cm	**Centimeter**
kΩ	**Kiloohms**
µW	**Microwatt**
mA	**miliAmphere**
mWm^{-2}	**Miliwatt per meter sequre**
mV	**Millivolt**
mA/cm^2	**Miliamphere per centimeter sequre**
Mj	**Milijoules**
mg/L	**Milligram per liter**
µA	**MicroAmpheres**
mg/L	**Milligram per liter**
mM	**Milimole**
Ω	**Ohms**
%	**Percentage**
rpm	**Rotation per minute**
V	**Volts**
Wm^{-3}	**Watt per meter cube**



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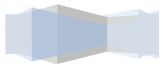
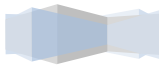


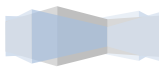


Abstract

Global energy demand is increasing rapidly with the development of science and technology. High operational cost and high rate of sludge generation in conventional waste water treatment plants demands a new alternate technology that could be operated efficiently at low cost and minimum sludge production. Microbial fuel cell could be alternative solution to this dilemma. Microbial fuel cells (MFCs) is a new approach, which electrochemically convert organic substrate containing chemical energy directly into electric current. The distinct advantage of this technology is to exploit the low-grade organic contents even waste water which otherwise not being used. The microbial power is being utilized in MFC to simultaneously generate electricity and treat waste water. Consequently, the high energy content of waste water is no more a waste, but seen as valuable energy reserve.

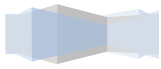
In the current research study, double chamber microbial fuel cell has been constructed with two different proton conducting material. In one type of MFC agar salt bridge was used while in second type Nafion membrane 115 was used for proton conduction in between the two chambers. Activated sludge and submerged soils (S2, S3) were tested for electrochemical activity. In sucrose fed salt bridged MFC, maximum voltage and power output was 347Ω and 40.136µW, while after enrichment the voltage and power output was dropped to 74.9mV and 1.87µW across 3kΩ. When the same cell was operated with Potassium acetate maximum voltage and power produced were of 28.8mV and 0.27 µW, after enrichment the voltage and power dropped to about 24mV and 0.192 µW. The voltage and power output was increased after enrichment to about 332mV and 3674.3µW in membrane MFC at 50rpm.From the diversity analysis of soil, activated sludge and the biofilm formed on anodic surfaces, it has been analyzed that initial samples (Soil and Activated) contain diverse range of bacterial consortia while after enrichment stage 2 only selective bacterial classes left, *α,β,γ- Proteobacteria* accounts for approximately more than 90% relative abundance, which have major contribution in

current generation. It has been observed that electrochemically active bacteria are better adopted in sucrose fed fuel cell. While membrane cell have greater power output than salt bridge MFC. It has been observed that resistance have a negative effect on current and power production while positive on voltage output



CHAPTER # 1

INTRODUCTON



Introduction

1.1 Background Research

Consumption of energy is increasing exponentially with the progression of science and technology. Developed as well as underdeveloped countries across the world are facing serious energy crises (3-5). Fossil fuels are being utilized and depleted faster than innovative alternate energy sources being discovered. An estimate highlighted the demand of energy that would grow more than 50% by 2025 (6, 7). Apart from energy crises, the global concern regarding environmental impacts due to consumption of fossil fuel is adding another challenge to the sustainability of the earth(8). High energy is required in conventional waste water treatment plants, which are demanding alternate energy solution which will be efficient and cost effective(9). So the concerned authorities are looking to find some solutions concerning these important issues by implying alternative green energy resources at small to large scales(10). It is generally recommended that no single energy solution is sufficient to solve energy shortage issues. A multi-dynamic approach is needed to be used to solve current problems(8). Amongalternate energy sources (wind, solar, nuclear, geothermal etc) , Microbial fuel cells is another growing promising technology(8, 11).

Microbial fuel cells are electrochemical devices, in which bacteria catalyze the conversion of chemical energy stored in organic (some inorganic) substrates directly into electricity(12-16). The concept of generation of energy from microorganisms is not new, it was known since 1970s.Microbial fuel cells have a distinctive advantage of utilizing low grade biomass and even waste water to generate electricity. MFCs simultaneously treat waste water and recover bio-energy in the form of electricity(12, 17-24). Domestic wastewater contain easily bioconvertible organic substrates that are used by bacteria in MFC and generate electricity (4, 9, 14, 25-28). It has been estimated that energy

generation accounts for approximately 25% of total operating cost of waste water treatment plants.

The main components of microbial fuel cells are anode, cathode and a proton exchange membrane(2, 15). Typically Microbial fuel cell is dual chamber fuel cell that consists of anode and cathode chamber connected internally by a proton conducting material and externally by a wire(15). Electrons and protons are released by the oxidation of organic substrate by microbial activity. Protons moved from anodic chamber to cathode through proton exchange membrane and electrons flow through an external circuit from anode to cathode donated on anode by biofilm in anodic compartment(29-34). The flow of electron from anode to cathode through an external circuit drives an external load. Primarily the electrical power output depends on the rate of electron transfer from the bacteria to the anode, the rate of substrate degradation, resistance of the circuit, the proton mass transfer in the liquid, electrode performance and the external operating conditions and so on(35).

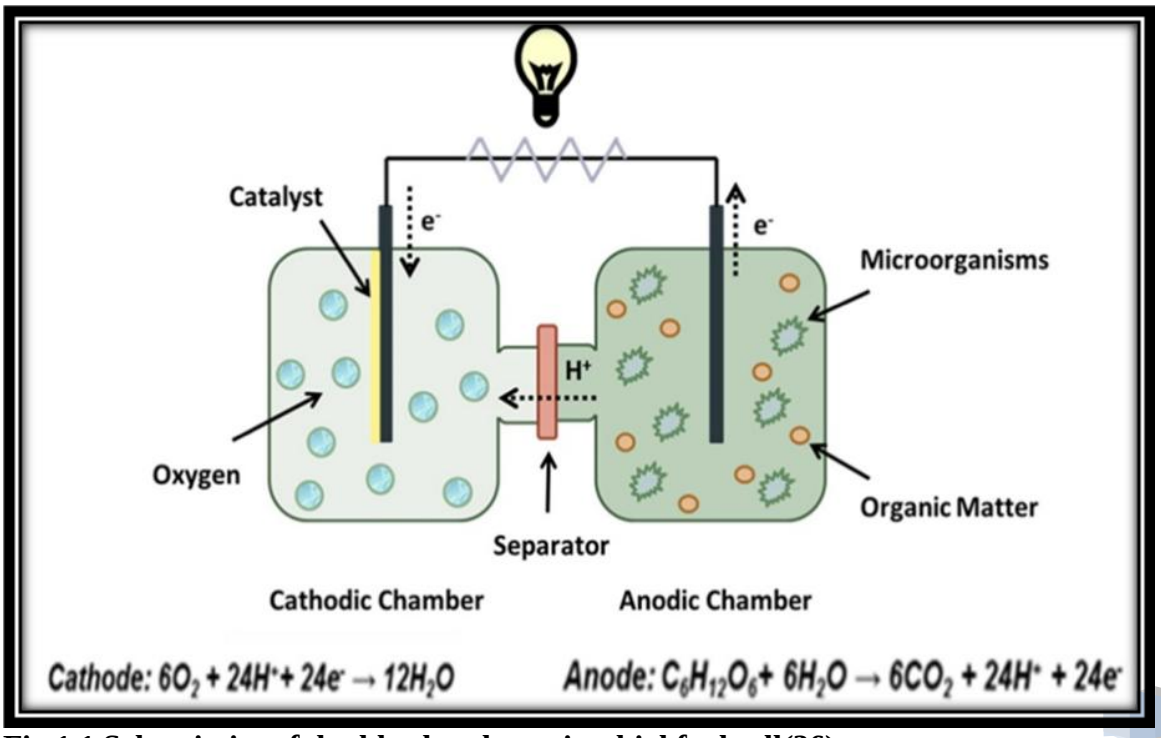
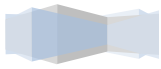


Fig 1.1 Schemiatics of double chamber microbial fuel cell(36)

Most of the bacterial surfaces contain non-conductive peptidoglycans, lipid membranes and lipopolysaccharides which hinders the direct electron transfer process(37). It has been reported that electrical current generation could be greatly enhanced by the use of electron mediators that helps the bacteria in anodic chamber of fuel cell to shuttle their electrons to anodic surface. These mediators could be naturally occurring or synthetic in nature. Naturally occurring mediators also known as endogenous mediators include microbial metabolites like phenazine and other pyocyanine compounds produced by *Pseudomonas sp.*while typical synthetic exogenous mediators comprises of metallorganics, dyes such as neutral red, thionine, methylene blue, naphtoquinone(14, 38-40). Unfortunately the instability and toxicity of synthetic mediators restrict their use in microbial fuel cell technology. A real breakthrough was made when it was found that some microorganisms were capable of directly transfer their electrons to anode(41, 42).

These bacterial cultures are found to be highly stable (42, 43). These bacterial cultures include*Shewanella putrificians*(27),*Geobacter metallireducens*(44),*Geobacteraceae sulferreducens*(4)and *Rhodoferax ferrireducens*(42)that are able to form biofilms and directly transfer their electrons by conductance through membrane on anode surface. Anode functions as the final electron acceptor in the respiratory chain of microbes in the biofilm. Therefore, the cost of mediators was subisided by, introducing mediator less microbial fuel cell in waste water treatment and power generation(45).

Apart from energy generation main element of Microbial fuel cell "Bacteria" are neglected considering the microorganisms as "black box"(11, 46). Recent research studies on MFC, the number of electricity producing bacteria has been expanded(30, 47, 48). These microorganisms are known as "electrochemically active bacteria in terms of electricity production." These microorganisms are proven to be capable of power generation and electron transfer to an electrode by degrading substrate without the help of external mediators. Some of the common representatives in MFC include *Rhodopseudomonas spp.* (Phototrophic bacteria),

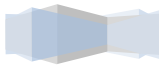


*Geobacter spp. (*metal reducing bacteria*)(49-53), Pseudomonas aeruginosa(34), Rhodoferax ferrireducens and Enterococcus Faecium(34).* Consequently, enrichmenttechique is a fundamental tool for the isolation of electrochemically active bacteria from different inoculums with greater current generation and biodegradation capabilities.

Anode is used for the isolation of electrochemically active bacteria from environment simply by enrichment technique under anoxic or anaerobic conditions(54). Anode material used for isolation of electrochemically active microorganisms must be chemically stable, biocompatible and conductive in nature. The most versatile material used for anode was carbon available in the form of compact rods, graphite plates, granules, as glassy carbon, fibrous material such as cloth felt, fibers, paper and foams. The simplest electrode material used are graphite rods or plates because they are relatively easy to handle, inexpensive and unambiguous surface area(55). It has been observed that current generation was increased with increase in surface area of anode as in the order of Graphite< Carbon foam< Carbon felt.(55)

Microbial fuel cell have important functional and operational advantages over the techniques currently used for electrical energy generation from organic substrates(17, 36, 56, 57). The specific uses of this technology include:

- It allows the conversion of substrate energy directly to electricity with a high conversion efficiency.
- It can be operated efficiently at ambient temperature which makes this technology more reliable than all other current generating technologies.
- Gas treatment is not required in microbial fuel cell because the off-gasses are enriched in CO_2 and usually have no energy content.
- Energy input is not required in microbial fuel cell for aeration as cathode is submissively aerated(17).



- Microbial fuel cells have wide spread applications at places lacking the electrical framework like as in operating tiny medical devices like microscopic drug-delivery systems, implantable medical devices(58) .
- As compared to conventional treatment plants of wastewater lower sludge is produced in microbial fuel cell(38, 57).

By continuous efforts of researcher's for more than a decade had led to the increase in output of power by several orders(30, 59), but still its too low to power the electronic devices. Therefore, one of the major tasks is the understanding of microorganisms that were most proficient in terms of power generation(11, 60).

1.2 Significance of Current Study

The current research will be significant in finding alternate solution to clean and sustainable energy generation Besides that, the problem of waste water treatment can also be solved because organic matter will be consumed by microorganisms that grow on anodic surfaces. The isolation of electrogenic bacteria will help in selecting best Bacterial strains that are highly proficient in energy generation

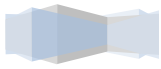
1.3 Aims of Current study

The aim of current study is to construct dual chamber Microbial fuel cell with different proton conducting material for simultaneous power generation and enrichment, isolation and characterization of electrochemically active bacteria from waste stream soils and activated sludge samples.

1.3A Research Objectives

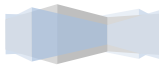
- To construct mediatorless double chamber Microbial fuel cells with salt bridge and proton exchange membrane as proton conducting material.

- To study power generation using mixed bacterial consortium from activated sludge and waste stream submerged soils.
- To study the effect of “Enrichment technique” on power generation and bacterial community composition.
- To isolate and characterize bacterial community from anodic biofilms and suspended cultures in MFCs.
- To study the effect of different resistors on Voltage and Current generation.
- To study the effect of different MFC designs on Microbial community development.



CHAPTER # 2

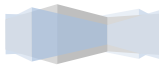
REVIEW OF LITERATURE



2.1 Review of literature

Demands of alternate energy sources has been increasing graduallybecause developed as well as underdeveloped countries like Pakistan, India etc are facing serious energy shortage issues(4, 5). High energy input required for conventional waste water treatment systems,however these systems demand alternate treatment technologies which operate efficiently at low energy input and will be cost effective(27). Along with concerned problems, environmental disquietslike global warming and air pollution with the usage of fossil fuels are also playing an important impetus for alternate energy generation technologies(61).The microbial fuel cellwas an endowed technology that utilizes the power of microorganisms forsimultaneous treatment of waste water with little sludge production along with the generation of renewable energy in the form of electricity(62-64). Various studies has been done to analyze the configuration, design of reactors, materials of electrode used and operational parameters (47, 48, 63)but the main element of MFC "Microorganisms" have not been well studied for maximum production of electricity(64). Over the past few decades,research in the field of electro-microbiology has been grown exponentially.

Electrodes were being utilized by the electroactive bacteria either as electron donors known as electrotrophs or as electron acceptors as electricigens. These organisms play a vital role in different fields like as in bioremediation, biosensors, energy and biofuel production by using different bioelectrochemical systems(65-67). In these systems, microorganisms often form biofilms on electrode surfaces. Microbial consortia include bacteria, archaea, and yeast(68). A number of factors affect the electrode-associated community composition such as the concentration of oxygen, influent substrate, temperature and pH(67, 69, 70). A variety of environmental inoculums has been used to enrich electroactive microbial consortia on electrode surfaces like as sewage sludge, soil, compost and aquatic sediments, etc.

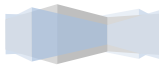


Initially, research focused on the study of electricigens for power production by using microbial fuel cell system. Although power production has been increased by several orders(30, 59, 64), but still restricted to power small devices(71). To overcome these limitations, a number of chemical, physical and biological approaches has been employed, but still a major limiting factor remains to be solved. Activitiesbetween and within the bacterial population and dynamics of bacterial community in the presence of electrodes remained to be distinguished(72).

2.2 Microbiological Aspects Of Microbial Fuel Cell Technology

Activity of bacteria inside the microbial fuel cell proved to be a source of energy production. So, microbial cultures have been the backbone of this technology. Some electrochemically active microorganisms like *Aeromonas hydrophila(73, 74), Shewanella putrefaciens(27), Geobacter metallireducens(4, 27, 29), Proteus vulgaris(75), Enterococcus gallinarum(76), Actinobacillus succinogenes(77, 78), Rhodoferax ferrireducens(79)* etc capable of accepting and donating electrons to and from an external source such as an electrode. These microbial flora named as electrogenic bacteria(30).

All bacteria are not electrogenic in nature but non electrogenic bacteria may also play an important role in providing organic nutrients to electrogenic bacteria, so they proved to be equally important in consortium(80-84).The basic catalytic activity of bacteria in anodic compartment was to oxidize the organic matter anaerobically, resulting in generation of electrons and protons. Electrons were transferred to the electrode in anode and protons transported to the cathodic chamber via a proton exchange membrane internally. While the electrons transferred to the anode surface were transported to the cathode compartment by an external circuit. Electrons stored as intermediates in the form of quinones and Nicotinamide adenine dinucleotide which become reduced and used as an energy source for living cells, while in cathode compartment oxygen reduction occurs by utilizing electrons and protons usually forming water(30, 31).



Various substrates including real and artificial wastewaters and lignocellulosic biomass has been investigated as feed for microbial fuel cell (31, 33, 85).Typical reaction on electrode using acetate as carbon source was as shown below in equation 1 and 2:

Reaction at Anode : $CH_3COO^- + 2H_2O \longrightarrow \text{microbes } 2CO_2 + 7H^+ + 8e^-$ (1)

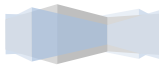
Reaction at Cathode : $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$ (2)

Substrate has been broken into water and carbon dioxide with electricity production as a by-product(35, 86).

The most abundant bacteria on anode surface depends on enrichment conditions. It has been reported that selective enrichment in sediment microbial fuel cell of *Geobacteraceae* on anode surface was examined not only in sediment fuel cell(68) but also in fuel cell fed with lactate, glucose or acetate inoculated with sewage sludge(87-89). However, with different inoculum sources major groups of bacteria include *Rhizobiales(90), β-Proteobacteria(91), γ-proteobacteria(92)* or *chlostridia(93)*.

2.3 Electron Transfer Mechanisms By Electricigens

For better understanding it's crucial to know how bacteria were capable of producing electricity. Substrate oxidized by bacteria and electron transfer from NADH to respiratory enzymes. Electrons flow down the respiratory chain and protons moved across the internal membrane. Enzyme ATPase pumps the protons back to the cell creating ATP energy. Finally the electrons were discharged to the soluble electron acceptors. In some cases bacteria obtain less energy because reduction potential of respiratory chain drops below than oxygen and electron leave the chain and transported outside the cell to a solid electron acceptor (94). Oftenly, soluble electron mediators have been depleted and the microorganisms turn to use non soluble electron acceptors or to fermentation by-products (95).

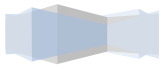


Microbial capability of generating electricity was correlated with their ability to transfer electrons to extracellular natural electron acceptors. This mechanism was known as extracellular electron transfer (EET) (96)and it has been best studied in metal reducingbacteria, as humic substances, Mn(IV) and Fe(III) were too large to enter into the cell(97, 98). The difference between natural biogeochemical cycle and electricity production by bacteria such as Fe(III) reduction is that electrons transferred to electrodes rather than natural electron acceptors(99).

Different mechanisms has been proposed for transfer of electron to an electrode like as electrons may be transferred via an endogenous redox-active metabolites or via nanowires or by membrane bound c-type cytochrome(49). Bioelectrochemical systems would lead to the study of extracellular electron transfer mechanisms to and from the indecipherable electron donors and acceptors(66). Currently, our knowledge of these interactions have been limited to only some of the well-studied bacteria including few species of ***Shewanella*** and ***Geobacter***(49, 100, 101)**.**

2.4Indirect electron transfer or Mediated electron transfer

It has been proposed that mediated electron transfer occurred via three different redox compounds: Primary metabolites, microbially generated mediators or by artificial (exogenous) redox mediators(102). Exogenous redox mediators include neutral red, methylene blue and thionine assist bacteria in electron transfer to anode(38, 45, 103, 104).These mediators scavenge electrons from reducing agents as NADH by penetrating the bacterial cells and get reduced(103). Then diffused out of the bacterial cell and get oxidized on the surface of electrode. Some electricigens have been shown to produce electron shuttling components like phenazine and other pyocyanine compounds produced by *Pseudomonas sp.* These compounds help in increased current production by bacterial communities or their own extracellular electron transfer(31, 34, 105).



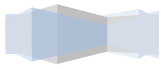
Shewanella oneidensis has been shown to release quinones and riboflavins(50, 106). A microbial fermentation of glucose to produce reduced metabolic products such as acids, alcohols and even hydrogen**,** which were oxidized abiotically at anode surface generating a pool of electrons and protons. However, the only portion of electrons available in organic matter were recuperated as electricity, which lead to the accumulation of organic products in anode chamber(99),so this mechanism wasnot efficient in electricity generation. Numerous microorganisms which have been reported to produce electric current by this mechanism include *Clostridium*, *Enterococcus* and *Alcaligenes(50, 107, 108).*

2.5 Direct Electron Transfer (DET)

Physical contact between bacterial pilus like structures or cell membranes and anode of MFC has been proposed to be required for DET(102). It has been suggested that in *Shewanella oneidensis* and *Geobacter sulfurreducens* outer-membrane C-type cytochrome redox proteins required for transport of electron to the anode(109, 110).Electrically conductive pilus-like structures known as “nanowires” were known to be produced by *G. sulfurreducens* and *S. oneidensis*that assist in electron transfer to distant electron acceptors(111, 112).It has been reported that nanowire are not only constrained to metal reducing bacteria but *Pelotomaculum thermopropionicum* (thermophilic fermentative bacterium) and *Cyanobacterium Synechocystis* PCC6803 also evolve nanowires(112).

2.6MFC Configurations

For lab-scale analysis, different configurations of MFC has been suggested(36). Double chamber or two-chamber type of MFC contained two chambers named as anode and



cathode substantially separated by a salt bridge or proton exchange membrane(36, 44, 113-116).

Dual chamber microbial fuel cell has found to be complex to handle and when up graded it create serious hurdles and problems. Alternatively, single chamber microbial fuel cell was constructed. It mainly consisted of one chamber anode while cathode remained exposed to the oxygen or air. This design was really alternative because it was cost effective along with offered operational savings. Another type of MFC was built in which anode was shaped into cylindrical reactor while cathode chamber remained outside the reactor (117). Rabaey et al. proposed a tubular shaped MFC with an inner anode and outer cathode using graphite electrodes(118). Attached fixed biofilm was usedin another type of fuel cell called as up-flow MFC continuously supplemented with substrate to the membrane across permeable anode(119).

2.7 Electrode Configurations

Electrodes were found to be the main constituents in determining the cost and performance of microbial fuel cell technology. Utmost challenge in making the microbial fuel cell technology scalable, lucrative and cost-effective is to select the design of an electrode(120, 121). Recently, in microbial fuel cell studies curiosity in examining the electrode material and its configuration have significantly been increased. A range of electrode has widely been explored for this technology. Two main categories of these electrodes have been explored: bio-electrodes (anode and biocathode) and chemical electrodes depending on whether a bacterium was used as catalyst or not.

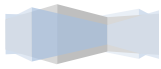
Physical and chemical properties of different electrode materials used, vary in electrical conductivity, surface area and chemical stability. They also differ in their influence of electron transfer, attachment of microbial consortia to electrode surfaces, rate of electrode surface reaction and the electrode resistance. Therefore, the selection of appropriate electrode materials for the optimization and better performance of microbial fuel cell was

of great importance. As a chief constituent of MFCs, the electrode material would determine the cost of MFC technology and ultimately influence the waste water treatment cost. Therefore, this field has engrossed ever-increasing concerns and lots of efforts has been made in designing and preparation of electrodes(35, 120).

2.8 Enrichment A Fundamental Tool

Inoculation and subsequently enrichment or acclimatization were found to be the fundamental processes in microbial fuel cell technology, but we do not know the diversity of species present in the inoculum and how they influence during the optimal performance of MFC operation(69, 122, 123).

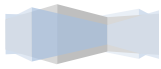
Unsurprisingly, in shaping the microbial community composition we do not have a rational means of control. In MFC waste treatment systems,it's neither unique nor essentially a barrier to their employment. In most of the open engineered biological processes, weather it was the metabolization of waste or the production of less detrimental or useful by-products like electricity, we rely on mixed microbial consortia(124). Up to many years of empirical research strategies have been developed for utilizing and improving the performances of these communities. However, microbial molecular ecology era provided a new probability of going beyond the black box approach to accelerate MFC design, engineering of microbial communities and predicting process performance. During the last decade, using the molecular approaches microbial consortia involved in waste water treatment has been interrogated. It has been shown that biofilm forming microbial communities were phylogenetically diverse and synergistically degrade a range of compounds(55, 59).



2.9 Mixed Bacterial Community Better Operating Option In MFC In Terms of Power Output

It has been observed that no single microbe predominate in MFC configuration(55). Microbial community composition was dependent on various factors such as sources of inoculum, substrate used, operating conditions and MFC configuration.*δ-Proteobacteria* predominates in marine sediment microbial fuel cell(114), in which most of them belong to Geobacteraceae family mostly *Desulfuromonas* or *Geobacter spp(114, 125)*. Greater part of Membrane-bound cytochromes were found to be bound on the outer membranes of these microorganisms which may take part in transfer of electrons from membrane surfaces to outer electron acceptors and it has been believed that in MFC these membrane-bound cytochromes function with carbon electrodes in the similar manner. However, α- and *β-Proteobacteria were*predominated in double chambered MFC when inoculated with river sediments(126). In another dual chamber MFC that was inoculated with activated sludge and fed with glucose-glutamate solution, it has been observed that 37% 0f 16S rRNA gene sequence clone library analysis of anodic biofilm comprised of γ-*Proteobacteria(127)* but activated sludge fed with acetate produced evenly distributed α-,γ- and δ-*Proteobacteria(128).*

In double chamber MFC anodic biofilm was developed using activated sludge as inoculum source fed with ethanol as substantial substrate, it has been revealed that 83% of 16S clone library were conquered by β-*Proteobacteria* and 17% *δ-Proteobacteria(91).* 41% of the sequences identified of α- and β-*Proteobacteria* when starch processing waste water was added as inoculum source(129). It has been detected from these studies that *Proteobacteria* prevailed but when fermentable or complex substrates were used, *Firmicutes* mainly gram-positive fermenters were present predominantly(130-132). Different reports suggest that different operating conditions selects for different microbial consortia.

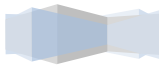


Axenic cultures have been developed for many MFC strategies, on the other hand MFCs operated with mixed cultures illustrate higher power output, larger substrate adaptability and higher resistance against process disturbance. Better understanding of electrochemical active bacterial community, improved electrode configuration had led to the better performance of MFC. Current generated with MFC operated under fed batch mode and supplied with synthetic wastewater was found to generate current of only 0.1A and the average power density of about $40Wm^{-3}$(35).

2.10 MFC As Waste Water Treatment Tool

Waste water based microbial fuel cell was first reported by Habermann and Pommer in 1991(133, 134). Considerable work was done by Logan and co-workers (18, 30, 135, 136)on the generation of electricity from microorganisms present in waste water. For the generation of continuous electricity by utilizing organic substrate and domestic waste water was done by Min and Logan with the operation of flat plate microbial fuel cell (FPMFC). The microbial fuel cell was designed by combining electrode/proton exchange membrane schemes so that reactor function as plug flow type reactor. The reactor was single chambered microbial fuel cell. Between the two non-conductive plates single channel was formed that alienated into two separate halves by the electrode/proton exchange membrane assemblage known as membrane electrode assembly (MEA). On the opposite side of PEM each electrode was placed. Anode was placed in chamber contained liquid and cathode in chamber containing air only. Anode chamber was continuously fed with feeding solution containing waste water or some other kind of organic substrate and the generation of electricity was analyzed.

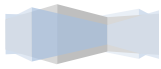
Initially the system was acclimatized for one month by feeding waste water and waste water enriched with a specific substrate media as acetate. With domestic waste water fed



at a flow rate of 0.39ml/min the average power density measured was 72 ± 1 mW/m^2 with 42% removal of chemical oxygen demand (COD)at 1.1h hydraulic retention time (HRT). At a longer of about 4.0 h HRT, average power density was 43 ±1 mW/m^2 and COD removal efficiency was improved up to 79%. According to a Monod-type relationship with a half-saturation constant of Ks = 461 or 719 mg COD/L the power output was established as a function of waste water strength.With various organic substrates at ˷1000mg COD/L, the power generation was found to be constant at high rates such as with acetate power density examined was 286 ± 3 mW/m^2, with glucose 212 ± 2 mW/m^2, 242 ± 3 mW/m^2 power density was measured with starch, with dextrin power density of about 150 ± 1 mW/m^2 and with butyrate power density measured of about 220 ± 1 mW/m^2. It has been deduced from these results that with various organic substrates power were generated and power generation at high rate can be possible in continuous flow reactors(133).

2.11 Effects ofelectrode spacing and flow rate through anode in MFC

Logon and co-workers(30) had studied the effect of continuous flow through anode and electrode spacing on the generation of electricity in microbial fuel cell. It was experiential that when space between the electrodes decreased from 2 to 1cm and glucose fed as organic substrate, the power density was decreased from 811 to 423mW/m2. When the space between the two electrodes was 2cm, the anode surface exposed to only one part of fluid but when reduced to 1cm it was opened to both sides of fluid. So, it has been observed that the power density was decreased with glucose 500mg/l from 811 to 684 mW/m^2. The power density was further reduced to 423 mW/m^2 when the space between anode and cathode was 1cm and exposed to one side of the fluid. Although the resistance by the interrupter technique (R_{int}) was reduced from 2cm (35Ω) to 1cm (16Ω) but still power density decreased. Power output should have to be increased with the decrease of

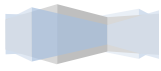


R_{int}, in spite of the fact that the Rint difference was insignificant. Insignificant difference because the magnitude level was incorrect according to several reports; probably the decrease was due to ohmic losses. But it has been noticed that with improving internal losses still then power density was reduced. 28% to 18% decrease in columbic efficiency was observed with electrode spacing reduction. The reason for the power density decline was due to the decreased bacterial activity on the surface of anode as it comes closer to the cathode(133).

However, power density was substantially increased by providing advective flow towards the cathode from porous anode. Maximum power densities were achieved in an air-cathode system fed with domestic waste water or glucose as organic substrate. In another system of MFC, the space between electrodes was 1cm fed with glucose operated under continuous flow conditions.Maximum power density measured up to 1540mW/m^2 and 60% increase in columbic efficiency was recorded. 464 mW/m^2 maximum power density was recorded by feeding domestic waste water of COD 255 ± 10 mg/L. However, with particular substrates like as domestic waste water the flow through the anode could led to the blockade. Still then the operation of system was continued for 42 days without any interruption. These results indicate that in single chamber air-cathode MFC, by decreasing the electrode distance and operating the reactor in continuous flow from the anode to cathode the power output could be enhanced(133).

2.12 Effect of stacked MFC configuration on current generation

Large-scale inefficiencies in waste water electricity generation in MFC was also been studied by Leropoulos et al. They tried to study the effect of scalability and stacked configuration on electricity generation(71). Power density was expressed as per unit area of electrode surface and as per unit volume of anode. For the 10 small units giving the same volume as one large 500ml of anodic unit, power output was measured.



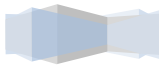
It gave probably a 10W/m^3 power output that was approximately 50 times elevated than power output produced by larger MFC. They also scrutinized the performance of fuel cell in water by connecting the cathode chamber to an artificial gill. At an ambient temperature the current generated was 32A which increased up to ~100 A (200%) at 52 ◦C. Output was also increased from 135% to 150% with increase in flow rate of water.

In single chambered membrane free microbial fuel cell, consequences of reactor architecture was studied by Liu et al. (17)carbon cloth as electrode containing large microbial fuel cell generate power density of about 16W/m^3at a 0.18 mA/cm^2 current densitywhile smaller MFC produce power density of 14W/m^3 which was slightly lower than LMFC. Orientation of anode, surface area of anode, type of reactor and biofilm study effectthe performance of MFC was also being studied by Liu et al(17). It has been observed that when the ionic strength of the solution was improved from 100 to 300mM with the decrease in internal resistance ($R_{int=}$ 7.3). The performance was also been improved up to 630mW/m^2 at 0.26 mA/cm^2 current density.

2.13 Internal resistance as limiting factor in Up-flow type MFC

He et al.(137)useartificial waste water in an up-flow type microbial fuel cell for the generation of electricity. For 5 months sucrose solution was continuously fed in reactor, as an electron donor. Electricity generation with 170mW/m^2 power density was produced. Up to 2.0g COD/L/day Chemical oxygen demand (COD) loading rate increased in power density was observed after that there was no increase in power generation indicating theexistence of limiting factors. The major limiting factor was the internal resistance, which at high power density shows readings around 85Ω and output of power was restricted by decreasing significantly the total potential of the operation.

Bicarbonate buffer was used to evaluate the functioning of MFC and along with that proton transfer mechanism also delineated by Fan et al.(138) Bicarbonate buffer

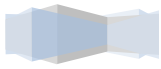


of pH 9 generated a current density of about 0.99 mA/cm^2 at a maximum power density $1550W/m^3$. Approximately 38.6% higher power density was produced than by using phosphate buffer of same concentration 0.2M of pH7.

2.14 Biofilm effect on electricity generation in single chamber MFC

Venkata Mohan et al.,(139, 140) studied the effect of growth of biofilm on the generation of bioelectricity by using chemical waste water and synthetically designed waste water in mediatorless single-chambered microbial fuel cell. Three separate cells of MFCs were run under acidophilic conditions of pH 6 at room temperature with different biofilm exposures, one with anode surface coverage (ASC) of 0% means no biofilm developed known as control, fully developed biofilm of 180 days and ASC of about ~96% and 90 days partially developed biofilm with ASC ~44%. It has been observed that in the absence of mediators, the formation of biofilm on anode surface increased the transfer of extracellular electrons. Analysis by cyclic voltammetry have shown that there was six fold increase in output energy from 1.812mJ control to 10.666mJ partially developed biofilm during operation and about eight-fold increase in energy production (86.856mJ) in fully developed biofilm from partially developed biofilm system.Configuration of biofilm developed during MFC operation has the potential to specifically encourage the electrogenic microbial growth with vigorous characteristics such as having the capability of higher power generation yield accompanied by degradation of complex substrates.

2.15 Simultaneous generation of electricity and degradation of xylose in dual chamber mediator less MFC



Mediator less dual chambered MFC was used by Huang et al. to study the generation of electricity generation along with the degradation of xylose(141). Maximum voltage output ranged from 55 ± 2.0 to 70 ± 3.0 mV and 41 ± 1.6 to 36 ± 1.2%

at the concentration of xylose from 0.5 to 1.5 mM. The maximum rate of degradation measured was up to 0.13 mM/h and Ks of 3.0 mM. In the anode compartment nitrogen stirring led to the maximum voltage of about 99 ± 2.3 mV with the coulombic efficiency of 5.9 ± 0.3% at the operational time of 180 hours, which is slightly higher than that of without agitation. With increase in transport of proton through the buffer solution would led to higher consumption of electron donor. Under stirring condition removal of COD measured of about 22.1 ± 0.3% which was 23.7 ± 0.4% somewhat lower than with no stirring operation. So, rate of xylose degradation was 59% lower under stirring conditions(133).

Logan et al. gave another configuration of MFC with fiber brush graphite electrodes in tubular cathode tumbled into a MFC tank(142). It was also been noticed that higher current and voltage can be achieved by attaching stacked MFCs in parallel or in series(143, 144). F.J. Hernández-Fernández et al.(36) connected six stacks of MFC in parallel and in series, obtained a current up to 255mA and voltage of about 2.02V. Membrane-less single chamber MFC was fabricated with colonized environment designed by Dong et al.(145) bioenergy was proved to implantable medical appliances.

2.16 Biofilm community analysis

Microbial fuel cell biofilm bacterial community analysis illustrated that no single winner in the microbial communities that develop biofilm on anode surfaces(69). The reason for this was because several different microorganisms especially bacteria are capable of producing electricity and most probably because of architecture of system, operational conditions, electron donors and electron acceptors at the cathode and anode.In microbial fuel cell electrochemically active bacteria are thought to be iron-reducers like *Geobacter*

species and *Shewanella(27, 114)*but whole community analysis showed that a variety of bacteria are persisting in biofilm community than these model iron-reducers(34, 126, 129, 146). Maximum power densities have been limited because of high internal resistance. Comparison of different microbial fuel cell systems using pure and mixed bacterial cultures cannot ascertain which microbial community or bacteria is capable of producing high power densities. Indeed, we do not yet know the highest upper power limit that is achievable using microorganisms.

2.17 Bacterial community analysis in different MFC configurations

In anode chamber contents were replaced after each cycle in fed-batch mode or in continuous flow systems, different configurations emerged in the development of microbial communities. In cathode, when oxygen was used for the chemical reaction diverse bacterial communities evolved in the microbial fuel cell system with compositions that differ with substrate and inoculum. Cloned PCR-derived 16S rDNA fragments sequences with unique restriction fragment length polymorphism (RFLP) patterns, a river sediment community dominated by *β-Proteobacteria*(similar to *Leptothrix* spp). When reactor was fed with glucose-glutamic acid mixture,*α-Proteobacteria* mainly *Actinobacteria* emerged(126). Sequences from DGGE-screened 16s rDNA clone library revealed that marine sediment used to inoculate an MFC fed with cysteine showed that 97% 0f bacterial communities were predominated by *γ-Proteobacteria* that's quite similar to *Shewanella affinis* KMM 3686,*Pseudoalteromonas* spp and *Vibrio* spp being the next most frequently noticed(146).

In some systems, a large number of clones were uncharacterized. In Microbial fuel cell using waste water as inoculum with dissolved oxygen at cathode, the microbial community composition shows that starch fed microbial fuell cell as carbon source consisted of 36% unknown clones,25% β- and 20 % *α-Proteobacteria. Cytophaga, Bacteriodes* and *Flexibacter* groups consisted of 19% of total composition on the basis of

RFLP-screened 16S rDNA clone sequences(129). With the same community analysis approach, acetate-fed reactor with same inoculum was similarly diversed with 24% α-, 7% β-, 21% γ- and 21% δ-*Proteobacteria* and 27% others(144). Isolation of γ-Proteobacteria from this reactor was capable of iron reduction and current production using glucose in microbial fuel cell(74).

2.18 Extracellular electron transfer and biofilm formation relationship

In microbial fuel cells biofilms were developed on electrode surfaces providing opportunities for extracellular electron transfer (EET) and allowed substantial substrate conversion capacity(147). Previously, our knowledge was centered around two model organisms *Geobacter* and *Shewanellaspecies* on extracellular electron transfer. It was believed that Gram-positive microorganisms cannot carry out EET by themselves as Gram-negative organisms(148). Pure and mixed culture experiments were performed to understand the process of biofilm formation on electrodes within the MFC and the influence of biofilm structure, developmental processes and viability on EET. During the current flow, in closed circuit the viability of biofilm was maintained highest near to anode surface. While, in open circuit operation, in which biofilm viability was maximum on top of the biofilm far apart from the anode surface.

Considerably, 30 ± 3 μm thin biofilm was formed by *Pseudomonas aeruginosa* in closed circuit anode as compared to open circuit anode of approx. 42 ± 3 μm respectively. Which was possibly due to the use soluble electron acceptors. Only a fraction of current was produced by two gram positive bacteria as compared to gram negative microorganisms. Power output was significantly increased by 30-70% with co-cultures of Gram-positive *Enterococcus faecium* and either Gram-negative bacteria. An about 1.8 ± 0.4mA of current was generated together by *Enterococcus faecium* and *Pseudomonas aeruginosa* however, it was 0.2 ± 0.05 mA and 0.9 ± 0.01 in the two bacteria respectively when used individually (147).

2.19 Bacterial diversity analysis in MFC using glucose as substrate

In one system of microbial fuel cell using glucose as carbon source without replacement of substrate, graphite as electrode with low internal resistance, aerated solution of

ferricyanide at the cathode generate high power density of 4.31W/$m^{2(34)}$. Chemicals produced by the cell were accumulated in the microbial fuel cell over many cycles because of lack of replacement of fluid. Bacterial community analysis that developed after some period of time using denaturant gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragment and dominant band sequencing demonstrate high phylogenetic diversity. Identification of sequences derived from bacteria of the taxa *α-,β-,γ-Proteobacteria* and *Firmicutes*(34, 69). Hydrogen production has been predominantly produced by facultative anaerobic bacteria such as Gram-positive *Enterococcus gallinarum* and Gram-negative *Alcaligenes faecalis*, possibly as a result of utilization of fermentable substrate with mixed bacterial culture inoculums. Isolates obtained were capable of producing electricity and highly coloured mediators in large concentration like pyocyanin production by *Pseudomonas aeruginosa*. Hence, it has been deduced that mediator production was the main reason for high power production along with low internal resistance of the system.

2.20Acetate as electron donor in MFC configurations

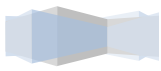
Under anaerobic conditions acetate was the most copious fatty substance in microbial fuel cell. It was utilized by anaerobic respiratory microbes as electron donor(128, 149). The best known anaerobes that were able to oxidize acetate were *Sulfidogens* and *Methanogens* but metal reducers also utilized acetate as a carbon source. Anaerobic bacteria either metabolized acetate directly or indirectly by synergestic associations with other microorganisms that were present in MFC but not taking part in electricity generation(150). To check the acetate utilizing capability of microorganisms,

marine sediment was added as inoculum and acetate was consumed as fuel in one system of microbial fuel cell(114). *Geobacteraceae* family was found to be most predominant bacteria in that system.

Jiyoung Lee et al. Used mediator-less MFC fed with acetate as electron donors to enrich electricity producing microorganisms. The enriched microbial community was characterized in fuel cells. MFC was fed continuously with artificial waste water and activated sludge having 5mM acetate at the rate of 0.15ml/min for microbial community enrichment that oxidize acetate in association with the current generation. In 10hrs the open circuit potential was reached up to 0.7V. During the first week, current was increased slowly when 500Ω resistors were connected through the electrodes. 1.5mA stable current was produced after inoculation within first three weeks of operation. Before lowering the resistance up to 10Ω the MFC was run continuously next for four weeks under the same circumstance.

The concentration of acetate in effluent was lower than the detection limit and the maximum current generated was about 5mA in these conditions. It was estimated that about 70% of electrons obtained from oxidation of acetate was recovered in terms of power production. After centrifugation of effluent from MFC showed a COD value of about 17mg/l. It has also been observed that when feeding of artificial waste water was hindered the current was gradually decreased to about 0.1mA and the current was immediately increased after subsequent start of feeding(128). Well-developed biofilm has been observed on the electrode surfaces in low vacuum electron micrographs obtained from MFC enriched with acetate. But clamps of bacteria was not present on the surface of electrode that are enriched with corn processing waste water. Rod-shaped cells of bacteria forming biofilms on the surfaces of electrodes has been observed under high magnification scanning electron microscopy(128, 129).

2.21 Symbiotic bacterial interaction in relation to power production



All the microbial consortia associated with anodic biofilm may not interact directly with the anode however, interact indirectly through other electrode community members(151). As in one system it has been found that ***Brevibacillus spp***.PTH1 was an abundant member of microbial community. ***Brevibacillus spp***.PTH1 produces very low power unless it is cultured along with *Pseudomonas sp*.(105) Electricity producing pure microbial strains in MFC include *Firmicutes* representatives(152), *Proteobacteria spp*.(34, 42, 125, 153-156), *Acidobacteria* along with that yeast strains like *Hansenula anomala(157)*and *Saccharomyces cerevisiae(158)*were also capable of current production(159). Varying degree of current was produced by these organisms by interacting with anode surfaces through direct or indirect mechanisms.

2.22 Substrate, bacterial consortia and coulombic efficiency

Columbic efficiency was a common measure of MFC efficiency, the measure ofcolumbic numbers recuperated as electricity as compared to the hypothetical maximum number of coulombs recoverable from the organic substrate added to the system. MFC columbic efficiency has found to be dependent on microbial consortia that carry out oxidation at anode and the organic carbon substrate from which electrons were derived(81, 89, 160). This has been found to be due to different metabolic pathways used by different bacteria and mechanisms by which electrons were transferred to the anode surfaces by these bacteria. The substrate should be completely oxidized to $C0_2$ with efficient transfer of electrons to electrodes to gain highest power output. Incomplete oxidation leads to energy loss from the system.

It has been studied that *Shewanella oneidensis* did not entirely oxidized the lactate organic substrate in MFC, unutilized electrons left as acetate, had a columbic efficiency

of about 56.2%(161). Columbic efficiency increased significantly with complete oxidation of substrate. Reported bacteria that are capable of organic substrate complete oxidation in MFC system are *Geothrix fermentans*with columbic efficiency of about 94%

utilizing acetate as carbon source,*Rhodoferax ferrireducens* oxidizing glucose having columbic efficiency of about 83% and *Geobacter spp.* having 100% columbic efficiency using acetate and with benzoate areof about 84%(4, 114, 162). Type of inoculums used in MFC system can also affect the columbic efficiencies like waste water after microbial enrichment maximum 65-89% has been reported(163).

2.23 Anode a mean to isolate electricigens

It has been observed that in absence of electron acceptors, the metabolism of *Shewanella putrefacians* was stimulated due to the presence of anode in MFC(41, 129, 164). Based on these observations it has been deduced that anode itself offer a pathway for the isolation of electrochemically active microbial consortia by means of anaerobic enrichment technology. Using microbial fuel cell system "Enrichment" acts as a tool for the isolation of electricigens. This method was explored using sludgeinoculum that was collected from corn-processing waste water treatment plant. Sludge was inoculated in anode sectionof MFC and fed it with waste water of the same source, while the cathode compartment contained buffer solution under aerated conditions. Cation exchange membrane separates the two compartments(129). After the inoculation the open circuit voltage of about 0.6V has been observed. When 10Ω resistor was connected the voltage potential dropped to 20mV with corresponding current of 20µA.The current was increased when solution in anode compartment replaced with new feed solution. COD reduction was concurrent with increase in current production. Repeated replacement of waste water feed solution led to increase in current production up to 1.2mA. Similar results were observed when MFC system was inoculated with anaerobic digester sludge or an activated sludge(129).

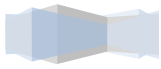
These results suggest that electrochemically active bacteria present at low concentration in sludge or waste water initially during enrichment and propagate in the MFC. Electrochemically active bacteria metabolized electron donor organic substrate in waste

water and shuttle those electrons on electrode surface in the absence of any other electron acceptor which results in electricity generation. Because it was considered that electrode reducing step was an energy storing step in microbial respiration step(114).

If the enrichment step is to be optimized then the metabolism of electron donors would be much faster than that of previous step. Additionally, diverse nutritional characteristics were used to enrich bacterial cultures like as oligotrophic cultures enriched with river water (126)or artificial waste water(165), copiotrophic cultures enriched with acetate containing artificial waste water(128), glucose or propionate retaining artificial waste water(127).16S rDNA analysis showed that fermentable substrates contain more diverse bacterial community than that of non-fermentable substrates.From enriched anodic populations DNA was extracted and evaluated using diverse nutritional characteristics. It has been revealed from denaturant gradient gel electrophoresis that microbial population is quite much different in enriched MFC system than that of original inoculums and the dominant bacterial populations were dependent on the type of substrate used(126, 128, 129).

16s rDNA sequence analysis showed that artificial waste water containing glutamate and glucose were dominated by *γ-Proteobacteria*accounts for 36.5% and 27% of *Firmicutes(127).* In most of cases Gram negative bacteria were prevailing more than Gram positive bacteria. In another MFC system, acetate containing artificial waste water had less diversity having approximately 70% of δ- *Proteobacteria* and *γ-Proteobacteria* accounts or only 17.3%(128). In contrast to that when enrichment was done with corn-processing waste water(129) the majority of bacterial clones amplified were of 40.9% β-*Proteobacteria* and α- *Proteobacteria* of 27.2%. It has been noticed that in marine environments(4) high percentage of *Firmicutes* were present than as compare to fresh water environments(128).

2.24 Soil bacterial diversity in double chamber MFC



Recently, a study was carried out to examine most probable bioelectricity producing microbial isolates from tea garden soil(61). Researchers tried to isolate and characterize microbial communities from tea garden soil samples(61). Three different soil samples were collected and total of 25 isolates were obtained. After 48 hours of incubation it has been observed that isolate named as S23 showed maximum cell density of about 0.89 and this isolate was considered for further analysis. On the basis of various biochemical and staining procedures the most probable isolate was identified as *Bacillus megaterium(Vos, 2009).* Commercially available biochemical kits named as HiBacillusTM KB013 were used for the biochemical analysis of potential isolate. Waste materials used to construct dual chambered microbial fuel cell for this experiment. The potential isolate S23 was inoculated in anodic chamber of MFC and incubated at room temperature for 7 days. After 48 hours of incubation period voltage was measured at an interval of 48hours(61). To observe the highest absorbance, standardization of minimal salt media was carried out using different carbon sources such as glucose, lactose, fructose, starch and maltose.

It has been evaluated that glucose as most suitable carbon source showing maximum absorbance. Glucose was acting as a sole carbon source followed by fructose. Utilizing carbon source maximum cell voltage was obtained after 48hours of incubation time of about 440mV and after 128 hours 66mV was recorded.Providing yeast extract and glucose as a carbon source along with external vitamin source,the voltage was again increased up to 698mV after 48 hours of incubation period.

Most of the previous literature showed that*Clostridium acetobutylicum, Clostridium thermohydrosulfuricum (166, 167), Enterobacter cloacae(167), Proteus vulgaris(168), Clostridium butyricum (169), Shewanella putrefaciens, Geobacter metallireducens, Rhodoferax ferrireducens, Geobacter sulfurreducens(4), Klebsiella sp.(170), Lactococcus lactis(171), Saccharomyces cerevisae(172), Tetrasilmis gracilis,*

Isochrysis sp., Synecocystis sp., Dicarteria sp., Chaetoceros calcitrans, Cholorella salina, Nanochloropsis sp., Pavlova sp., Dunaliella sp(173), Shewanella sp.(27, 129, 174), Corynebacterium sp. (175), as a potential isolates for the bioelectricity production

but in the current study out of 25 strain *Bacillus megaterium* as best strain for the production of bioelectricity. After the addition of yeast extract in the media the voltage was rapidly increased up to 698mV. These characteristics were the most "green" feature of microbial fuel cells. Organic matter and biowastes was being used directly for the generation of electricity. The generated energy can be sold to the energy market or used for the functioning of waste water treatment plants.Additionally, hydrogen gas can be produced from the current generated in fuel cell.

Hydrogen or a buffer were desirable for energy storage temporarily, since water flows were often variables. It has been reported that in anaerobic processes, during the thermal combustion of biogas the high yield of electrical energy generation was only one third of the input energy. The recovery of energy can be obtained by heat exchange mechanism but the overall effective yield still to be remains of the order of 30%(61)**.** There were no intermediate substantial processes in microbial fuel cells so, if about 30% conversion efficiency was obtained in MFC than it would be the best efficient bioelectricity generating process.

Approximately 0.5V power was obtained per fuel cell. Consequently, in order to obtain acceptable voltage considerable amount of MFCs will be desirableeither in separated or stacked in series. Another very important aspect of this technology is that as in conventional type batteries microbial fuel cells need not to be charged before operation but they can work within little time after the addition of inoculums, until and unless the starvation time period was too long to support active biomass. It has been observed that the *Bacillus megaterium* generate a voltage of about 690mV constantly for long period of time for approximately 100 hours(61).

2.25 Role of surface area of anode in power production

Mixed microbial consortia incorporated different combination of mechanisms of electron transfer through which stable and high densities of current have been produced.

Functionality of pure cultures has been more limited. Power output was boosted due to the presence of *Geobacteraceae*in the community of bioanode(59, 176). Structure and material of electrode detrimentally affect the performance of bio-electrochemical systems(BES). It has been observed that current output was considerably high with rough surface graphite electrodes as compared to smooth ones(177). Similarly, when the surface area of electrode material was increased current density increased ultimately.

Microbial electrical connections and adhesion between the electrode and bacteria increased significantly with the increase in surface area of electrode material. So, modifications in surfaces of anode materials will lead to the improvement of current densities.

2.26 Role of electrode surfaces in higher power yield

An electron transfer by exoelectrogens to metal electrodes could also be possible like as tantalium and iridium oxides that are dimensionally stable anode, platinum and stainless steel. It has been shown that with *G. sulfurreducens* current densities increased considerably when thin Pt wire was used as anodic ultra-microelectrode(178). It was also been reported that current densities usually higher with porous carbon than metal attained but recent reports emphasized on the importance of stainless steel electrodes(179). Apparently performance of current density was increased when planer electrodes were switched to three-dimensional electrodes having optimized microstructures.

With Corrugated fiberboard electrode current was measured up to 70 A m^{-2} when used as single sheet electrode but with six layered electrode current density was drastically increased up to 390 Am^{-2}[180]. Therefore, due to optimal macrostructure it was considered

as high performance electrode. With highly conductive material such as porous carbon felt electrodes current density of about 60-85Am^{-2} has been achieved(181, 182).In conventional electrochemical methods, higher performance of the systems was ensured

by conductivity of electrolyte alternately in microbial fuel systems this parameter was restricted to the salinity level of tolerance of microorganisms in operating system. In few studies it has been shown that halophilic bacteria showed higher ionic conductivity approximately 1.5 times more than seawater(181). Bacteria in anode compartment utilize acetate more frequently as this substrate was easily biodegraded by microbes. Bioanode polarization potential was approximately ranged from -0.25V to +0.4V vs SHE(33).

Although the functioning of bioelectrochemical systems was affected by the density and activity of anode associated microorganisms, but it was also been seen that power density was usually restricted by the cathodic activity(59). One critical factor was the electro kinetic rate of oxygen reduction reaction in cathode, helped in the improvement of the performance of all cathode configurations. With the projected surface area of cathode, power densities were varied from 2 to 3 Wm^{-2} with neutral to slightly alkaline electrolyte at $30^{o}C$(183). It has been reported that the upper limit value of power density at lowest internal resistance was about 17 to $19Wm^{-2}$(184).

2.27 Advance techniques in characterization of bacterial communities from soil and water samples

Commonly denaturing gradient gel electrophoresis (DGGE), 16S rRNA gene sequencing via clone libraries, terminal restriction length polymorphism, analysis of automated intergenic spacer analysis and pyrosequencing more recently have been used to find out the diversity of microbial communities associated with MFCs (185). These techniques provided information on bacterial community profile associated with electrodes from different water and soil samples(186, 187). It has been identified that *Geobacter* was the

dominant genus on the surfaces of anode. However, power production was not prevented in the absence of *Geobacter spp.* 16S rRNA clone libraries and DGGE analysis provide a greater understanding of qualitative assessment of the relative abundance in mixed community of microbial consortium. Althoughthese studies were very limited in the

detection of microbial diversity in depth(188). Some more modern techniques such as Phylochip and GeoChip have allowed better investigation of bacterial community diversity. High-density oligonucleotide microarray PhyloChip analysis has identified Gram-positive *Firmicutes*, mostly the members of *Thermincola* and *Geobacillus* as anode-reducing bacteria (70). Phylochip and GeoChipanalysishave allowed to track out dominant as well as minor bacterial population and could also assist in uncoveringapproximately 35 times the bacterial community diversity as compared to clone libraries and DGGE techniques. Gene specific probes in GeooChip have allowed to find the structural and functional examination of different stochastic processes takes place in bioelectrochemical systems(BES), although this technique is restricted in number of taxon-specific oligonucleotide probes (189).

It is now possible to analyze the community diversity in depth and detailed "snapshots" of electrode-associated bacterial population. It has been observed that among the anodophilic microbial population 3277 phylotypes representing 25 distinct phyla and 39 bacterial classes were identified(190). Syntrophic interactions between phylogenetically diverse microbial communities could also be speculated because of the diversity among microbial populations through the consumption of organic matter. Methanol is converted into acetate by *Sporomusa(*a new genus of *Firmicuties)(191)* which is then utilized by *Geobacter* for electricity generation(192).

2.28 Microscopic analysis of electrode associated bacterial communities

Scanning electron microscopy (SEM) has been used more often in order to visualize the population of bacteria within a bioelectrochemical cells. This technique simply requires dehydrated sections of electrode surface. Because of dehydration of electrode samples, pili and exopolysaccharides may be erroneous for nanowires and individual specie identification was difficult in mixed microbial community. It has been revealed from SEM images that on anode biofilms in landfill BES predominantly bacilliform

microorganisms were present and cells firmly attached with nanowire-like filamentous appendages to the anode surfaces(193).

Confocal scanning laser microscopy (CSLM) has been used to determine the architecture, viability profile, thickness and spatial distribution of microbial biofilms. Appropriate cell wall or DNA stain was used for the staining of electrode surface samples for this technique. With the help of this technique, it has been observed that increased current generation was co-related with thicker biofilm structures(194). Microcolonies of Gram positive and Gram negative organisms has been observed throughout an electrochemically active biofilms(92).

Fluorescence in situ hybridization (FISH) technique using rRNA-targeted oligonucleotide probes, genus-specific in nature allowed the temporal and spatial visualization, quantification and identification of microorganisms in an electrode associated microbial population. With the help of non-PCR based techniques, on the anode surfaces of sludge in BES *Geobacter sulfurreducens* was shown to be present(193), dispersed homogenously on anode fed with potato waste water in BES(176). It has been shown to contribute to about 60% of the anodic biofilm formation fed with waste water of different sources (195).

2.29 Physiological analysis of bacterial population forming biofilms

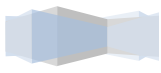
Transcription profiling analysis technique helped to identify the physiology of microbial community forming biofilms(196).Thegenomicexpression methods has been used to relate with specific physiological functions of the electrochemically active bacterial communities. *Ishii et al*. (2013) used metatranscriptomic techniques to characterize bacterial communities exposed to high and low extracellular electron transfer(EET) rates. Microorganisms belonging to the family *Desulfobulbaceae*has been identified as predominant bacteria(197). These bacteria contained c-type cytochromes encoded by a number of EET-related genes characteristically related to those reported in *Geobacter.*

mRNA/DNA ratio showed that methanogens and sulfate reducers were abundantly present and consistently active bacteriaharbored in bio-electrochemical systems(BES) . Conversely, the electron transfer stimulus was not related with the methanogenesis or sulfate reduction pathways indicating that although the genes were present but not expressed(185).

2.30 Need of new advance techniques for whole electrode community analysis at genome level

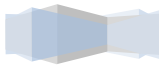
It was expected that in the near future with a whole genome shotgun metagenomic technique highly diverse anodophilic microbial consortia will be identified. Along with that we were able to highlight the molecular potential within the anode associated microorganisms. It was not completely understood that which type of deterministic and stochastic processes will influence the structure of microbial consortia forming biofilms on the surfaces of anode within the microbial fuel cells. In these biofilms different microbial population may be established while keeping the deterministic factor constants like temperature, influent substrate and applied voltage (189).

The highly diverse nature of microbial community dynamics highlights the need for new techniques that are capable of tracking the microorganisms and their interactions in such type of systems.



CHAPTER # 3

MATERIAL AND METHODS



Material And Methods

The current study is related to the enrichment of electrogenic bacteria from activated sludge and soil samples in dual chamber microbial fuel cells with different proton conducting materials. The research was carried out in Molecular Research laboratories(MRL), department of Microbiology, Quaid-i-Azam University,Islamabad, Pakistan.

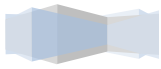
3.1 Culture medias and chemicals

All the chemicals and medias used in current study was purchased from Oxoid chemical Company Uk, BDH Laboratory Chemical Division (Poole, Dorset, Englant), Fluka granite CH-9470 buchs, Sigma Chemicals Co., St. Louis, E.Merck (dermstadt, Germany), ICI 9211 North harborgate street Portland.

All the Media's were prepared acoording to manufacturar's recommendation. Chemicals and media's were prepared and autoclaved at 121^{o}C for 20mins for complete sterility. The sterility of media's and prepared chemicals were checked by incubating at 37^{o}C for 24 hours.

3.2 Construction of microbial fuel cell

Double chambered microbial fuel cell was used in the present study. Two different types of dual chambered microbial fuel cells were constructed: one with salt bridge and another with proton exchange membrane separating the two chambers "Anode and Cathode" of the cell. The two cells were operated under static and agitation conditions with different inoculum source.



3.2.1 Construction of doublel chamber microbial fuel cell

A double chambered Microbial fuel cell was constructed using the material available in the laboratory. Polyacrylic bottles of 500ml volume were utilized for the assemblage of anode and cathode chambers of a fuel cell with inlet and outlets for the addition and removal of samples. The two chambers were connected with glass rod having the length of 14cm respectively containing agar salt bridge. The total working volume of two chambers was approximately 300ml. To prevent the leakage the joints of chamber were sealed with epoxy glue and silicon sealant. The anodic chamber was kept air tight throughout the incubation period(61).

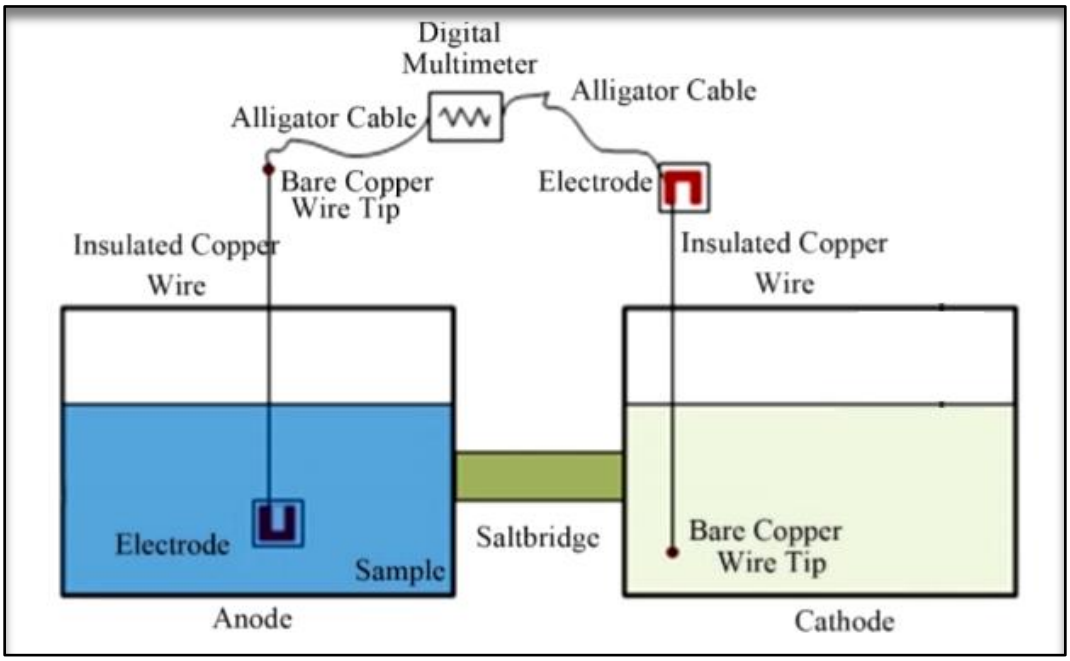


Fig 3.1 Schematic diagram of salt bridged double chamber Microbial Fuel cell(1)

3.2.2 Preparation of salt bridge

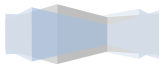
Salt bridge was prepared using 11.18g of KCl with 2% agar technical in distilled water. To make the sterile solution the mixture was autoclaved at 121^{o}C for 20mins. A hot solution was poured in glass rod and allowed it to solidify for some time. The glass rod was fixed between the two polyacrylic bottles (anode and cathode chamber) and the ends were covered with parafilm to avoid leakage(61).

3.2.3 Platinum catalyst coating on cathode surface

For Pt catalyst coating on cathode surface Cheng et a.,(2006)(136, 198) procedure was followed. For 15cm2 carbon cloth, 10% Pt/C of about 52.5mg was taken in sample plastic vial. Drop wise 43.57µL of deionized water was added. 367.5 µL of 5% Nafion R solution and pure iso-propanol of about 174.8 µL was added and for 20 seconds was vortexed. By using paintbrush catalyst paste was homogeneously coated on the cathode surface. The coating was allowed to air-dry for 24 hours at room temperature before being used.

3.2.4 Construction of membrane H-shaped microbial fuel cell

Membrane H-shaped cell was constructed using polyacrylic bottles for anode and cathode chambers of total 500ml volume each. The two chambers were separated by a Nafion115(Gas hub pte Ltd, Du Pont Company, 30cm*30cm) cation exchange membrane fixed in two plexiglass slabs. Holes of diameter….. was drilled in plexi glass slabs and membrane sandwiched between the two slabs. The slabs were fixed with the help of screws and join with chamber bottles. The joints were sealed with epoxy glue and leakage stopped with the help of silicone sealant(2).



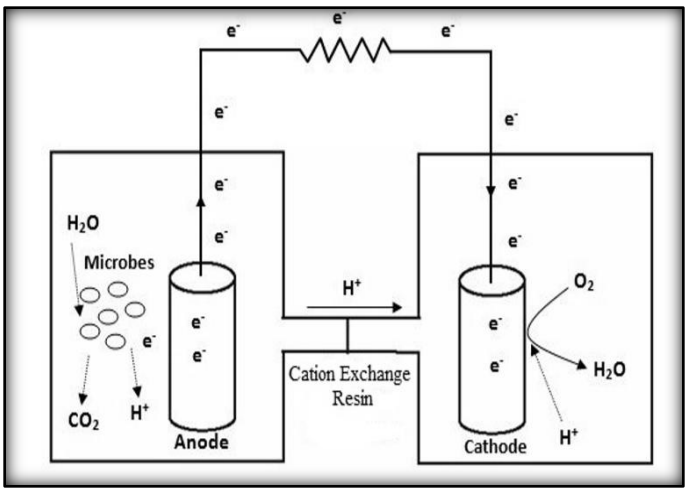


Fig 3.2.4A Schematic diagram of double chamber microbial fuel cell with salt bridge(2)

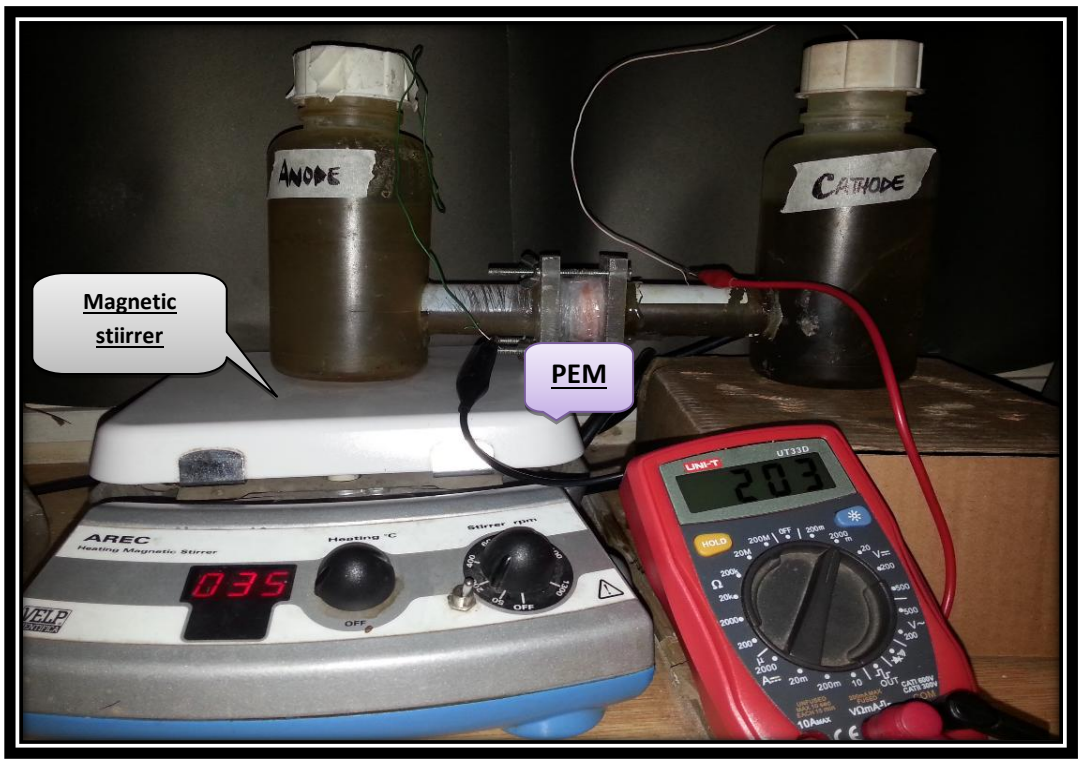


Fig 3.2.4B Double chamber microbial fuel cell with membrane as proton conducting material

3.2.5 Electrode used in MFC

Electrodes used in the present study was:

- Carbon cloth (EC-CC1-060 ,no wet proofing)

Caron cloth (EC-CC1-060, no wet proofing) was used as electrodes in few Microbial fuel cells. The size of electrodes was kept 5 *5 cm. Carbon cloth was autoclaved before processing at 121^{o}C for 20 mins.

3.2.6 Anode and catalyst coated cathode assembly

5 by 5 centimeter pieces of carbon cloth was cut. Two pieces of copper wire of diameter 0.8mm were collected and by using wire stripper one end of copper wires was stripped about six inches long while the other end one cm of wire was stripped. Bare 6 inch copper wire was bent into the square shape like the shape of carbon cloth pieces. To stick the copper wire to the carbon cloth piece conductive epoxy glue was applied and let it dry for some period of time. All work done under completely sterile conditions.

Fig 3.2.5A Carbon Cloth

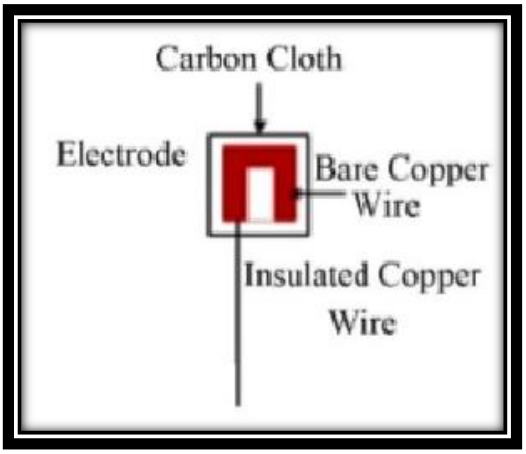


Fig 3.2.5B Schematics of Carbon cloth Assembly(1)

3.3 Sample collection

Two submerged soil samples (S2, S3) were collected from three distanct places of waste stream in Gujar Khan. Soil was digged with the help of sterile spatula up to 1 and half an feet down the earth and taken into a new sterile bucket. The sample buckets were tightly closed and brought into the laboratory. The samples were placed into refrigerator at $4^{o}C$ before use.

Fig 3.3 Submerged soil sample collection view

Activated sludge sample was collected from Waste water treatment Plant I-9 Islamabad, Pakistan in a sterile container. The samples were placed at $4^{o}C$ before use.

3.4 Cell feed solution

Anolyte Solution: Two different types of anolyte solution were prepared for different collected sample.

- For activated sludge, artificial waste water was used as the feed solution for microbial fuel cell. Synthetic waste water with two different carbon sources sucrose and acetate was used in the present study, to determine the effect of carbon source on energy generation and microbial activity.

Table 3.4(a) Composition of Synthetic waste water(9)

Components of synthetic waste water	Concentations (mg/L)
Sucrose	450
Potassium acetate	450
NaHCO3	480
NH4Cl	95.5
K2HPO4	10.5
KH2PO4	5.25
CaCl2.2H2O	63.1
MgSO4.7H2O	19.2

Trace metals were added as:

Trace metals were added as FeSO4.7H2O = 10 mg/L, NiSO4.6H2O = 0.526 mg/l, MnSO4.H2O = 0.526 mg/l, ZnSO4.7H2O = 0.106 mg/l, H3BO3 = 0.106 mg/l, CoCl2.6H2O = 52.6 µg/L, CuSO4.5H2O = 4.5 µg/L, and (NH4)6Mo7O24.4H2O = 52.6 µg/L.

- For soil samples bacterial growth media was prepared.

Composition of bacterial growth media was as followes:

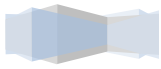
Table 3.4(b) Composition of bacterial growth media

Chemical's Name	Concentration
Acetate	25mM
NH4Cl	0.33g/l
Na2HCO3	50mM
FeCl2	1ml/L
Minerals and vitamins	10ml/L

Mineral and Vitamin solutions were prepared according to the ATCC medium:

Table 3.4(c) Minerals and Vitamins solution composition

Wolfe's Mineral Solution:	• Nitrilotriacetic acid 1.5 g • MgSO4 . 7H2O 3.0 g • MnSO4.H2O 0.5g • NaCl 1.0 g • FeSO4 . 7H2O 0.1 g • CoCl2 . 6H2O 0.1 g • CaCl2 0.1 g • ZnSO4 . 7H2O 0.1 g • CuSO4 . 5H2O 0.01 g • AlK(SO4)2.12H2O 0.01 g • H3BO3 0.01 g • Na2MoO4.2H2O 0.01 g • Distilled water 1.0 L
Vitamins Solution	• vitamin B12 vitamin tabltes



Catholyte solutions: In cathode chamber Potassium permanganate (KMnO4) 0.6mM was used as catholyte along with Phosphate buffer solution(PBS).

PBS contains:

- NaH_2PO_4 4.904g/L,
- Na2HPO4 9.125g/L,
- NH_4Cl 0.62g/l,
- and KCl 0.26g/L in 1L distilled water
- pH was adjusted to 7.

All the prepared medias (Anolyte and Catholyte solutions) were autoclaved at 121^{o}C for 20mins to remove all the comtaminants.

3.5 Operation of Microbial fuel cells

3.5.1 Working of Microbial fuel Cell 1

Initially cell was operated with activated sludge named as MFC 1. Salt bridged Double chambered microbial fuel cell was assembled. Carbon cloth electrodes (anode and pt catalyst coated cathoode) were inserted into the cell. Total working volume of cell was 300ml. 240ml of autoclaved synthetic waste water containing sucrose as a sole carbon source was added as a feed solution and 60ml of inoculum containing activated sludge as bacterial source was injected into the cell. Cell was sparged with N2 gas for 20mins to create anaerobic conditions for bacterial growth. COD of synthetic waste water was about 237mg/L. when inoculum was added to the feed solution, COD goes beyond the level of 1500mg/L. pH was adjusted to 7.3-7.6. In the first operation of the cell, sucrose was acting as a carbon source. The cell was placed in incubator at 37^{o}C for 18 days.

After few hours, Open circuit voltage was measured with the help of precision

multimeter (UT33C; UNI-T). Ciruit was closed by applying 3KΩ resistor and the voltage of microbial fuel cell was continuously monitored with the help of precision multimeter (UT33C; UNI-T) for 18 days and data was recorded. During 18days, biofilm was formed on anode surface. In 18 days of working operation of fuel cell samples were taken anaerobically in glovebox to check the type of microorganisms were present. Samples were serially diluted and spreaded on nutrient agar (prepared as prescribed by the munfacturer) plates anaerobially in glovebox. After 24 hours of incubation at 37^{o}C, different type of colonies were obtained that were purified and processed further. After 18 days, biofilm was preserved in PBS for further analysis and cell was enriched by adding fresh media (synthetic waste water) about 260ml in new cell and. 30ml of previous cultures from already running cell was added in new cell. The new enriched cell was placed in incubator at 37^{o}C for next 19 days again. Voltage was again recorded with precision multimeter (UT33C; UNI-T) as previously. Biofilm was again developed on anode surface of carbon cloth in next 18 days and preserved in PBS. Samples were again taken and cultured as described previously(129, 199, 200).

Small pieces of developed biofilm on anode surface were cut with autoclaved sterile scissor and sonicated briefly to detach the bacterial cells from anode surface and cultured by following serial dilution method of microbiology. The difference in the number and type of microorganisms at enrichment stage 1 and 2 was observed.

3.5.2 Working of Microbial fuel cell 2

In second cell carbon source was changed to potassium acetate intead of sucrose source. 240ml of potassium acetate containing synthetic wastewater of pH7.3-7.6 was added to double chamber, salt bridged microbial fuel cell. 60ml of activated sludge was added and spurged with N2 gas for 20mins to develop anaerobic conditions in anode chamber. While in cathode chamber, 150 ml Potassium permanganate and 150ml of PBS was added to accept electron acceptor. Cell was placed at 37^{o}C for 16 days. After few hours of stabilization open circuit voltage (OCV) was taken.

After cell was stable the circuit was connected through 3kΩ resistor and voltage was continuously recorded for 16 days. Different resistors were also connected to check the maximum output of cell at different resistance. After 16 days of operation, cell was enriched by adding fresh medium in new salt bridged double chambered cell along with that small piece of biofilm on anode surface was cut with sterile autoclaved scissors and sonicated briefly to detach the cells and added to the fresh medium. The new enriched cell was placed in incubator at 37^{o}C for next 16 days again. OCV was taken after 24 hours of incubation and connected through 3kΩ resistor. Voltage data were recorded for 16 days. Both biofilms formed during enrichmen stage 1 and enrichment stage 2 were preserved for further analysis. Samples were taken during operation of enrichment at stage 1 and 2,cultured by the serial dilution procedure of microbiology anaerobically in glovebox. Difference in type and number of cultures was also been determined.

3.5.3 Working of Microbial fuel cell 3

H-shaped microbial fuel cell was assembled by connecting anode and cathode chambers through proton exchange membrane along with carbon cloth (anode and pt catalyst coated cathode) as electrode. All leakage points were sealed before operation of MFC. In third cell, soil sample was tested for the current generation. All the solutions used in MFC were autoclaved at 121^{o}C for 20mins to remain contamination free. 290ml of bacterial anolyte solution was added as the feed solution to anodic chamber and potassium permagenate along with phosphate buffer solution, total of about 300ml of solution was added to the cathode chamber. 10g of soil (S3) was added as bacterial source in anode chamber. The anode chamber was sparged with N2 gas for 20mins to remove all oxygen and to create anaerobic growth conditions for microorganisms. The cell was continuously operated under agitation at 50rpm with the help of magnetic stirrer. The temperature was set at 35^{o}C.

After a few hours, open circuit voltage (OCV) was measured, but the call was not

showing any kind of voltage. The cell was allowed to remain at open circuit in order to obtain stable voltage. After 2 days OCV was again measured. After obtaining a stable OCV, the circuit was closed by applying 100kΩ resistor.

Closed circuit voltage was continuously monitored for 16 days with the help of precision multimeter (UT33C; UNI-T) and data was recorded. Samples were taken and spread on nutrient agar plates (prepared according to manufacturer's recommendations) by following serial dilution method of microbiology. After 24 hours of incubation at 37^{o}C different colonies were observed, that were purified and processed further.

After 16days, biofilm on anode surface was preserved and the cell was enriched by adding fresh media along with 10% of previous inocula in newly assembled MFC. Again the cell was sparged with N2 for 20mins and operated under agitated condition 50rpm at 35^{o}C. Open circuit was measured after two days of stabilization of fuel cell. closed circuit voltage was measured for next 16 days again as done previously. To check the difference in the number and type of microorganisms at enrichment stage 2, samples were taken and microorganisms were allowed to grow on nutrient agar plates by the serial dilution method. Similarities and differences between enrichment stage 1 nad 2 was recorded. Biofilm was preserved after completion of MFC operation and analyzed further.

3.6 Effect of external resistance

Different resistors (3kΩ, 100Ω, 170Ω, 68Ω, 57Ω, 10Ω) were also tested in all experiments to determine the effect of resistance on current generation. Voltage data were recorded and analyzed(201).

3.7Current measurement

Voltage during enrichment V1(mV) stage 1 and 2 V2 (mV) was recorded for 15-19days for all microbial fuel cells. Readings were monitored during day time continuously with precision multimeter (UT33C; UNI-T). Current (I) was calculated by using ohm's law:

$V = I*R$

So,

I=V/R

Where,

I = Current

V = Voltage

R= Resistance

There was direct propotional relation between current and voltage while current and resistance were inversely proportional to each other.

3.8 Calculating power

Power (P or W) was calculated from voltage (V) and current (I) by using the formula:

P=I xV

3.9 Calculating Power Density

Power density (P_D) was calculated by measuring the surface area of anode along with voltage (V) and Current (I). The formula used for calculating Power Density was:

P_D = I xV/ surface area (A) of anode in (m^2)

As both sides of anode was exposed to microorganisms. So, both sides were used for calculating surface area, this area was known as projected or geometric surface area(44, 202, 203).

So,

Surface area of anode (A) = 2 xL xW

Where,

L= length

W= width

3.10 DNA Extraction

DNA was extracted from activated sludge, soil (S1), soil (S3) and biofilms formed by all these samples. Two different protocols were followed for the isolation of DNA. For DNA

isolation from biofilms soil DNA isolation kit (Norgen biotek corp, product 26500) was used.

3.10.1 DNA extraction steps

- Small pieces of carbon cloth on which biofilm developed was cut and added into few ml of phosphate buffer solution (PBS) and properly labeled each sample. All samples were sonicated for 20min in a sonicator to detach all the cells from the anode surfaces.
- 1ml of sample was added into DNase free microcentrifuge tubes, properly labbled and centrifuge at 10,000 rpm for 4-5mins to get cells in pellet.
- Supernatant was discarded and pellet cells were resuspended in 750µL of lysis buffer and vortex briefly.
- Add the votexed solution into the provided bead tubes and vortex briefly again.
- 100µL of Lysis Additive A was added and briefly vortex the solutions.
- Beat tubes were votexed for 5mins at maximum speed.
- Tubes were centrifuged at 14000rpm for 2mins.
- 450µL of supernatant was transferred to new DNase free microcentrifuge tubes. Labelled properly.
- Binding buffer of about 100µL was added and mixed well by inverting the tubes and incubate on ice for 5mins.
- To pellet out soil particles and proteins, the lysate was centrifuged at 14000 rpm for 2mins.
- 450µL of supernatant was transferred to new DNase free microcentrifuged tubes. Lanbelled each tube properly. Make sure that there was no contact with the pellet.
- 50µL of OSR solution was added to above supernatant containing tubes, mixed well and incubate on ice for 5mins.
- Then centrifuge at 14000 rpm for 2mins to pellet remaining proteins and soil particles.
- Without any contact with pellet 450µL of supernatant was transferred to humic

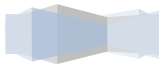
acid removal column (clear O-ring)

- The column was centrifuged at 8000 rpm for 1min. DNA was in flow through.
- 230µL of 96-100% ethanol was added directly to the flow through.
- Grey O-ring spin column along with collection tube was assembled. All of the lysate along with ethanol was added on to the column and centrifuged at 8000 rpm for 1min.
- Flow through was discarded and column was re-assembled with the collection tube.
- Buffer SK of about 500µL was added to the columns and centrifuge again for 1min at 8000 rpm. Make sure that whole wash solution has been passed and collected into the collection tube.
- Flow through was discarded and column was re-assembled with the collection tube.
- 500µL of wash solution A was added to the column and centrifuge again for 1min at 8000 rpm.
- Flow through was discarded and column was re-assembled with the collection tube.
- The column was spined at 14000 rpm for 2mins. Collection tubes were discarded and columns were placed into fresh 1.7ml elution tube and properly labeled each tube.
- Elusion buffer B of approx. 100µL was added to the column and incubated for 1min at room temperature.
- Centrifuge the elusion tubes at 8000 rpm for 1min.
- The purified genomic DNA was stored at 4^{o}C for a few days.

3.11.2 C-TAB method for DNA extraction

Geo et al., 2009(204) method of DNA extraction was followed for soil samples.

3.11.2.1 Preparation of reagents for DNA Extraction



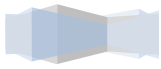
- DNA Extraction Buffer: 100mM/L, tris HCl pH 8,1.5mol/L NaCl, 1% cTAB.
- Proteinase K 100µL
- 20% SDS
- Chloroform isoamylalcohol (24:1,V/V)
- Chilled isopropanol
- TE buffer: 10mM/L tris HCl pH 8, 1mM/L EDTA pH 8

3.11.2.2 Steps of DNA extraction

- 5g of soil samples were added into 13.5ml of DNA extraction buffer and 100µL of proteinase K in centrifuge tubes.
- Shaked the centrifuge tubes horizontally at 225 rpm/min at 37^{o}C for 30mins.
- After shaking, 1.5ml of 20% SDS was mixed and incubate the samples for 2hrs at 65^{o}C in water bath.
- Invert the tubes after every 15-20mins.
- Centrifugation was done at 6000 rpm at room temperature for 10mins.
- Supernantant were separated into new DNase free microcentrifuge tubes.
- Equal volume of chloroform iso-amylalcohol were added.
- By centrifugation aqueous phase was recovered and precipitated with 0.6 volume of iso-propanol for 1 hr or over night at room temperature.
- Pellet was obtained by centrifugation for 20mins at 10,000 rpm.
- Resuspended the pellet in TE buffer.
- Concentration of DNA was then checked through nanodrop.

3.12 Pyrosequencing Analysis

DNA samples were send to Molecular Research (MRDNA) 503 Clovis Road Shallowater, Texas for 454 pyrosequencing analysis.



3.13 Scanning electron microscopy

Biofilm formed during enrichment stage 2 with activated sludge and soil(S3) on carbon cloth in anode chamber. A small piece of carbon cloth was cut with sterile scissors and placed in sterile buffer solution. The sample were sent to centralized resource laboratory, university of Peshawar, Khyber Pakhtunkhwa.The samples were fixed for 2 h in 2% (v/v) glutaraldehyde and washed three times, for 20 min per wash, in 0.1 M sodium cacodylate.The fixed samples were successively dehydrated with ethanol and stored overnight at 4°C in 100 % ethanol. These samples were dried by critical-point drying, coated with gold and examined with a scanning electron microscope at 20kV at different resolutions.

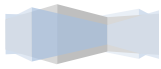
3.14 Microbial analysis

Samples were taken from anode chamber before and after enrichment in each experiment. Along with that microbial biofilm flora were also subjected to characterization and identification.

3.14.1 Sub-culturing of bacterial colonies

Morphologically different bacterial colonies appeared on nutrient agar plates. These colonies were further separated on nutrient agar plates. Sub-culturing was carried out until individual separated colonies were obtained. Plates were incubated in an

anaerobic jar for 24 hrs at 37^{o}C. Individually separated colonies were grown on different differential and selective media's for pure culture isolation. Differential and selective media used are: blood agar, MacConky agar. For the identification and characterization of these individual colonies, further biochemical tests, morphological characterization and microscopic analysis were carried out.



3.14.2 Morphological characterization

Pure culture bacterial colonies were differentiated initially on the basis of colony morphology. In colony morphology, different colony characteristics were taken into the consideration like as

Table 3.14.2(a) Morphological Characterization Comparision chart.

Colony Form:	Circular, Rhizoid, Irregular, Filamentous.
Colony Margins:	Undulate, Lobate, Entire, Erose.
Colony Elevation:	Raised, Flat, Convex.
Colony Size:	Pin pointed, Small, Medium, Large
Colony Texture:	Dry, Moist, slimy, Watery
Colony Opacity:	Transparent, Translucent, Opaque

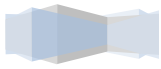
3.14.3 Microscopic analysis

Gram staining was done for the characterization of bacterial isolates as Gram positive and Gram negative.

3.14.3.1 Smear Preparation

Smear was prepared by adding a drop of saline solution on slide, small amount of colony was transferred to slide and spreaded evenly with the help of inocutating loop. Heat fixed the slide by gently passing over the flame.

3.14.3.2 Staining



Crystal violet was added on smear for 45-60sec and gently rinsed the slide with water. Iodine solution was added for 1min and then rinsed off gently with a stream of water. Few drops of decolorize were added for only 10-15secs. Time was noted with the help of stop watch. Washed the decolorized with water gently. At the end safranin as counter stain was added for 45secs. The slide was gently washed with water and let it to air dry for few mins.

3.14.3.3 Microscopy

The slide was observed under a light microscope by adding a drop of emulsion oil. Gram negative appeared Pink while Gram Positive microorganisms appeared Purple in color.

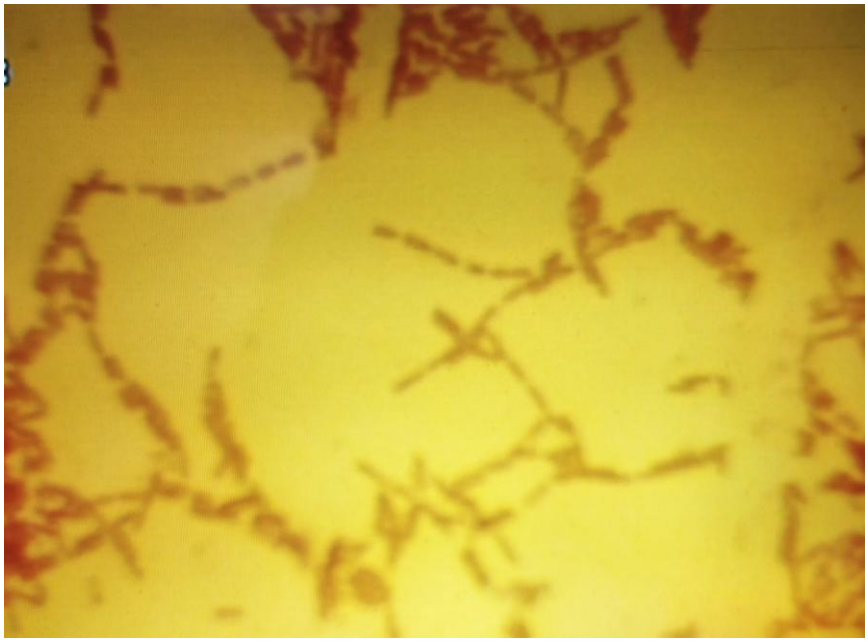


Fig 3.14.1 Gram negative rod shaped bacterial image in light microscope

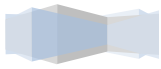
3.15 Microtiter Dish Biofilm Formation Assay(205, 206)

3.15.1Material Required

ISOLATED BACTERIAL STRAINS.
LB media or TGB (prepared according to manufacturer's recommendations).
0.1% crystal violet in water
70% ethanol
30% acetic acid in water
96 well microtiter plates
Microtiter- plate reader

3.15.2Procedure

- Bacterial strains were grown in a general purpose media over night.
- For biofilm formation assay overnight cultures were diluted 1:100 into fresh medium.
- 100µL of the dilutions were added in to 96 well plates.
- Incubate the microtiter plate for 24hrs at 37^{o}C.
- After incubation, cells were dumped out by turning the plate over and over.
- Gently rinsed the plates with water. This will help to remove and prevent from staining the media components and unattacted cells.
- 0.1% CV solution of about 125µL were added in each well of plates.
- Microtiter plates were incubated for 10-15mins at room temperature.
- Gently rinsed the plates 3-4 times with water.
- Microtiter plates were turned upside down and let the plates dry for a few hours or overnight.
- 125µL of 30% acetic acid were added in each well to solubilize the CV.
- Microtiter plates were incubated for 10-15mins at room temperature.
- Absorbance were taken in microtiter plate reader at 630nm.



3.16 Classification of Strains

According to Christensen et al. (1985) the isolates were divided into four categories(207, 208):

Table 3.16(a) average OD values to check the capability of biofilm formation by bacteria

Biofilm formation capability	Average OD values
non-adherent	(OD < ODc)
weakly-adherent	ODc < OD < 2xODc)
moderately-adherent	(2xODc < OD < 4xODc)
strongly-adherent	(4xODc < OD)

While Optical density cut-offvalue is calculated from the following formula(209):

ODc= Average OD of negative control+ 3x Standard deviation

3.17 Biochemical testing

Different biochemical tests were performed by conventional methods for further characterization of microbial isolates.

3.17.1 Oxidase test

A small piece of filter paper was soaked in 1% Kovács oxidase reagent. With the help of wire loop small well isolated colony was picked and rubbed onto oxidase reagent containing filter paper. The color change was observed.

Oxidase Positive Microorganisms

Dark Purple Color appeared within 5 to 10sec.

Late Oxidase Positives

Apperance of Purple color in 60 to 90sec.

Oxidase Negative Microorganisms No color change or change of color after 2mins.

3.17.2 Catalase test

Adrop of hydrogen peroxide was put on to the slide with the help of dropper. A signle bacterial isolate was taken with wire loop and inoculated in to the drop of hydrogen peroxide.

Positive results

In case of catalase positive bubbles were formed.

Negative results

While in negative no bubble formation was observed.

3.17.3 H2S and Indole Test

Kovac's Reagent Preparation

50ml of Hydrochloric acid was mixed with 10g of p-dimethylamine benzaldehyde and 150ml of Amyl Alcohol.

Method Used

SIM agar tubes were prepared (According to Manufacturar's Recommendations). Inoculation of individual bacterial isolates was done into SIM agar tubes. Test tubes were incubated at 37^{o}C for 24hrs. The microbial ability to degrade tryptophane amino acid that was converted into indole pyruvic acid and ammonia was tested in this tested. Indole presence was inveterated by the addition of Kovac's reagent.

Indole Positive Reaction

cherry red color indicates the presence of indole.

Indole Negative Reaction

No cherry red appearance was observed.

Hydrogen Sulphide production

H2S production was confirmed by the appearance of black precipitates along the slab inoculation line.

Motility Indication

Motile organisms were moved away from inoculating side and were indicated by the foggy appearance away from inoculating line.

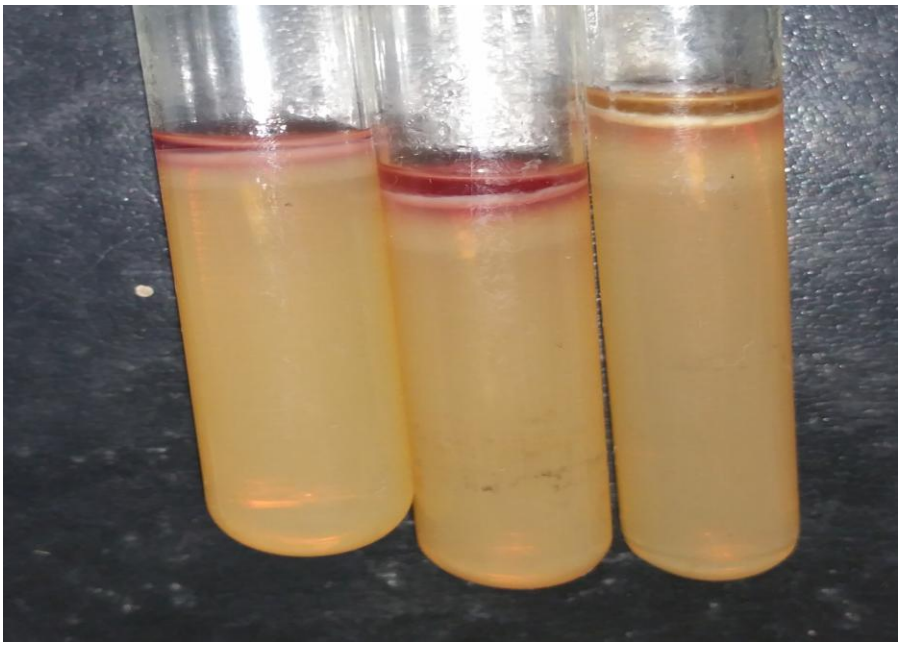


Fig 3.17.1 SIM image

3.18.4 Triple Sugar Iron Test (Glucose/Lactose fermentation)

TSI agar slants were prepared after autoclaving the media and test tubes at 121^{o}C for 20mins. By stab-streak inoculation bacterial colonies were inoculated into TSI slants. Test tubes were incubated at 37^{o}C for 24hrs.

Slant Color	
RED (R)	No lactose/Glucose Fermentation
YELLOW (Y)	Lactose or Sucrose Fermentation

Butt Color	
RED (R)	No fermentation, The Bacterium was obligate aerobe
YELLOW (Y)	Some fermentation occurs. Facultative anaerobes.
GAS FORMATION (YG)	Agar was cracked at some places or butt may be pushed out of the tube.
BLACK	H2S production

3.19 API 20E Kits

Api 20E kits were used for the quick biochemical identification of strong and moderate biofilm formers.

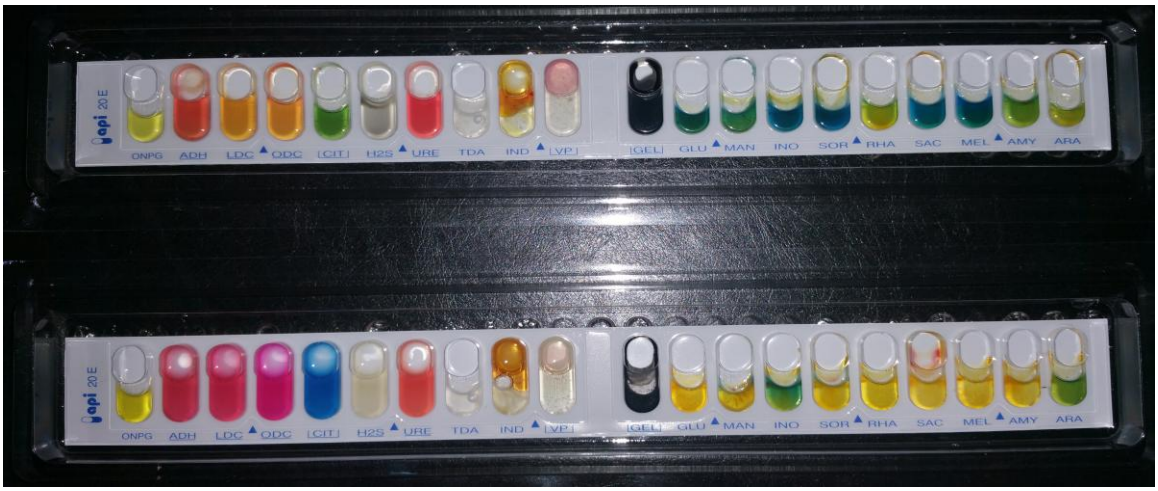
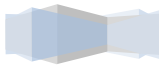


Fig 3.19.1 Api 20E Images showing positive and negative results

CHAPTER# 4

RESULTS



Results

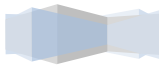
4.1 Enrichment Of Electrogenic Bacteria From activated Sludge In Sucrose Fed Salt Bridge MFC

In first experiment, salt bridged Microbial fuel cell was inoculated with activated Sludge as a bacterial source with a feed solution containing sucrose as a sole carbon source and operated at 37^{o}C. Maximum Voltage generated before and after enrichment was 347mV and 74.9mV. Subsequently maximum current produced was 0.115mA and 0.024mA respectively.

4.1.AEnrichment Stage 1

4.1.1A Voltage Data Analysis

An open circuit potential of about 107.5mV was recorded within few hours of inoculation. This open circuit potential development showed that electrochemically active microbial consortia were present in the sludge. When the circuit was connected through 3kΩ resistor, the voltage was dropped to 54.5mV which correspond to a current of 0.018mA. Maximum voltage of about 347 across 3kΩ resistor was observed on 11^{th} day of operation as shown in graph 4.1.1a. Another sharp increase in voltage was observed on 17^{th} day to about 274mV after that voltage dropped to 18.1mV. It has been observed that a large time that microorganisms took to reach the maximum voltage. Initially small rise in voltage to about 109mV was seen on 2^{nd} day but it started to decline till 10^{th} day of working of fuel cell.



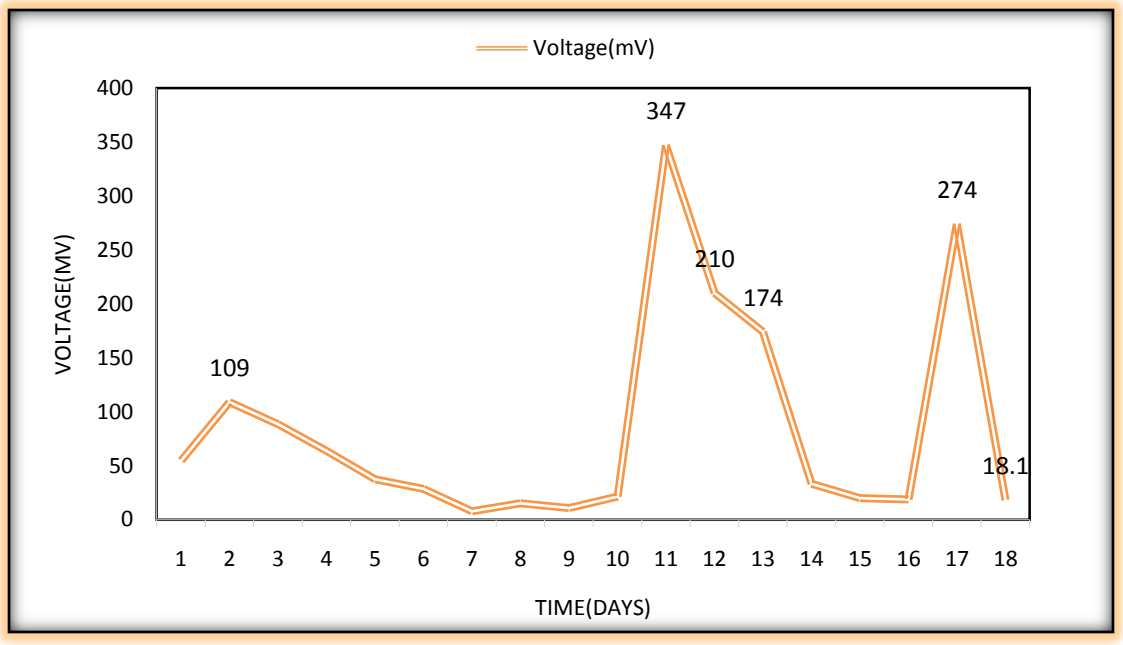


Fig. 4.1.1(a). Voltage obtaing with activated sludge fed with sucrose in salt bridge double chamber microbial fuel cell(Appendix A)

4.1.2A Effect Of External Resistance On Voltage Generation

To check the effect of resistance on voltage production, different high and low resistor than 3kΩ were connected through the circuit. It has been observed that when the 10Ω resistor was connected, minimum voltage of 0.5mV of the cell was generated. Subsequently, by increasing the resistance step wise the voltage was also enhanced gradually. At 100Ω the voltage of 17.2mV while at 170Ω, 32.4mV was recorded.

When the resistance increased from 3kΩ to 100kΩ, the voltage was also increased from 347mV to 597mV on 11th day as shown in graph 4.1.3a, on the very same day maximum voltage was recorded across 3kΩ resistor. By Ohm's Law:

V=I*R. So, by keeping the Current (I) constant, Voltage (V) is directly proportional to Resistance (R). With increase in resistance, voltage would also be increased.

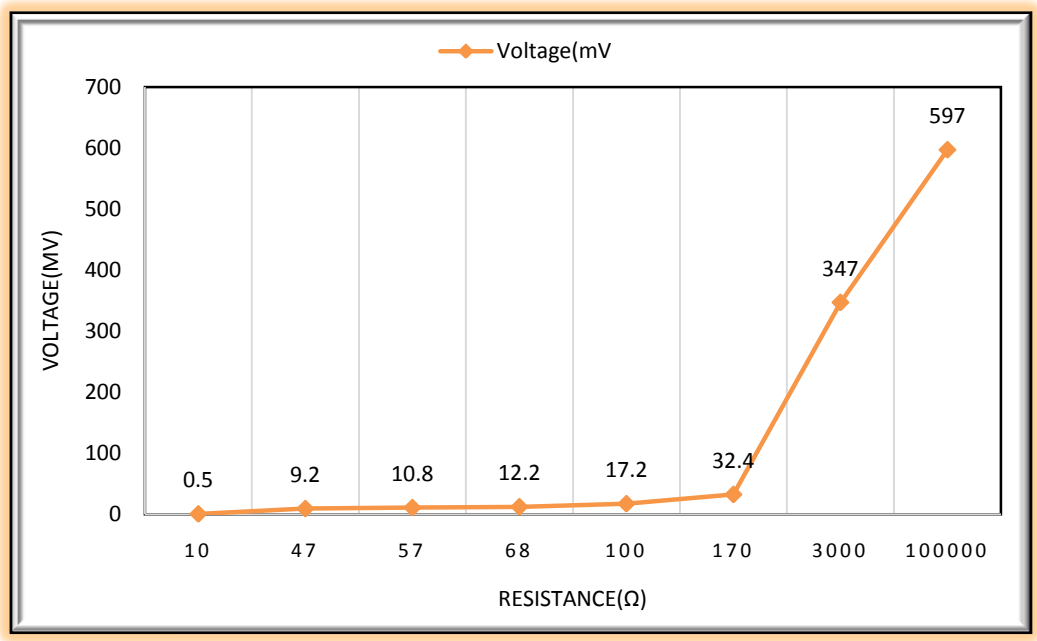


Fig 4.1.2(a). effect of external resistance on voltage production at enrichment stage 1 of electrogenic bacteria from activated sludge fed with Sucrose in salt bridge double chamber microbial fuel cell(Appendix B)

4.1.4A Polarization Curve

To analyze the effect of resistance on current and voltage simultaneously, a polarization curve was drawn as shown in fig 4.1.4(a). Maximum current 0.19mA was generated across 47Ω. Mainly current decreases with increase in resistance while conversely, voltage increases with increase in resistance. But some anomalous behavior was also been observed at some points like as initially when 10Ω resistor was connected,0.05mA of current generated as compared to the current produced at 47Ω resistor.

Secondly, when resistor was switched from 100Ω to 170Ω the current was increased from 0.172mA to 0.19mA. While, the voltage was continuously increasing with increase in resistance. Current production was drastically decreased with increase in resistance after 170Ω resistance. Minimum current of 0.005mA at 100kΩ was observed. As in Ohm's Law Voltage (V) is directly proportional to Resistance (R) but the Current (I) is inversely proportional to resistance. I=V/R

Therefore, increased resistance, drop in current production has been observed. At 100kΩ resistance, the current was dropped to 0.00597mA while maximum voltage of 597mV was recorded.

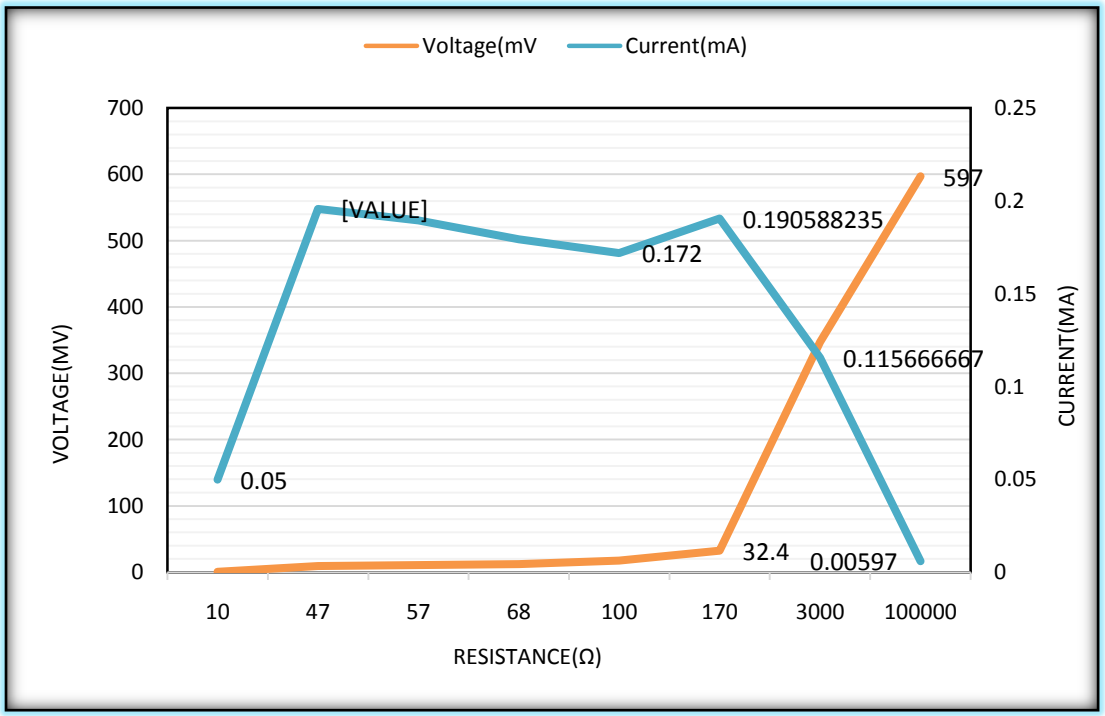


Fig 4.1.3(a). polarization curve at enrichment stage 1 of electrogenic bacteria from activated sludge in sucrose fed salt bridge double chamber microbial fuel cell

4.1.5A Current Vs Voltage At Stage 1 of Enrichment In Double Chamber Salt Bridge Microbial fuel Cell

With increase in voltage, current was also increased as shown in fig 4.1.5a. 0.115mA of maximum current was observed at 347mV voltage on 11th day of working of fuel cell. Second maximum current was observed when voltage reached up to 274mV, showing current value of about 0.0913mA. Initially, a little bit increase in current production from 0.018mA to 0.036mA was obtained. Then the current and voltage starts to decrease constantly up to 0.0007mA and 21.5mV respectively. Then suddenly, a sharp increase in voltage and current production was observed showing maximum values 0.115mA Vs 274mV. After showing maximum potential, voltage and current started to decrease slowly. Second abrupt rise and fall was observed on 16th to 18th day showing current of 0.09mA and voltage of 274mV.

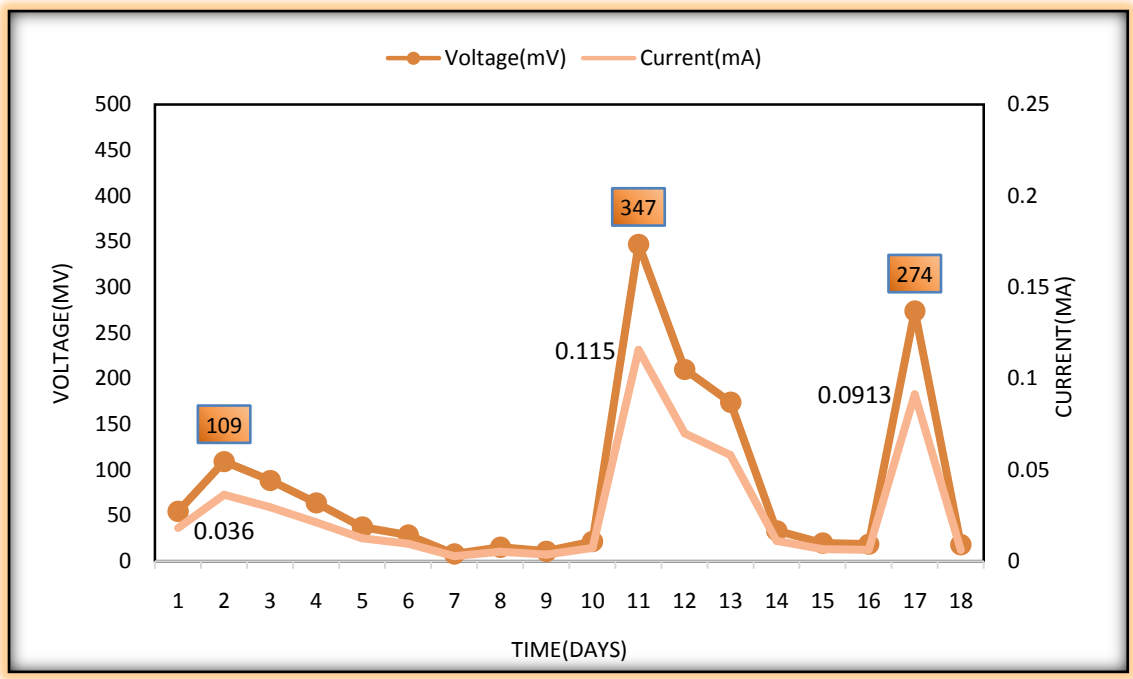


Fig.4.1.4(a). Current Vs Voltage at enrichment stage 1 of electrogenic bacteria from activated sludge in sucrose fed double chamber microbial fuel cell(Appendix D)

4.1.6A Voltage and Power Relationship

To determine the linkage between power and voltage, a curve was drawn from the recorded data and analyzed. It has been observed that Power, voltage trend was same as seen with voltage and current. First rising peak of voltage and power were seen on second day at the start of experiment. It was about 3.96µW at 109mV then a decline phase was observed till 10th day of operation. A threshold peak was analyzed on 11th day, maximum Power of 40.136µW at maximum voltage 347mV produced while keeping the resistance constant at3kΩ throughout the experiment. A third rising peak was analyzed at the end of experiment on 17th day and the power of 25.02µW was produced at 247mV voltage. Then, again started to decrease up to 0.109µW at 18.1mV. The minimum voltage (10.8mV) and minimum power of 0.0388µW was recorded on 9th day of experiment across 3kΩ resistor.

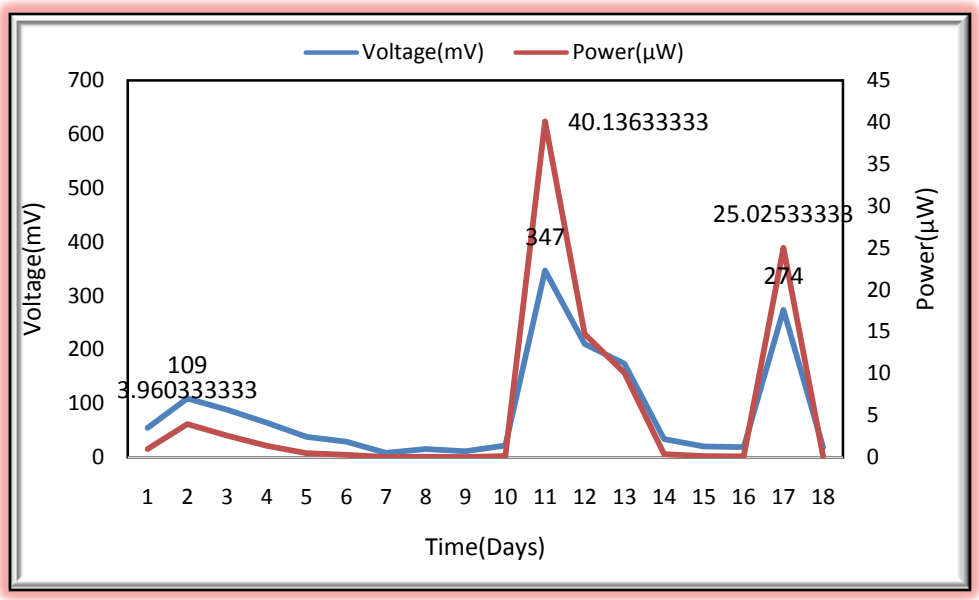


Fig 4.1.5(a). Voltage and power analysis curve at enrichment stage 1 of electrogenic bacteria from activated sludge in double chamber microbial fuel cell (Appendix E)

4.1.7A Current and Power density Relationship

It has been observed that with increase in current production, power density was also increased. Maximum power density produced was 0.00802μW/m^2 at 0.115mA current. Current and power density are directly proportional to each other. With increase in one variable other variable increased automatically.As observed with current and voltage, voltage and power graph, similar pattern was noticed with current and power density graph as shown in fig. 4.1.7(a).

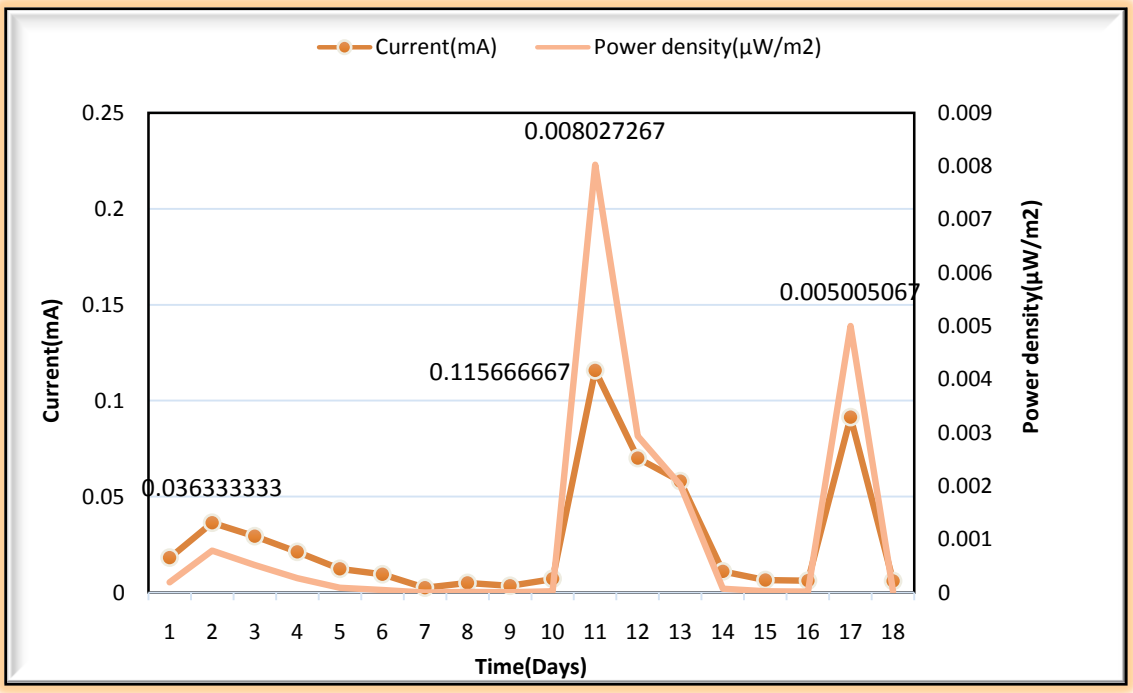


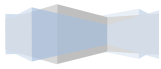
Fig 4.1.7(a). current and power density relationship at enrichment stage 1 of electrogenic bacteria from activated sludge in salt bridge double chamber microbial fuel cell (Appendix F)

4.2B Stage 2 Of Enrichment with Activated Sludge In Sucrose fed Salt Bridge Double Chamber Microbial Fuel Cell

After 18 days, the cell was enriched by successively transfering10% of previous inoculum from stage 1 of enrichment to fresh anolyte in new salt bridgedouble chamber microbial fuel cell. The cell was placed in incubator at 37^{o}C and data was recorded for next 18 days.

4.2.1B Voltage Data Analysis

After few hours of inoculation open circuit voltage was measured that was around 98.2mV. The cell's anodic solution pH was 7.03 when inoculated. The circuit was closed by applying 3kΩ resistor across the circuit. By applying resistance, initially the voltage drops then started to increase. Voltage data was recorded for 18days. The maximum voltage recorded at stage 2 of enrichment was about 74.9mV on 4th day. Voltage obtained at stage 2 was comparatively lower than at stage 1of enrichment. Initially, there was slow rise in voltage production from 44.3mV to 52.3mV reaching the maximum voltage potential of 74.9mV. After reaching the highest potential voltage drops constantly. The resistance was kept constant throughout the experiment at 3kΩ unless mentioned. The minimum voltage generated at stage 2 of enrichment across 3kΩ was 4.3mV in sucrose fed double chamber microbial fuel cell.



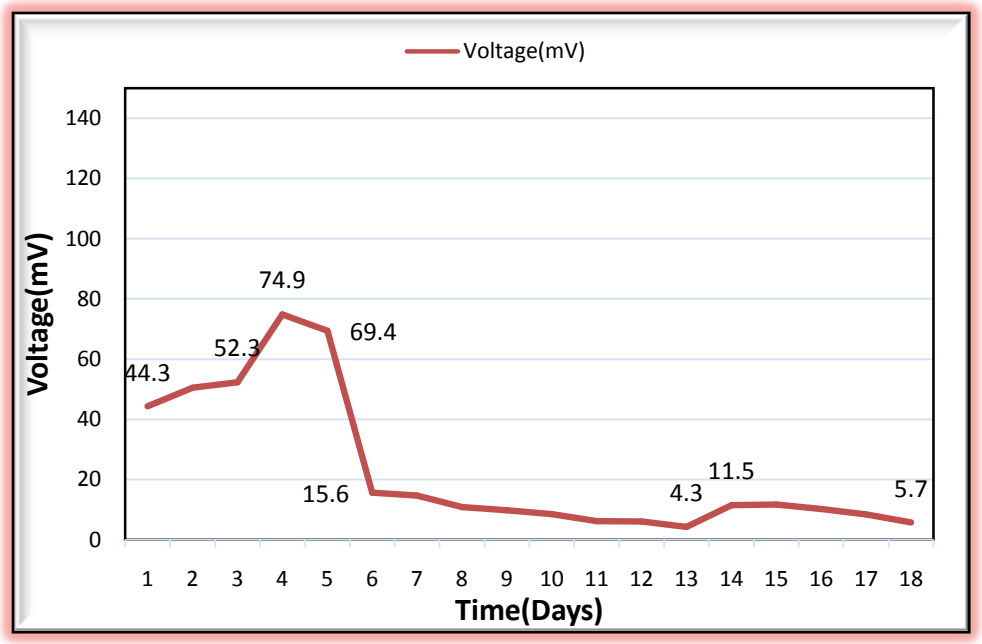


Fig 4.2.1(b). Voltage recorded at enrichment stage 2 of electrogenic bacteria from actvated sludge in double chamber microbial fuel cell (Appendix A1)

4.2.2B Effect Of Resistance On Voltage Production

To check the effect of resistance on voltage production and to find the differences in voltage generation across different resistors at stage 1 and 2 of enrichment. Resistors of 10Ω, 47Ω, 57Ω, 68Ω, 100Ω, 170Ω, 3kΩ, and 100kΩ were connected one by one and voltage data was recorded by pausing at each resistor for at leaset 20min. Voltage increased with increase in resistance same as observed at stage 1. Maximum voltage obtained at 100kΩ was about 345.7mV on 4^{th} day of operation of fuel cell. At 10Ω minimum voltage (0.1mV) was produced while as the resistance was increased voltage was also increased steadily and gradually. As noticed earlier at 10Ω, 0.5mV was generated at stage 1 while at stage 2 it was further decreased to 0.1mV. At 100Ω, 2.5mVs were generated while it was about 17.2mV at stage 1 of enrichment.

When resistor switched from 3kΩ to 100kΩ the voltage was increased about four and half times from 74.9mV to 345.7mV. While before enrichment upon switching of resistors, one and half times increase in voltage was recorded from 347mV to 597mV.

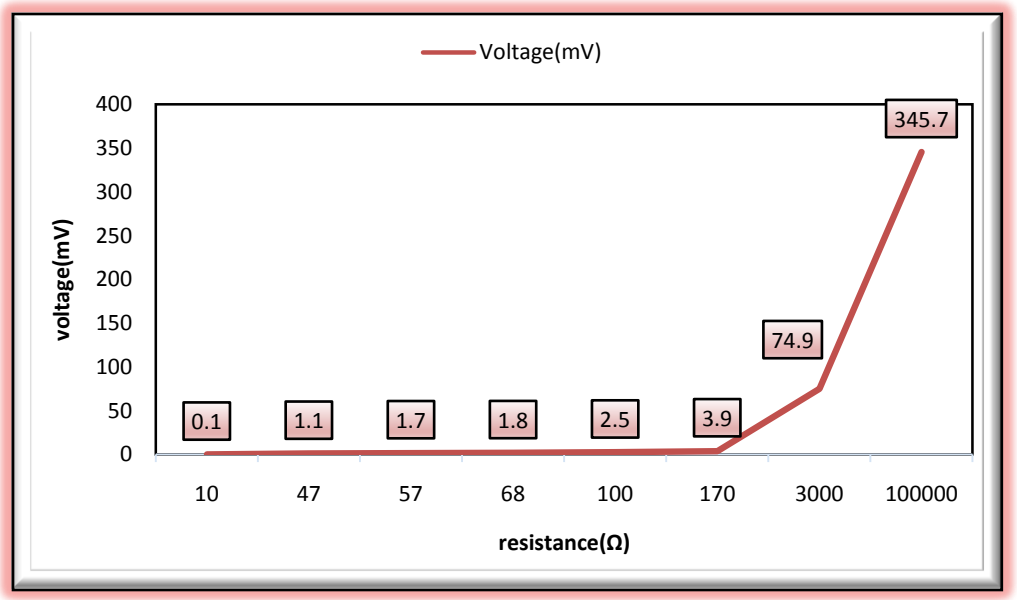


Fig 4.2.2(b). Effect of resistance on voltage generation at enrichment stage 2 of electrogenic bacteria from activated sludge in sucrose fed salt bridge double chamber microbial fuel cell (Appendix B2)

4.2.3B Polarization Curve

To determine the effect of resistance on current and voltage, a polarization curve was drawn as shown in fig.4.2.3(b). At start of cycle a little fluctuations were seen in current generation. A maximum current of 0.0298mA was recorded across 57Ω resistor. As described earlier with increase in resistance, current would decreased according to ohm's Law. As the resistance was increased to 100kΩ from 3kΩ, current was decreased to 0.0034mA from 0.024mA while alternately maximum voltage 347.5mV was produced. In the 1st part of experiment at stage 1 of enrichment, maximum current 0.195mA was produced at 47Ω. While at stage 2, maximum current of 0.0298mA was recorded,

when circuit was closed by connecting 57Ω resistor. But generally, it has been observed that current decreases with increase in resistance while voltage was increased with increase in resistance in both stages of experiment.

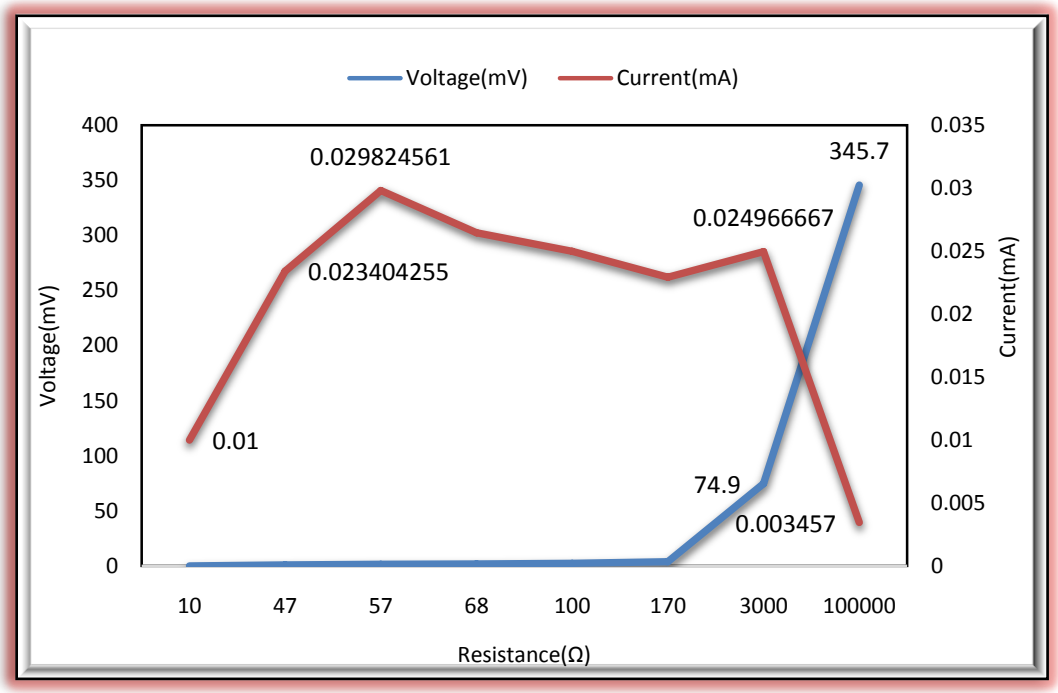


Fig 4.2.3(b). Polarization curve between current, voltage and resistance at enrichment stage 2 of electrogenic bacteriafrom activated sludge in double chamber microbial fuel cell (Appendix C2)

4.2.4B Current and Voltage in one relationship

Alternatively, with increase in voltage current was also been increased by keeping the resistance constant at 3kΩ. As shown in fig.4.2.4(b), with increase in voltage from 44.3mV to 74.9mV from day 1 to day 4, current was also increased from 0.014mA to

0.024mV across 3kΩ resistor. Maximum current of 0.024mA at a maximum voltage of 74.9mV was recorded. Minimum current generated across 3kΩ was of 0.001mA at minimum voltage of4.3mV throughout the operation of fuel cell at stage 2 of enrichment in sucrose fed salt bridge double chamber microbial fuel cell.

At 3kΩ, maximum current and voltage generated at stage 1 was about 0.115mA at 347mV, while maximum current and voltage recorded at stage 2 was about 0.024mA at 74.9 mV.

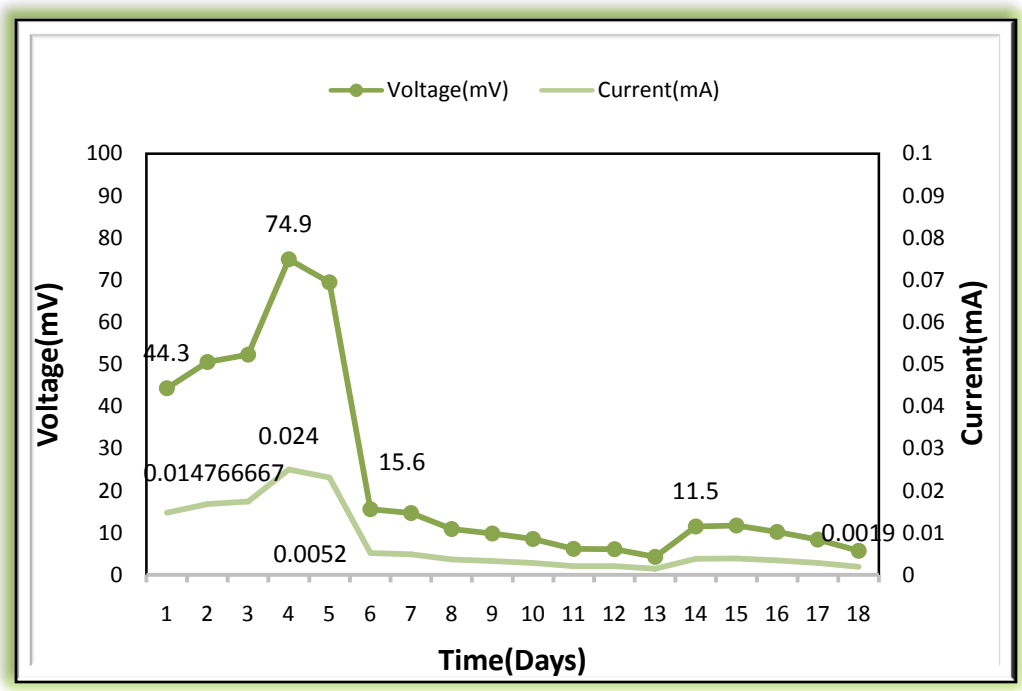


Fig 4.2.4(b). Voltage, current relationship at enrichment stage 2 of electrogenic bacteria from activated sludge in salt bridge double chamber microbial fuel cell (Appendix D2)

4.2.5B Voltage and Power Relationship

It has been observed that voltage and power have strong relationship, with increase in voltage Power was also been increased. At the start of experiment, voltage and power slowly increases. Initially, voltage and power of 44.3mV and 0.654µW was recorded. A sharp rise was seen after 3rd day of operation, reaching maximum potential of cell.

Maximum Power of about 1.87µW at maximum voltage of 74.9mV was observedin fuel cell at stage 2. After maximum potential a long decline phase was observed. Minimum power potential of 0.006µW was recorded during the experiment.

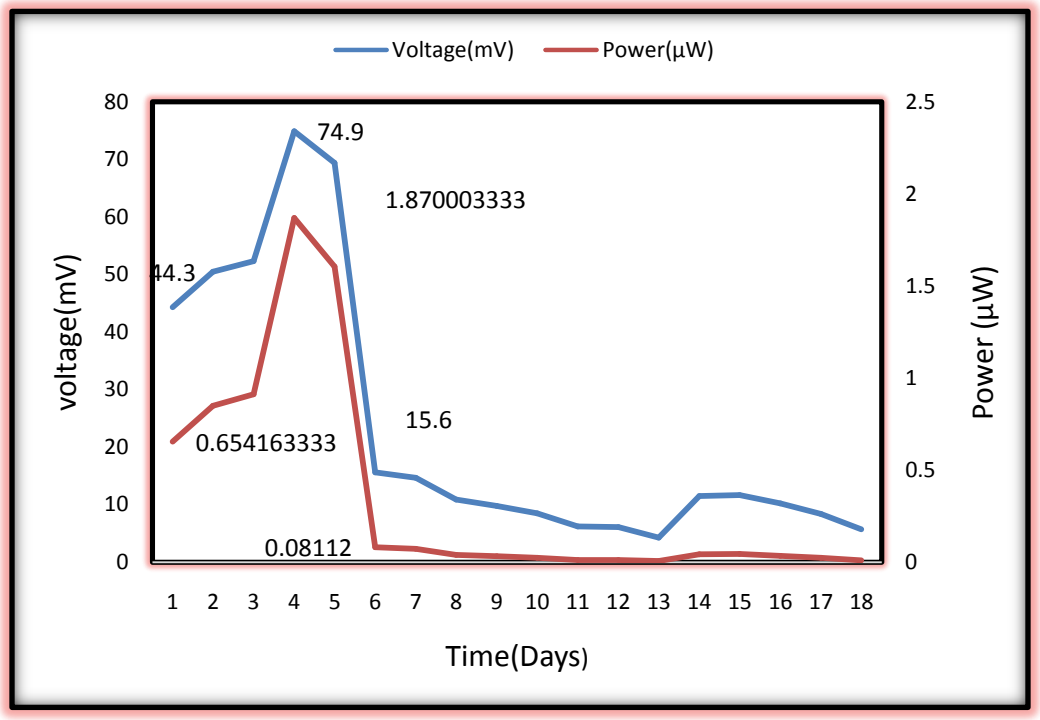
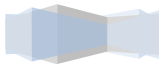


Fig 4.2.5(b). Voltage and power relationship at enrichment stage 2 of electrogenic bacteria from activated sludge in sucrose fed double chamber MFC (Appendix E2)

4.2.6B Current and Power density relationship

As seen in 1st stage of enrichment, with increase in current, power density was also been increased. Same trend was observed in second stage of enrichment. Current and Power density are directly proportional to each other.At the start of experiment, current increases constantly reaching its maximum value 0.024mA. Like as current, same behaviour was observed with power density. Maximum power density 0.0037µW/m2 was produced at maximum current 0.024mA on 4th day of experiment at 2nd stage of enrichment in double chamber salt bridge microbial fue cell.

Maximum Power density and current produced in stage 1 was 0.0080µW/m2 and 0.115mA across 3kΩ respectively. Consequently maximum power density recorded at stage 2 of enrichment was of 0.003µW/m2, which was comparatively less.



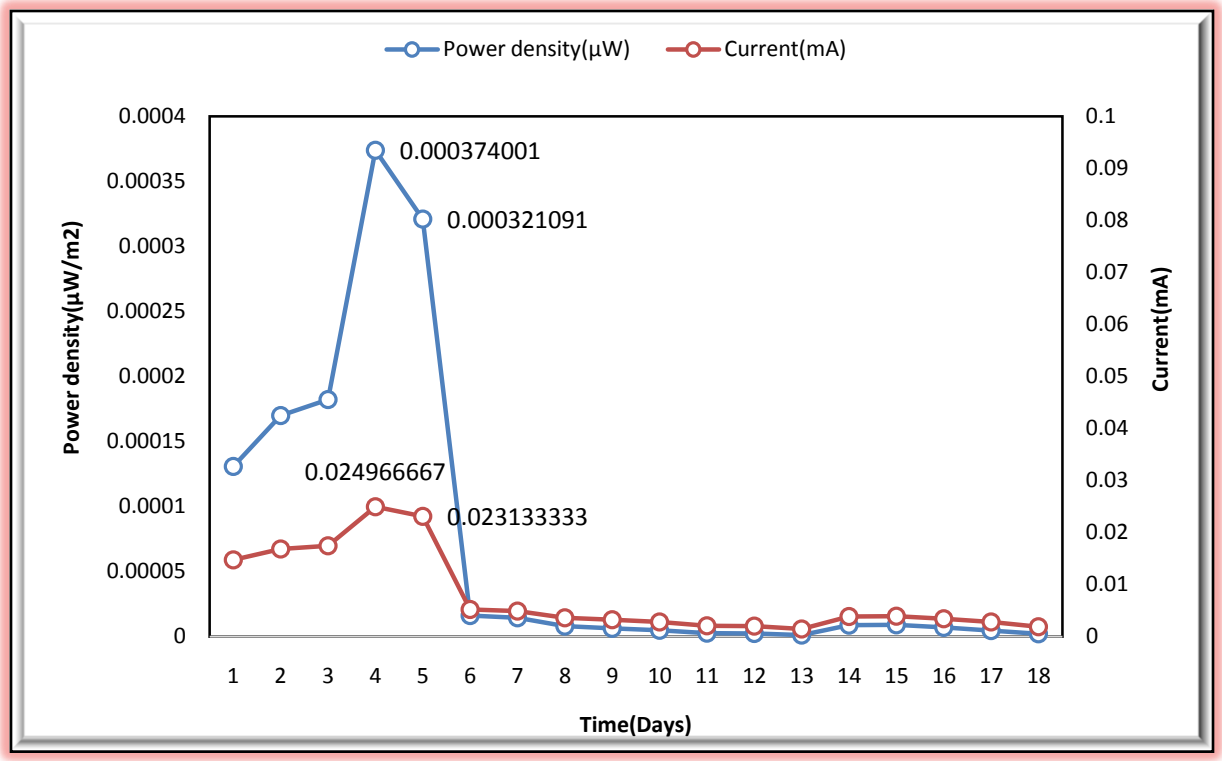


Fig 4.2.6(b). Power density Vs Current at enrichment stage 2 of electrogenic bacteria from activated sludge in sucrose fed double chamber microbial fuel cell (Appendix F2)

4.2.7 COD removal efficiency

Chemical oxygen demand (COD) was monitored to enumerate the probability of microbial fuel cells to function as unit for wastewater treatment. It has been observed that on 18th day of operation, %age COD removal efficiency reached up to 86.045% which indicates the role of bacteria in metabolizing the source of carbon as electron donor. Experimental data showed that COD removal and current production were relatively compatible.

Table 4.2.7(a). COD Removal efficiency during operation at stage 1 in sucrose fed salt bridge double chamber MFC

Days	COD_{int}(mg/l)	COD_{out}(mg/l)	%age CODRemoval efficiency
1-5	1555	979	37.0418
6-13	1555	517	66.75241
14-18	1555	217	86.04502

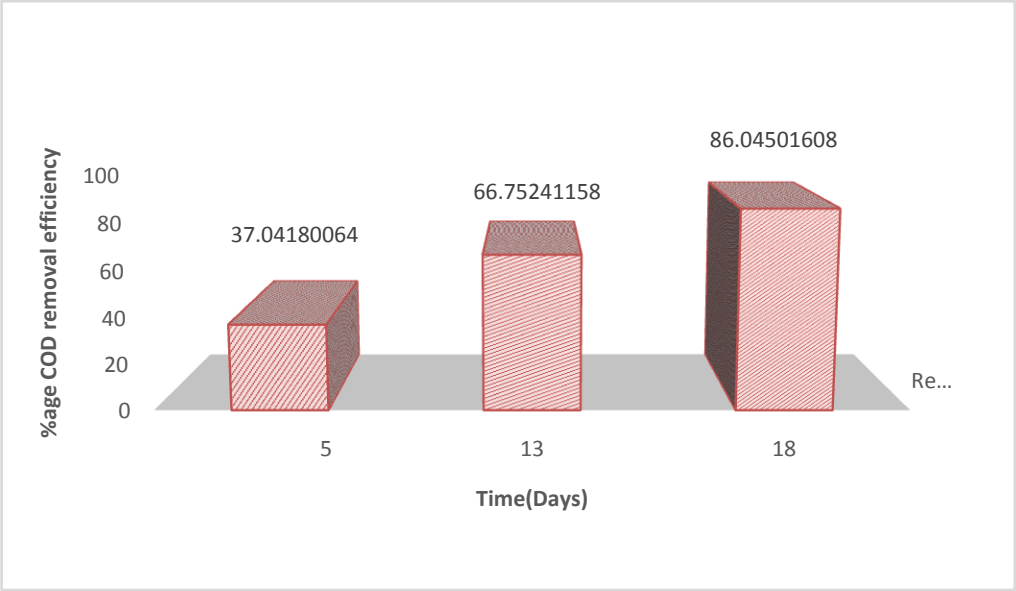


Fig 4.2.7(b). %age COD removal efficiency at enrichment stage 1 of electrogenic bacteria from activated sludge in sucrose fed double chamber microbial fuel cell

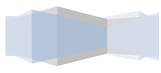
4.2.8%age COD Removal Efficiency at Enrichment Stage 2

At stage 2 of enrichment, COD was also monitored and it has been observed that at the end

of experiment %age COD removal efficiency was 77.85%, which indicates that microbes are efficiently metabolizing the electron donor organic contents in waste water. But COD removal efficiency was about 8 times less after enrichment.

Table 4.2.8(a) %age COD removal efficiency at enrichment stage 2 in sucrose fed salt bridge double chamber MFC

Days	COD_{int}(mg/l)	COD_{out}(mg/l)	%age COD removal efficiency
5	971	417	57.05458
13	971	310	68.07415
18	971	215	77.85788



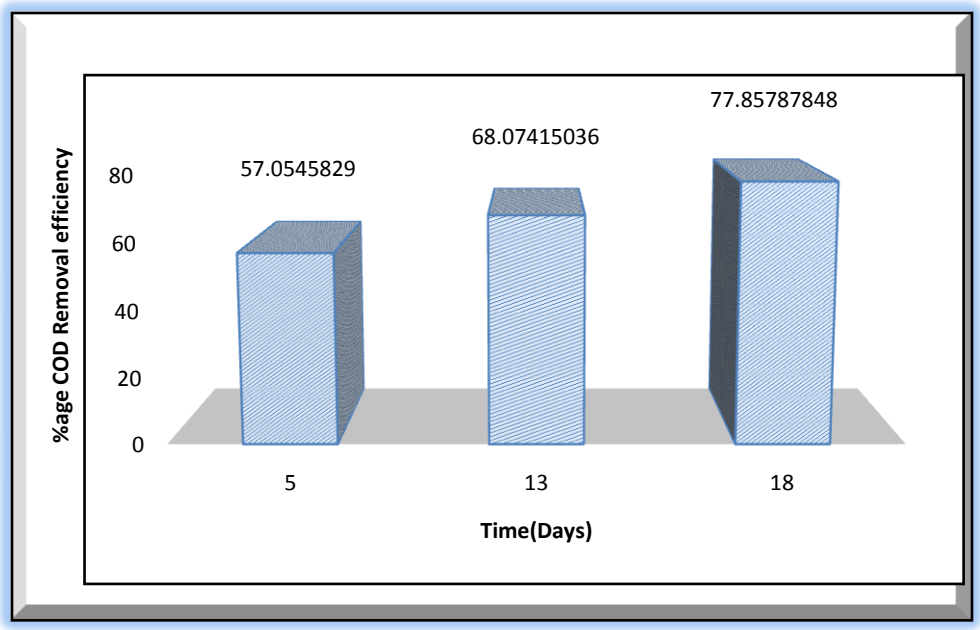
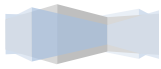


Fig 4.2.8(b). %age COD removal efficiency at enrichment stage 2 of electricigens from activated sludge in sucrose fed double chamber salt bridge microbial fuel cell

4.3 Enrichment Of Electrogenic Bacteria From Activated Sludge Supplemented With Potassium Acetate In Salt Bridge Double Chamber Microbial Fuel Cell

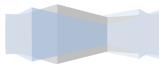
Second salt bridge double chamber microbial fuel cell was operated with activated sludge fed with potassium acetate as sole carbon source. Cell was run for 16 days, data recorded and analyzed further. In second experiment maximum voltage obtained was 28.8mV and 24mV in 1st and 2nd stage of enrichment, while current of about 0.096mA and 0.024mA respectively was recorded in double chamber salt bridge microbial fuel cell.



4.3A Enrichment Stage 1 In Acetate Fed Microbial Fuel Cell

4.3.1A Voltage Data Analysis

After few hours of inoculation open circuit voltage reading was observed but the cell was not showing any voltage generation. The cell was incubated at 37^{o}C for two days and let the microbial consortia to establish. After two days, open circuit voltage (OCV) was measured with the help of precision multimeter (UT33C; UNI-T). OCV was found to be 229mV. After OCV generation the circuit was closed by connecting through 3kΩ resistor and voltage generation was recorded for 16 days. It has been observed that after connecting the circuit through resistance the voltage drops to about 19.8mV. Voltage started to increase to about 22.5mV on 2nd day which increased to 28.8mV on 3rd day of operation. The maximum voltage generated during 16 days of working was about 28.8mV. After maximum voltage generation, voltage started to decrease till 5th day. Then again rise in voltage was observed. Voltage raised to about from 2.6mV to 8.7mV on 6th day of operation. After that again a decreasing trend was found till 13th day and this time voltage dropped to 0.8mV. From onwards increasing trend in voltage generation was analyzed. The voltage increased to about 21.2mV on 16th day working of fuel cell.



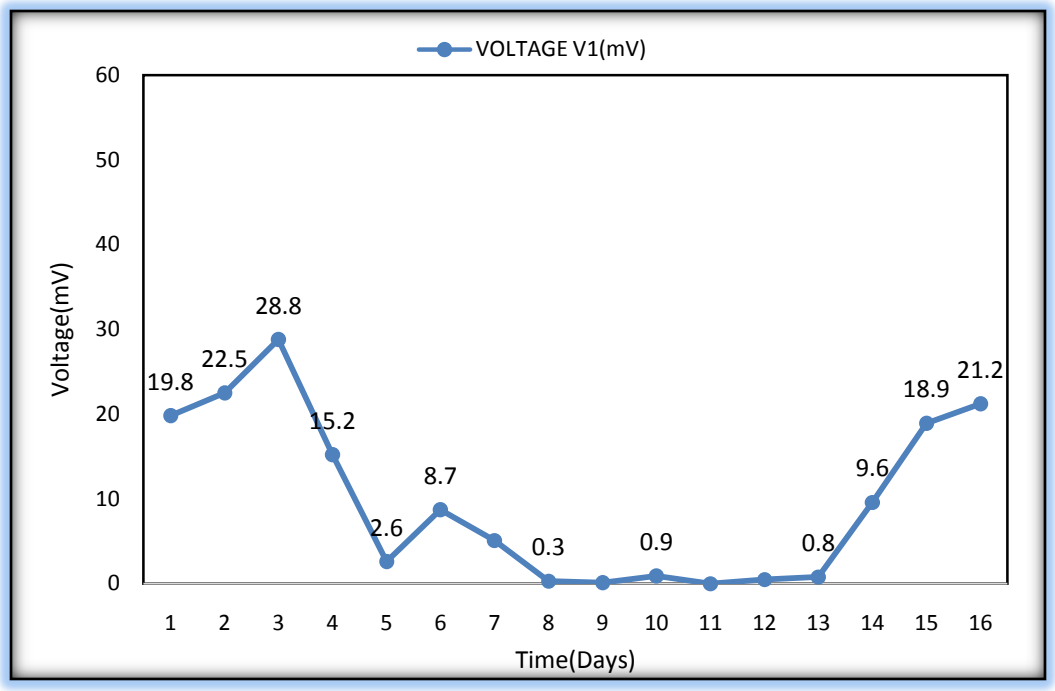


Fig 4.3.1(a). Voltage generation at enrichment stage 1 of electrogenic bacteria from activated sludge in K acetate fed double chamber salt bridge microbial fuel cell(Appendix G)

4.3.2A Effect Of Resistance On Voltage Generation

To check the effect of resistance on voltage generation different resistors (10Ω, 47 Ω, 57 Ω, 68 Ω, 100 Ω, 170 Ω, 3kΩ, and 100kΩ) were connected through the circuit. Voltage data was recorded by pausing at each resistor for about half an hour to get the stabilized readings. It has been observed that with increase in resistance, voltage was also been increased as reported in literature.

It has been observed that when 10Ω resistor was connected the circuit only 0.1mV voltage produced. With increase in resistance voltage was also been increased, as at 47Ω

voltage of 0.3mV while at 57Ω and 68Ω voltage of about o.3mV was generated. Further increase in resistance to 100Ω the voltage of about 0.6 was recorded. At 170Ω, 1.2mV while at 3kΩ voltage increased to 28.8mV which was the maximum voltage generated

during satge 1 of enrichment with potassium acetate as carbon source. To check the effect of higher resistance than 3kΩ, resistor of 100kΩ was connected. A tremendous increase in voltage to about 136.2mV was observed as shown in fig. 4.2.2(a).

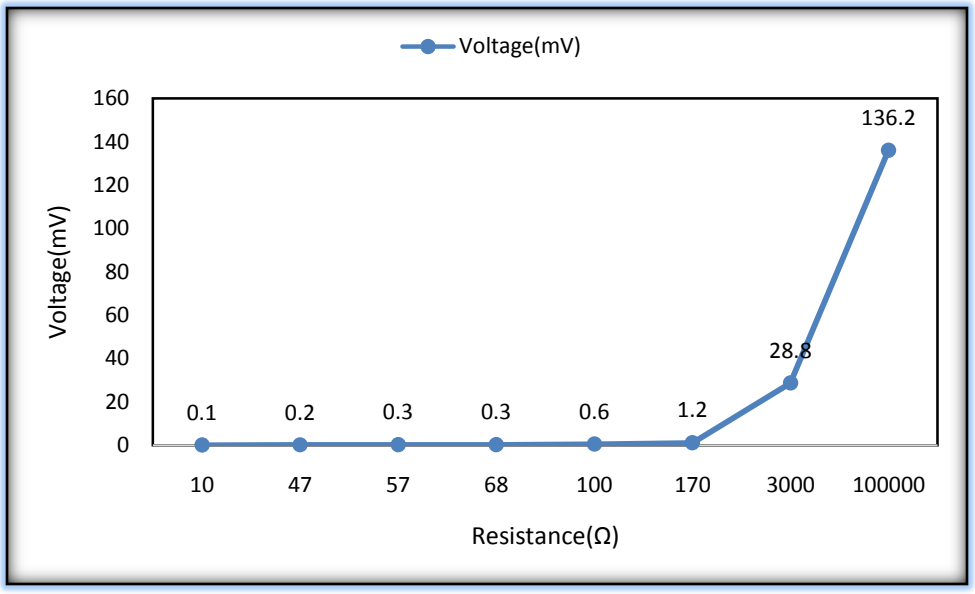


Fig 4.3.2(a). Effect of different resistors on voltage generation at enrichment stage 1 of electrogenic bacteria from activated sludge fed with K acetate in salt bridge double chamber microbial fuel cell(Appendix H)

4.3.3A Polarization Curve

Effect of resistance on current and voltage was observed simultaneously by drawing a polarization curve. As it has been observed that with increase in resistance, voltage was also increased. While maximum current of 0.01mA was generated at 10Ω.

Current dropped with increase in resistance. Current decreased from 0.01mA to 0.004mA from 10Ω to 47Ω. Anomalous behavior was observed from 68Ω to 3kΩ,

current generation increased from 0.004mA to 0.009mA. At 100kΩ the current was again dropped to about 0.001mA.

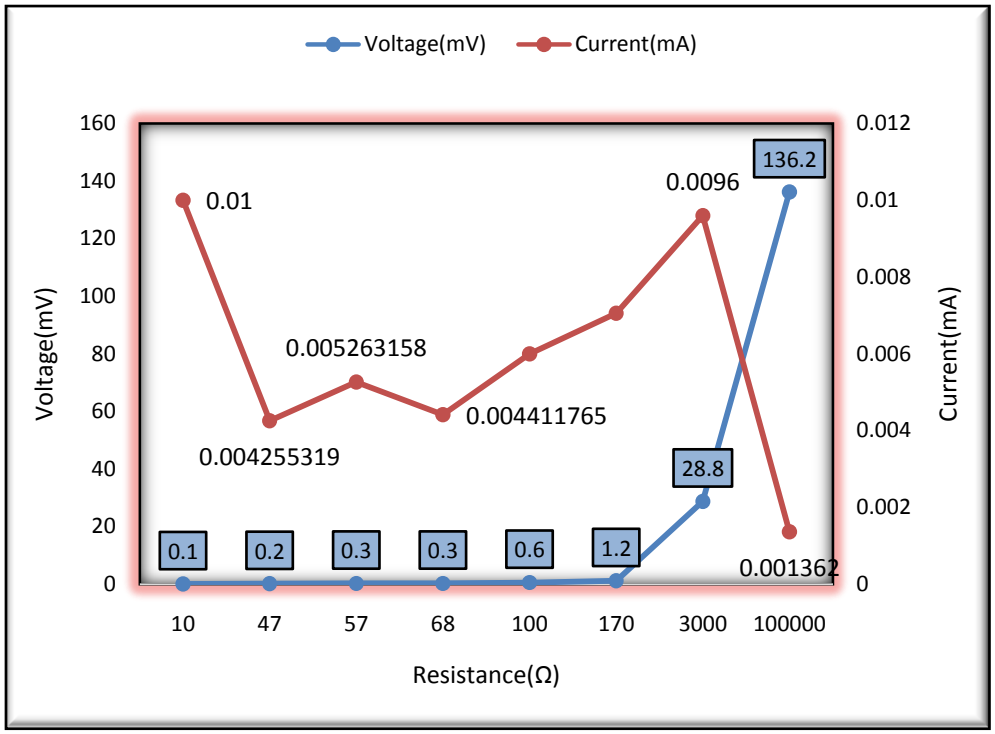


Fig 4.3.3(a). Polarization curve between Resistance, Voltage, and Current at enrichment stage 1 of electrogenic bacteria fro activated sludge fed with K acetate in double chamber salt bridged microbial fuel cell (Appendix I)

4.3.4AVoltage Vs Current Relationship

Voltage and current effect was observed by keeping the resistance constant at 3kΩ. It has been observed that voltage and current are directly proportional to each other. With increase in voltage current was also been increased as shown in fig 4.2.4(a).

Initially, current and voltage increased continuously till 3rd day of operation. Voltage increased from 19.8mV to 28.8mV while current increased from 0.006mA to 0.0096mA.

Maximum current generated during 1st stage of enrichment was about 0.0096mA. After reaching a maximum value, voltage and current drops to 2.6mV and 0.00086mA respectively. Again, an increasing peak was observed on 6th day. Voltage increased from 2.6mV to 8.7mA while current increased from 0.00086mA to 0.0029mA. Third time rising peak was observed on 13th day till 16th day. On last day of working of fuel cell. maximum current production was measured to about 0.007mA while voltage of about 21.2mV respectively.

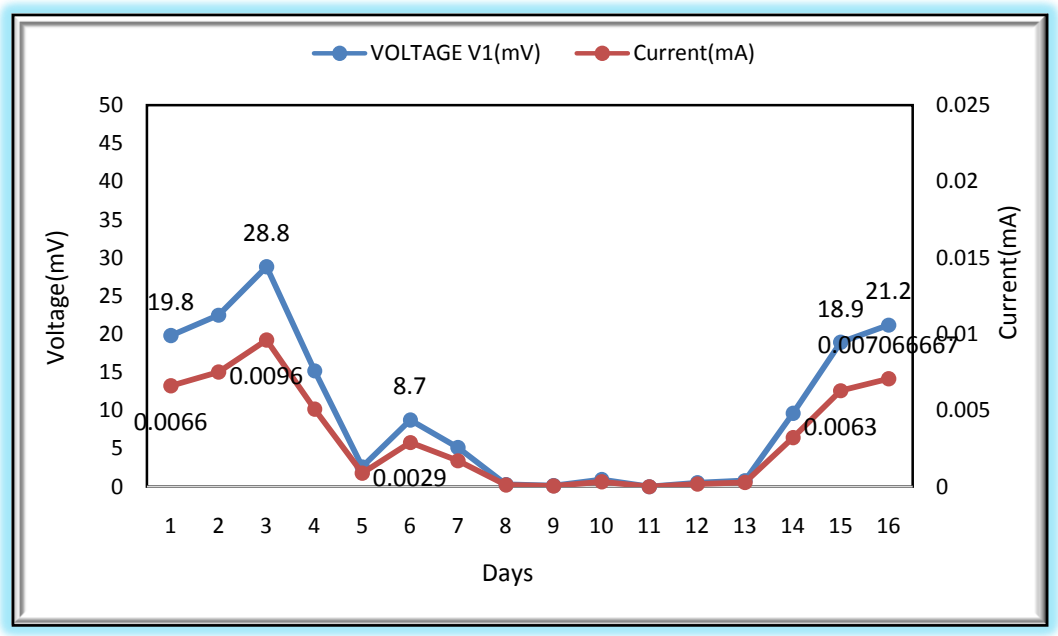


Fig 4.3.4(a). Voltage Vs Current relationship at enrichment stage 1 of electrogenic bacteria in k acetate fed salt bridge double chamber microbial fuel cell(Apendix J)

4.3.5A Voltage And Power Relationship

To find the relationship between voltage and power, a graph was drawn as shown below. It has been analyzed from the recorded data that voltage and power are directly linked to each other while keeping the resistance constant at 3kΩ. With increase in voltage, power was also been increased. Maximum Power recorded during the whole operation was 0.27µW, while other maximum values observed were 0.02µW, 0.119µW, 0.14µW on 6th, 15th and 16th day of operation respectively.

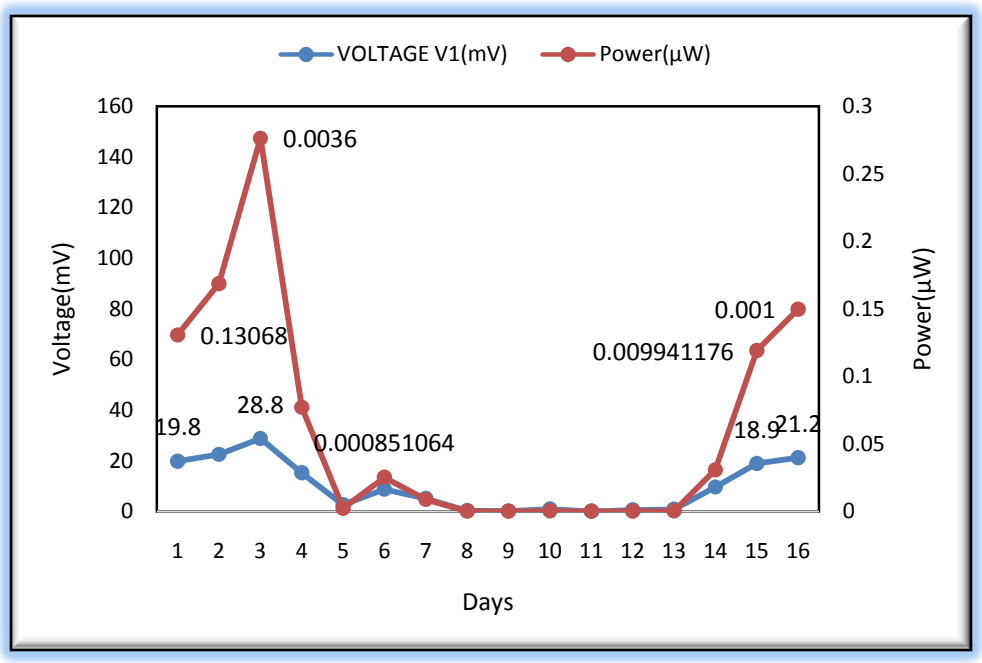


Fig 4.3.5(a). Voltage, power relationship at enrichment stage 1 of electrogenic bacteria fed with K acetate in salt bridge double chamber microbial fuel cell (Apendix K)

4.3.6A Current And Power density Relationship

It has been observed that Current, Voltage, Power and Power density are directly proportional to each other. With increase in current, power density was also increased while keeping the resistance constant at 3kΩ. Maximum Power density recorded was about 0.000055µW/m2 with maximum current of about 0.0096mA during the whole operation of cell. Initially, Power density was continuously raised till 3rd day as seen with current than dropped and again raise to about 0.0000050µW/m2. The 3rd rise in power density was observed at the end of operation to about 0.0000029µW/m2.

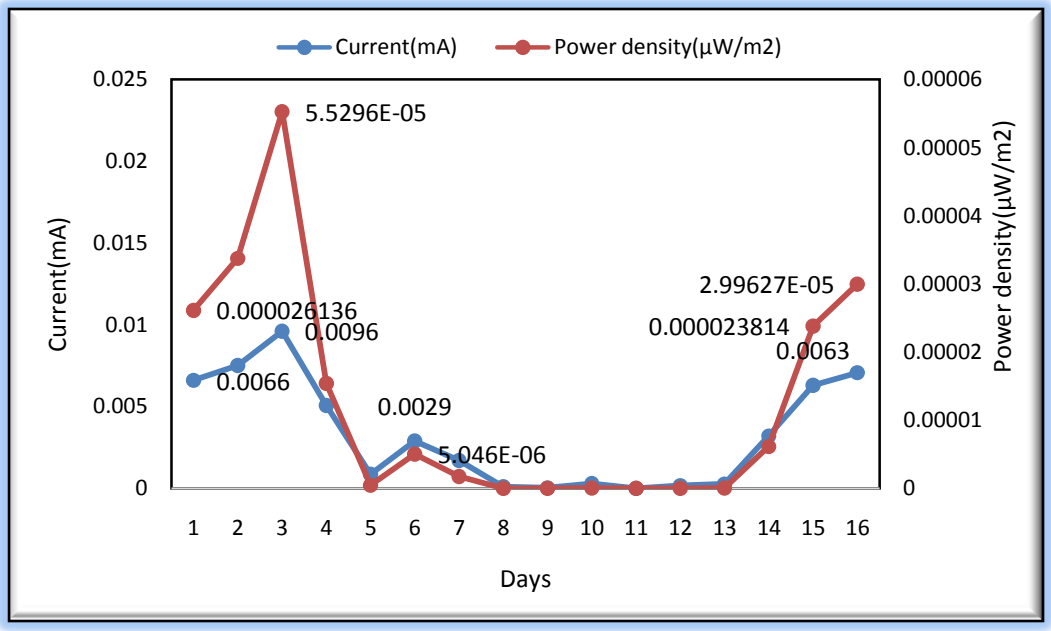


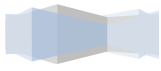
Fig.4.3.6(a) Current Vs Power density relationship at enrichment stage 1 of electrogenic bacteria from activated sludge fed K acetate in salt bridge double chamber microbial fuel cell (Apendix L)

4.4B Stage 2 Of Enrichment With Potassium Acetate Fed Salt Bridge Double Chamber Microbial Fuel Cell

After 16 days of working of fuel cell, enrichment of electrochemically active bacteria was carried out by successively transferring 10% of inoculum from stage 1 of enriched cell to new fresh anolyte media. The cell was placed in incubator at 37^{o}C for next 16 days. Voltage data was obtained and analyzed.

4.4.1B Voltage Data Analysis

After few hours, Open circuit voltage (OCV) was checked but the cell was not showing any kind of voltage. The fuel cell was placed at 37^{o}C, on next day OCV was again taken with the help of precision multimeter. The cell produced an OCV of 119mV. The circuit was closed by connecting through 3kΩ resistor and voltage was again measured. It was found that voltage instantly drops to 12.1mV. The voltage slightly increased to 13mV on next day but started to decrease until 5th day of experiment. The voltage constantly dropped from 13mV to 5.5mV. On 6th day of experiment, the high peak of voltage was observed. The voltage started to increase from 5.5mV to 20mV, which was further rise to its maximum value of 24mV. A third rising peak of Voltage was observed on 10th day. The voltage increased from 13mV to 18mV. Subsequently, on 11th day a little rise was again observed, voltage increased from 8mV to 11mV. A decline phase was observed at the end of experiment as shown in fig.4.4.1(b).The maximum voltage generated during stage 1 and 2 of enrichment was 28.8mV and 24mV. The voltage dropped on 2nd stage of enrichment in salt bridge double chamber microbial fuel cell.



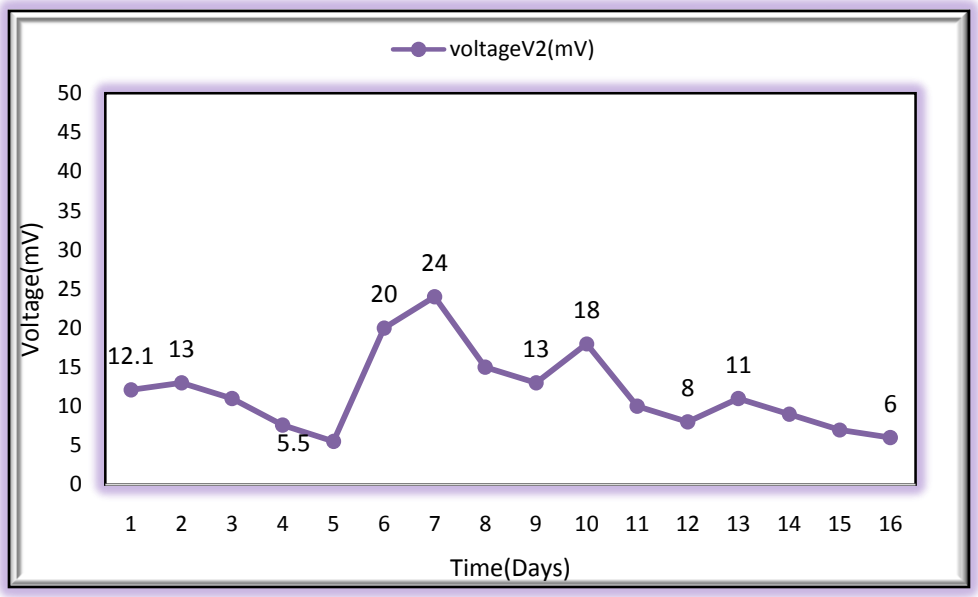


Fig 4.4.1(b) Voltage obtained at enrichment stage 2 of electrogenic bacteria from activated sludge fed with K Acetate carbon source in double chamber Microbial fuel cell (Appendix G2)

4.4.2B Effect Of Resistane On Voltage Production

To check the effect of resistance on voltage production different (high and low resistance) resistors (10Ω, 47 Ω, 57 Ω, 68 Ω, 100 Ω, 170 Ω, 3kΩ, and 100kΩ) were connected one by one through the circuit. It has been observed that resistance have a significant effect on voltage production. Initially, when 10Ω resistor was connected through the circuit, no voltage was shown while by switching from 10Ω to 47Ω, 0.6mVs were produced. Similarly by increasing the resistance step wise, voltage production was also be enhanced. At 100Ω, 2mV were produced. When higher resistor (100kΩ) than 3kΩ was connected across the circuit, sharp rise in voltage from 24mv to 76mV was recorded as shown in fig.4.4.2(b). That was about three times more than at low resistance. While the voltage generated at 1st stage of enrichment was about five times higher at 100kΩ than at 3kΩ. The maximum voltage generated at 100kΩ at 1st and 2nd stage of enrichment was 136.2mV and 76mV.

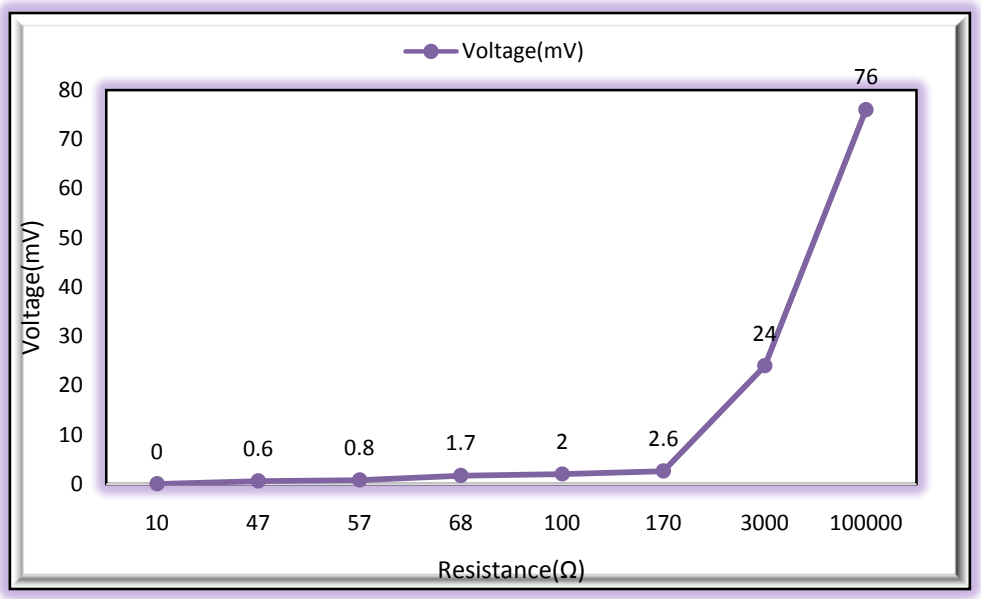


Fig 4.4.2(b). Voltage across various resistors at enrichment stage 2 of electrogenic bacteria from activated sludge fed with K Acetate in double chamber microbial fuel cell (Appendix H2)

4.4.3(b) Polarization Curve

Resistance is inversely proportional to Current described in ohms law. But at 2nd stage of enrichment, initially an unusual behavior was observed at low resistance. At low resistance, current and voltage was also low. On the 7th day of experiment, when maximum voltage was recorded, maximum current of 0.025mA produced across 68Ω. After 68Ω, the current starts to decrease with increase in resistance as reported in literature. Minimum current of 0.0007mA while maximum voltage of 76mV was produced at 100kΩ. Maximum current generated during 1st stage of enrichment was 0.01mA at 10Ω while at 2nd stage of enrichment, it was about 0.025mA at 68Ω at the day when maximum voltage production was recorded. While the minimum voltage recorded on the same day across 100kΩ during 1st and 2nd stage of enrichment was 0.001mA and 0.0007mA. The effect of resistance is very clear from the fig 4.4.3(b) shown below.

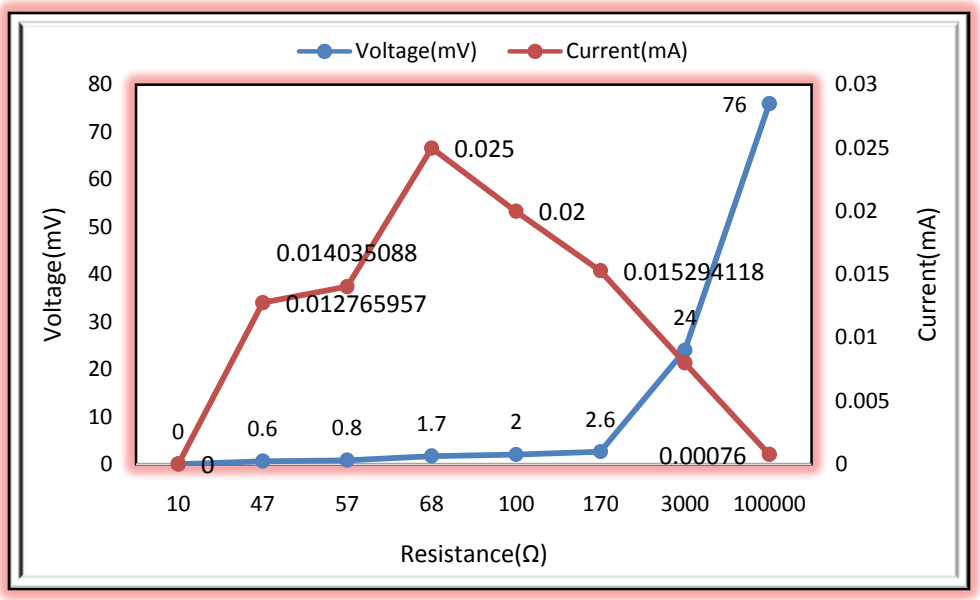
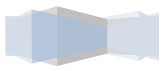


Fig 4.4.3(b). Polarization curve of Current, Voltage across different resistors during enrichment2nd stage of electrogenic bacteria from activated sludge fed with k acetae in double chamber microbial fuel cell (Apendix I2)

4.4.4B Voltage Vs Current Relationship

It has been observed that during experiment with increase in voltage, current was also been increased. Current trend was same as shown by voltage but resistance was kept constant at 3kΩ. It has been analyzed from the collected data of current and voltage that maximum current of 0.008mA was produced when maximum voltage of 24mV was recorded across 3kΩ. When voltage increased from second decline from 13mV to 18mV, current of 0.006mA was recorded. At the end of experiment a little rise in voltage from 8mV to 11mV with rise in current from 0.002mA to 0.003mA was seen.



Maximum voltage 28.8mV and maximum current of 0.0096mV was produced at 1st stage of enrichment during experiment while at stage 2 of enrichment, current and voltage decreased to 24mV and 0.008mA.

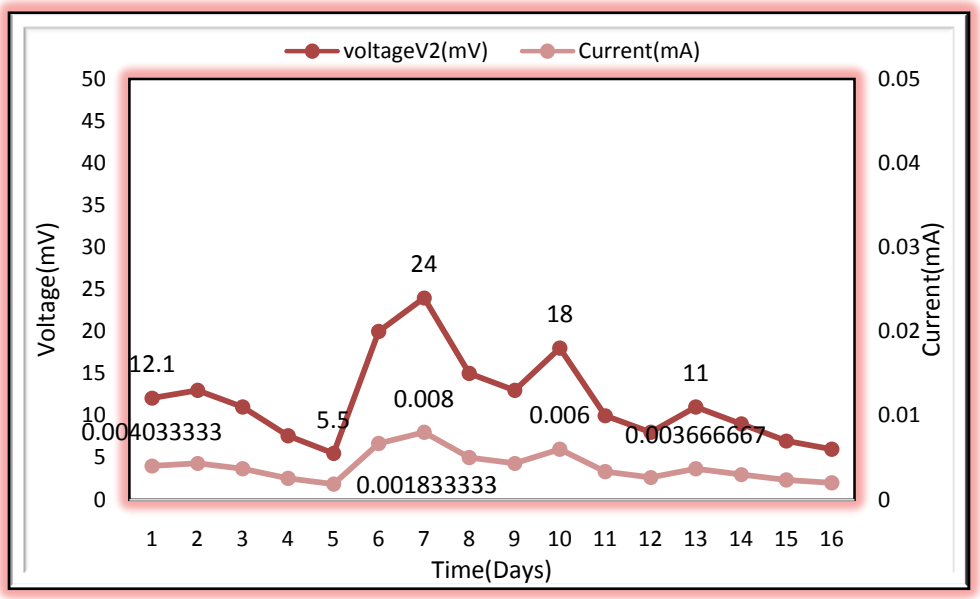


Fig 4.4.4(b). Voltage Vs Current relationship at enrichmentstage 2 of electrogenic bacteria from activated sludge fed with k acetate in double chamber microbial fuel cell (Apendix J2)

4.4.5B Effect of Voltage on Power Production

Voltage and Power are directly linked with each other. With increase in voltage, power was also increased. Initially voltage and power was about 12.1mV and 0.0488µW. in 2nd stage of enrichment cell took a long to increase the potential output. After 5th day voltage and power output started to increase gradually reaching their maximum values. Maximum Power produced after enrichment was about 0.192µW at maximum voltage

output 24mV on 7th day of experimental operation. After maximum power generation decline phase was again observed.

Another rising peak was observed as seen with voltage. On 10th day of working second maximum power potential 0.108µW at second maximum voltage output 18mV. Maximum Power generated during 1st stage was 0.276µW at maximum voltage of 28.8mV while power output during 2nd stage of enrichment was 0.192µW at maximum voltage of 24mV.

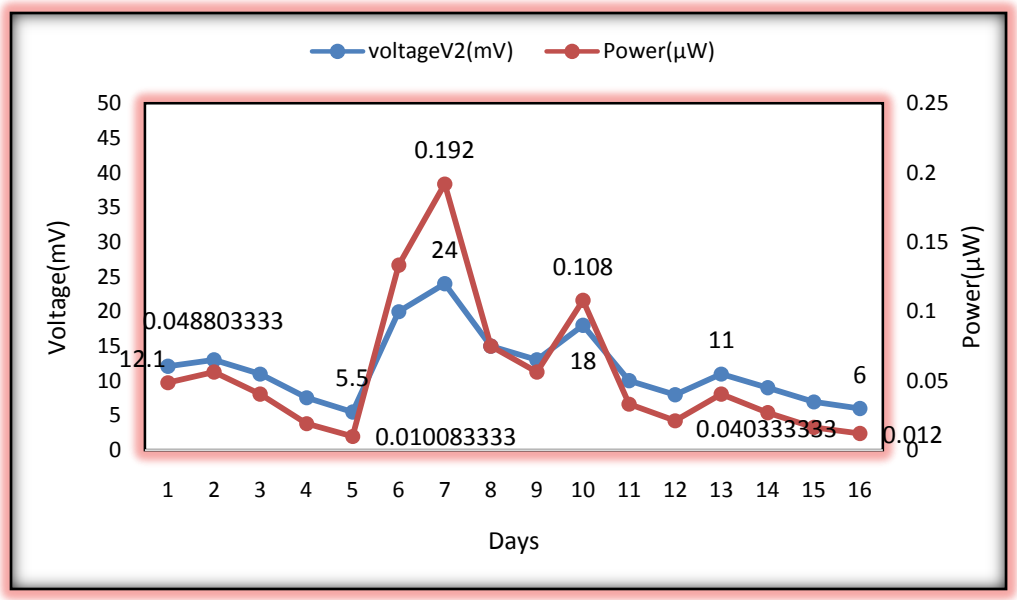


Fig.4.4.5(b). Voltage Vs Power relationship at enrichment stage 2 of electrogenic bacteria from activated sludge in K acetate fed double chamber salt bridge microbial fuel cell (Apendix K2)

4.4.6B Current Vs Power density Relationship

Power density is the amount of power generated per unit surface area. Power density and current have a strong co-relation, both are directly proportional to each other. Increase in

one thing will lead to the increase in other automatically. It has been observed from the recorded data that with increase in current, Power density was also increased. The trend was almost same as seen with current, voltage graph. At the start of experiment cell took a long time to develop current.

After 5th day, increase in current and power density has been observed. Maximum power density recorded during the experiment was 0.0000384µW/m^2 at a maximum current 0.008mA across 3kΩ. Resistance was kept constant throughout the experiment. Second rising trend was observed after 8th day of experiment. Second maximum power density was found to be 0.0000216µW/m^2 at a current value of0.006mA. While minimum Power

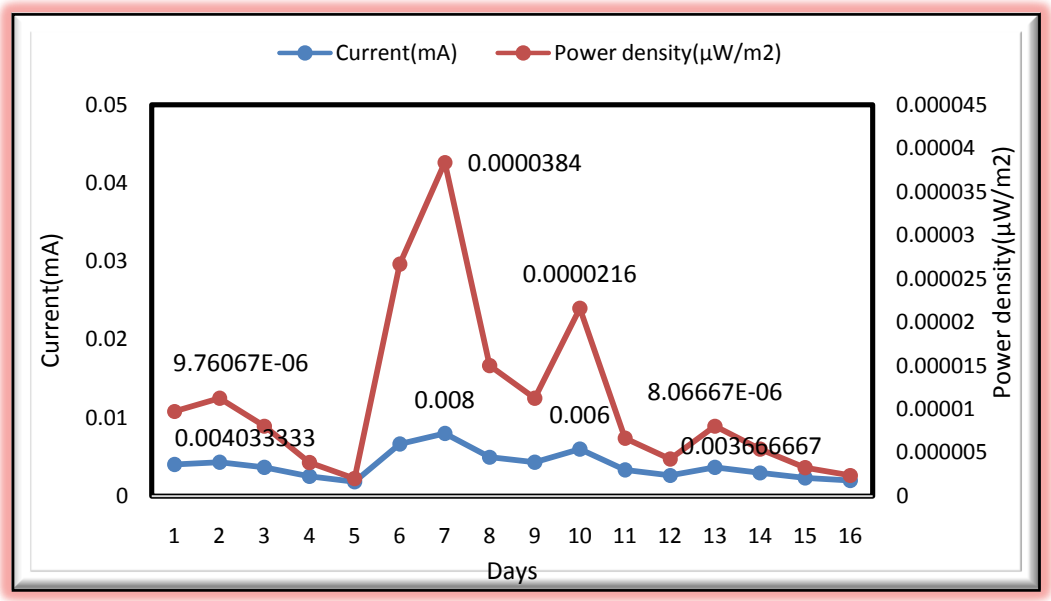


Fig 4.4.6(b). Current and power density co-relation at enrichment stage 2 of electrogenic bacteria from activated sludge fed with k acetate in double chamber microbial fuel cell (Apendix L2)

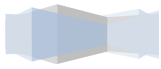
density recorded on 5th day of about 2.01*10^{-6} µW/m^2 at a minimum current (0.001mA) production point. Maximum power density of 0.00005 µW/m^2 and maximum current of 0.0096mA was generated before enrichment while after enrichment it was about 0.000038µW/m^2 at a maximum current 0.008mA across 3kΩ resistor.

4.4.7 Effect of Carbon Supplements During Enrichment With Activated Sludge In Salt Bridge Double Chamber Microbial Fuel Cell

To determine which carbon source (sucrose or potassium acetate) is better utlilized by the electrochemically active bacteria in both experiments, voltage generation was compared during 1st and 2nd stage of enrichment from both the cells.

It has been observed that thethe different carbon sources amended into the anolyte, the maximum voltage generated in sucrose fed fuel cell was greater than the fuel cell operated with Potassium Acetate. Maximum voltage generated with sucrose fed fuel cell during 1st stage f enrichment was 347mV while during 2nd stage of enrichment was about 74.9mV across 3kΩ resistance. Subsequently in 2nd cell, containing Potassium acetate carbon source generates a voltage of 28.8mV during 1st stage of enrichment and 24mV during 2nd stage of enrichment as shown in fig.4.4.7(a).

So it has been deduced from the above experiments that voltage generated in sucrose fed fuel cell was about three times greater than voltage generated in K acetate containing fuel cell. So, sucrose is better utlilized by the bacteria than potassium acetate.



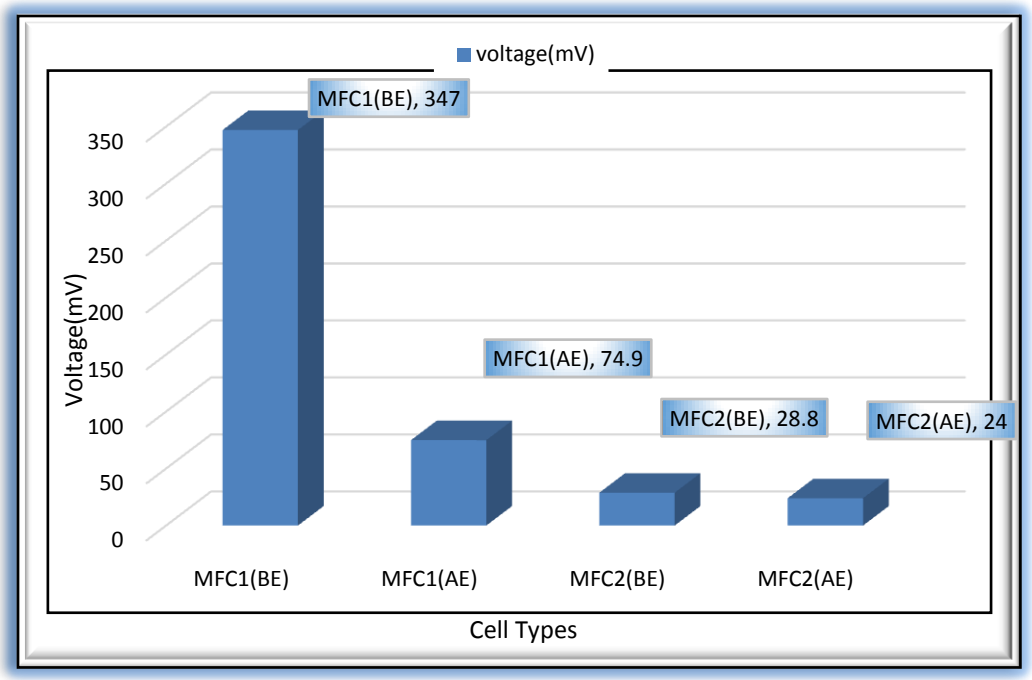


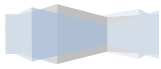
Fig 4.4.7(a). Comparision of during 1st and 2nd stage of enrichment of electrogenic bacteria from activated sludge fed with sucrose and k acetate in salt bridge double chamber microbial fuel cell. MFC1(BE) represents Sucrose fed MFC During stage 1, MFC2(AE) represents sucrose fed MFC during 2nd stage of enrichment, MFC2(BE) represents K Acetate fed MFC During 1st stage of enrichment while MFC2(AE) represents K Acetate fed MFC During 2st stage of enrichment (Apendix L2A)

4.5A Enrichment Of Electrogenic Bacteria From Soil(S3) In Proton Exchange Membrane Containing Double Chamber Microbial Fuel Cell

In Proton exchange membrane containing double chamber fuel cell, soil (S3) as bacterial source was tested for the presence of electroactive bacterial cells and current generation. MFC was continuously operated under stirring condition at 50rpm. The maximum voltage and maximum current generated in 1st stage of experiment was 105.7mV and 0.00074mA, while during 2nd stage of enrichment, about 332mV and 0.110mA of voltage and current was produced when the circuit connected through 3kΩ. So, it has been deduced that voltage output increases about three times than 1st stage of enrichment in PEM containing double chamber microbial fuel cell.

4.5.1A Voltage Output Analysis

An open circuit voltage of about 206mV was recorded after stabilization of cell. Then the circuit was closed by connecting through 3kΩ resistor and data was recorded. After connecting the circuit through the resistor, the voltage was again measured. It has been observed that closing the circuit decreases the voltage output to 105mV. At the start of experiment, the voltage output dropped continuously. The fuel cell took a long time to increase the voltage production and get stabilized. After 8th day of working of fuel cell the voltage production started to increase. After getting stabilized, a sharp rise in voltage was observed reaching its maximum value. The resistor was kept constant at 3kΩ. To continue the maximum voltage output enrichment of fuel cell was performed. Maximum voltage of about 105.7mV was recorded across 3kΩ resistor as shown in fig: 4.5.1(a):



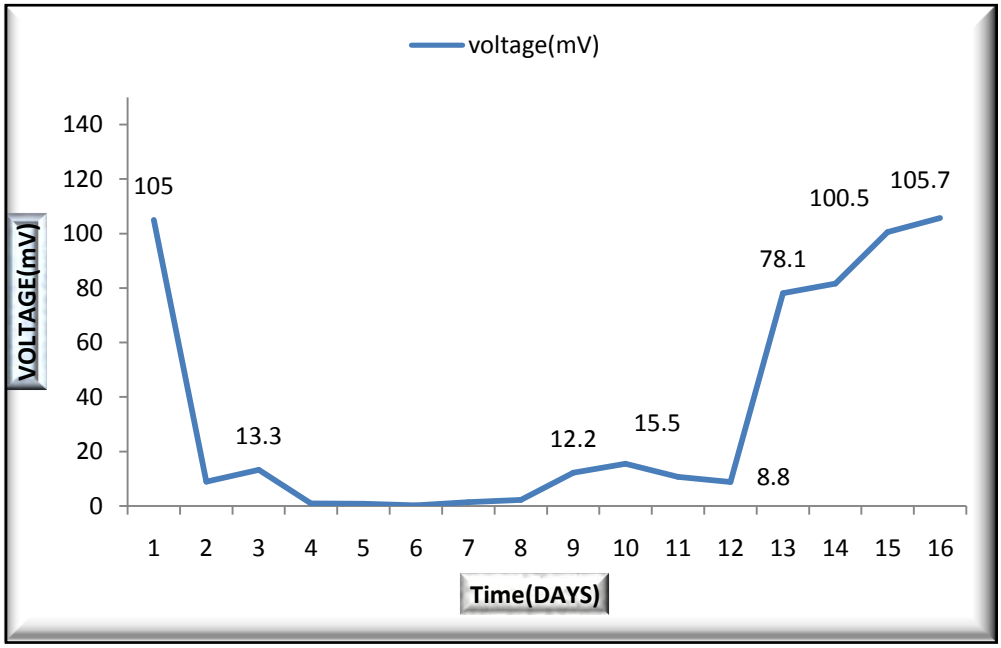


Fig 4.5.1(a). Voltage (mV) output with soil(S3) in PEM containing double chamber microbial fuel cell at enrichment stage 1 of electricigens (Apendix M)

4.5.2 Effect of Resistance on Voltage Output

To check the effect of resistance voltage data, was recorded across different resistors (10Ω, 47Ω, 57Ω, 68Ω, 100Ω, 170Ω, 3kΩ and 100kΩ) one by one. It has been observed that with increase in resistance voltage was also increased. When 10Ω resistor was connected across the circuit, minimum voltage of 0.2mV was recorded. Increasing the resistance from 10Ω to 47Ω, voltage was also increased from 0.2mV to 0.4mV about 2 times enhanced. At 100Ω, voltage of 3.8mV was measured. While increasing the resistance from 3kΩ to 100kΩ, voltage was increased from its maximum value from 105.7mV to 179.9mV.Maximum voltage of 179.9mV was recorded across 100kΩ resistor on 16th day of operation of cell as shown in fig 4.5.2a:

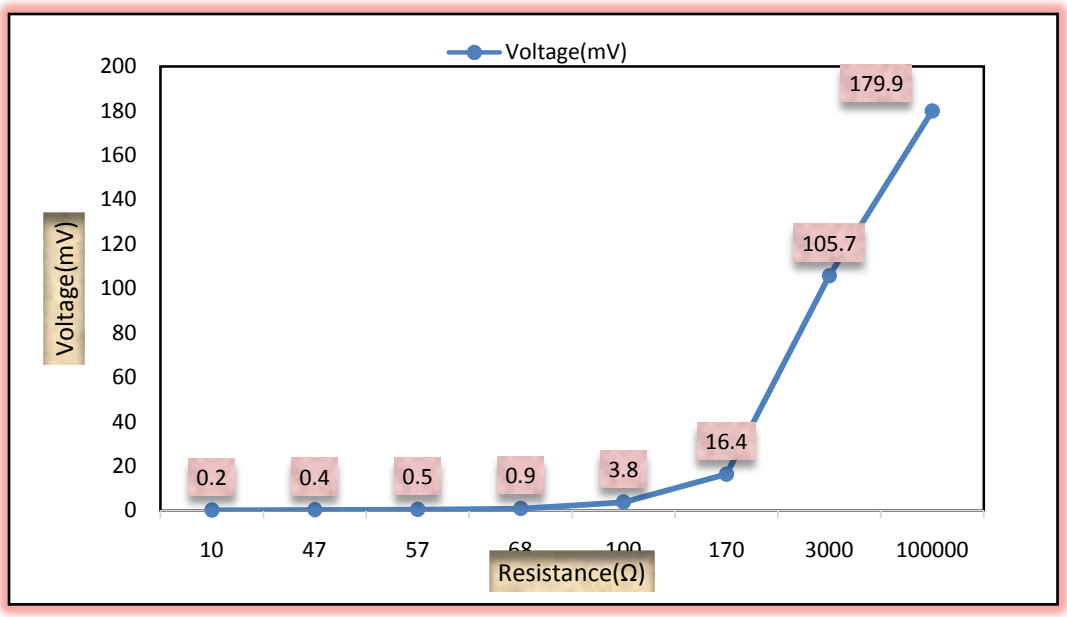


Fig 4.5.2(a). Voltage (mV) across different resistors(Ω) with soil(S3) in PEM containing double chamber microbial fuel cell at 1st stage of enrichment of electrogenic bacteria(Apendix N)

4.5.3A Polarization Curve

Resistance have a negative effect on current while positive on voltage. With increase in resistance current decreases while increases alternatively. An unusual behavior was observed at the start of experiment, current increases with increase in resistance up to 170Ω while voltage was continuously increased with increase of resistance. Maximum current of 0.096mA was produced at a resistance of 170Ω. Then current started to decrease with further increase in resistance. At 3kΩ the current production was decreased from 0.096mA to 0.035mA. While further increase in resistance to 100kΩ, current was further decreased from 0.035mA to 0.0017mA.

Maximum current of about 0.096mA was recorded across 170Ω. Fluctuations in current production was observed but mainly current drops with increase in resistance.

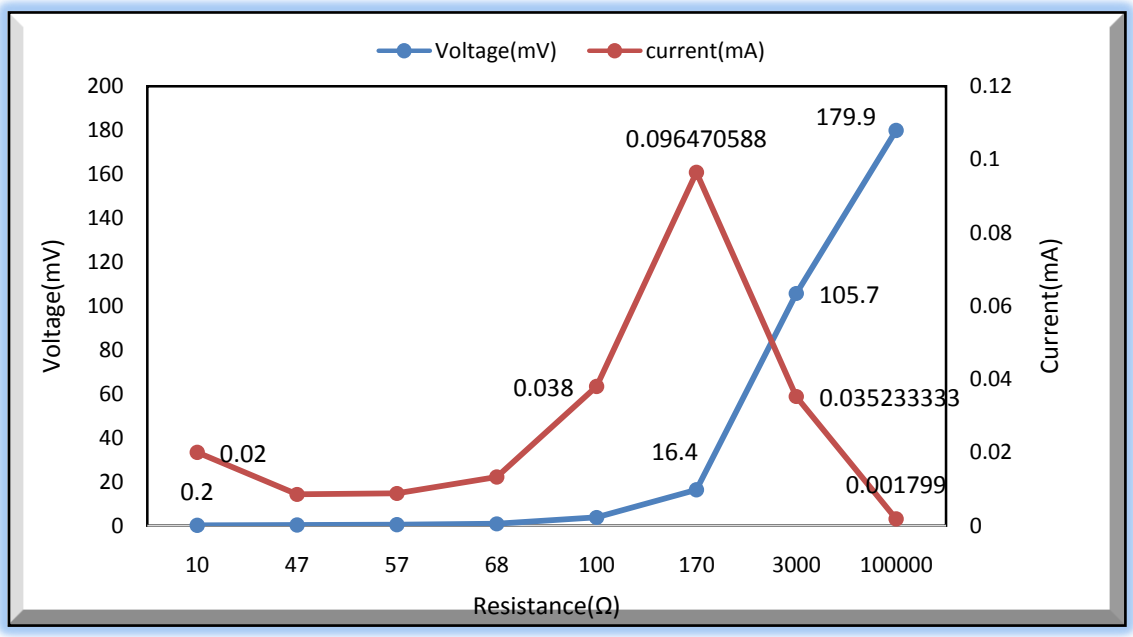


Fig 4.5.3(a). current and voltage across various resistors at enrichment stage 1 of electrogenic bacteria from soil(S3) in PEM containing dual chamber microbial fuel cell (Apendix O)

4.5.4A Voltage and Current Relationship

It has been observed that with increase in voltage, current was also increased. Current and voltage are directly proportional to each other. At the start of experiment current of 0.035mA at a voltage of 105mV was recorded. Then, it started to decrease with decrease in voltage as seen earlier. The current trend as almost same as of voltage. As it has been observed that voltage increase started after 8th day of experiment, current was also started to increase with voltage.

Maximum voltage of 105.7mV on 16th day of operation while maximum current of 0.033mA was recorded. Minimum current of 0.0001mA was measured at minimum voltage of 0.3mA during operation at 3kΩ.

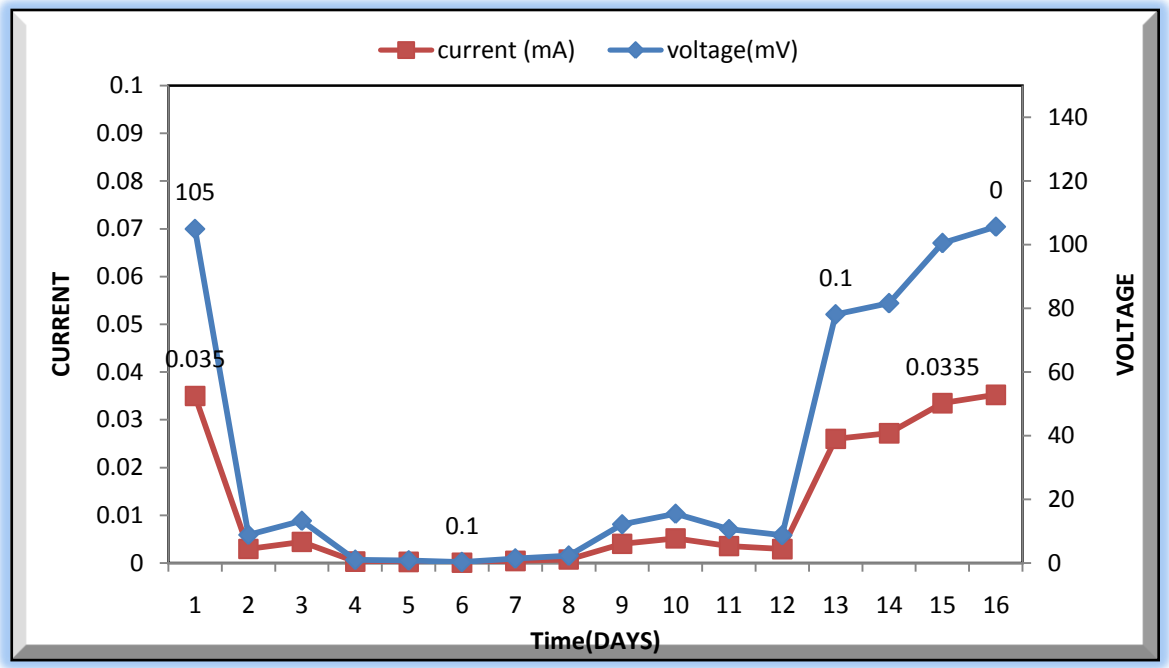


Fig 4.5.4(a). Voltage Vs Current at enrichment stage 1 of electrogenic bacteria from soil(S3) in PEM containing double chamber MFC(Apendix P)

4.5.5A Voltage And Power Relationship

Direct relationship was found between voltage and power. Initially, the Voltage and power was high of about 105mV and 3.6µW but sharply decreased to about 0.06µW. after few initial fluctuations, the voltage and power output starts to get stabilized. It has been observed that after 12th day of operation, the voltage and power output increased constantly up to 105.7mV and 3.724µW.Maximum Voltage of 105.7mV and Maximum Power of 3.72µW was recorded while minimum voltage and power of about o.3mV and 0.0003µW was seen.

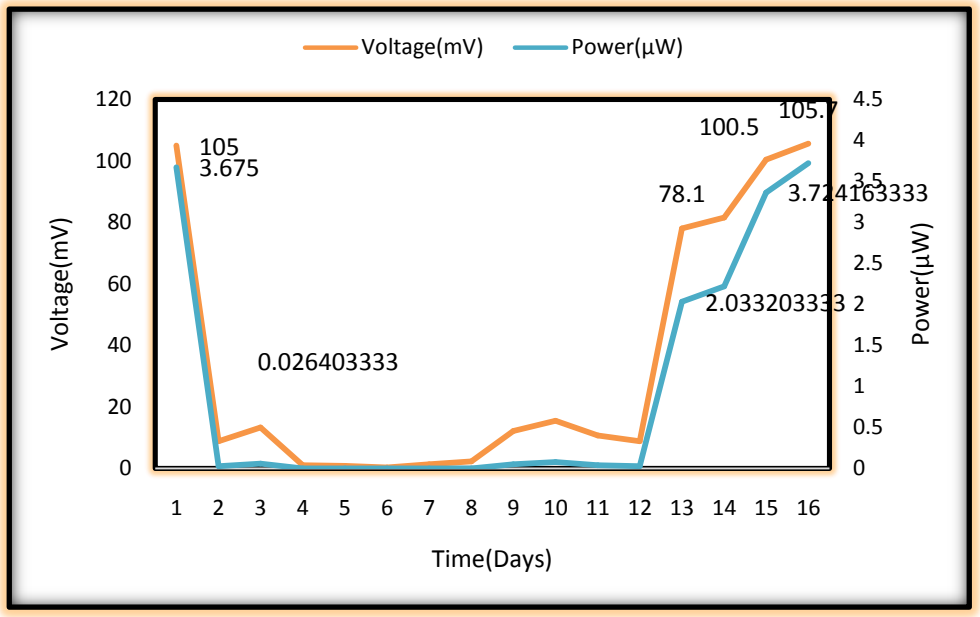


Fig 4.5.5(a). Voltage and power relationship at enrichment stage 1 of electrogenic bacteria from soil (S3) in PEM containing MFC (Appendix Q)

4.5.6A Current And Power Density Relationship

With increase in current, power density was also increased. At the start of experiment,power density decreases with decrease in current. Initially, power density was 0.00073µW/m^2 at a current output of 0.035mA. It decreases to 5.2x10^{-6} at a current of 0.002mA. Power density and current continuously decreases till 8^{th} day of experiment. Minimum power density 6E^{-9} was recorded at a minimum current of 0.0001mA.

Maximum power density of 0.00074µW/m^2 was observed at maximum current production 0.033mA.

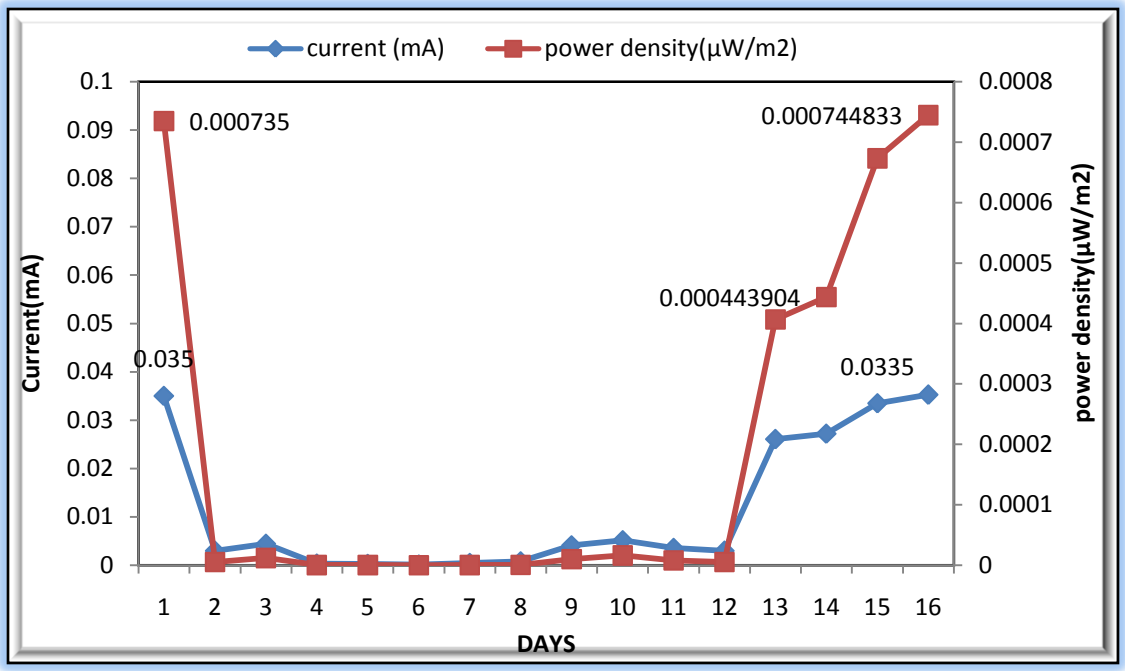
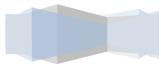


Fig 4.5.6(a).Current(mA) Vs Power density(µW/m²) relationship at enrichment stage 1 of electricigens from soil(S3) in PEM containing double chamber microbial fuel cell (Apendix R)

3.6B 2nd Stage Of Enrichment With Soil(S3) In PEM Containing double chamber Microbial Fuel Cell At 50rpm

Enrichment of soil (S3) was done to reach close to more specific type of electrogenic microorganisms and to increase the voltage output. After successive transfer of 10% previous inoculum into fresh anolyte media, it has been observed that maximum voltage generation was increased from 105.7mV to 332mV in PEM containing double chamber MFC under agitation at 50rpm.



4.6.1B Voltage data Analysis

Open circuit voltage was found to be 110mV after two days of enrichment, before that fuel cell was not showing any kind of voltage. After obtaining stable OCV, the circuit was closed by connecting through 3kΩ resistor. The voltage dropped after closing the circuit to 0.1mV, which started to increase gradually to 32.6mV by next day. After 3rd day of experiment, voltage increases constantly till 8th day of working of cell. Maximum voltage of 332mV was recorded on 6th day at 2nd stage of enrichment across 3kΩ. Second maximum value of voltage was found to be 305mV. Minimum voltage of 0.1mV was produced during the experiment as shown in fig: 4.6.1(b).

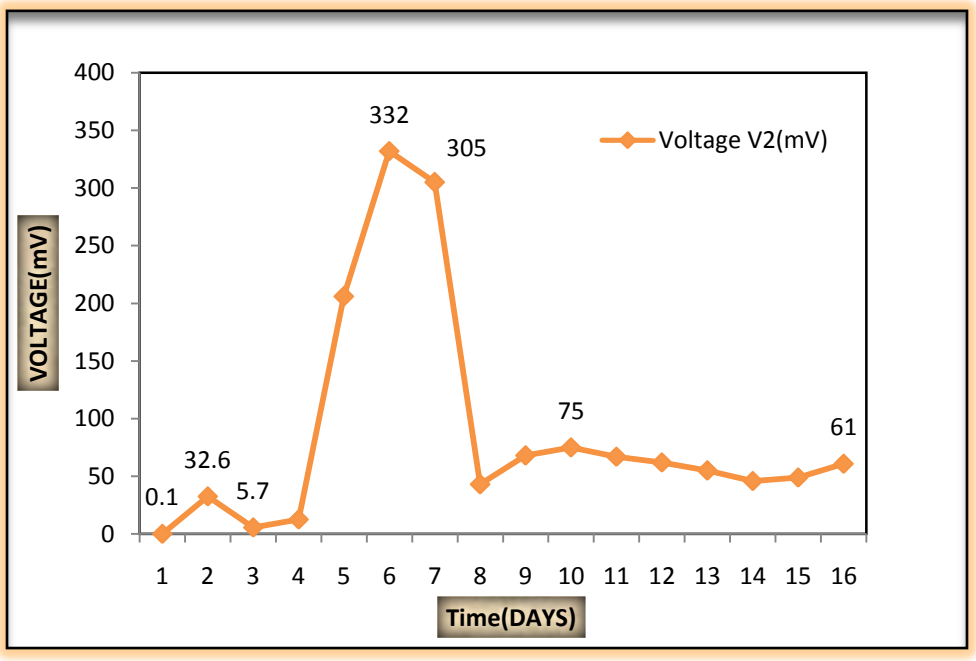


Fig 4.6.1(b). Voltage(mV) obtained at enrichment stage 2 of electrogenic bacteria from soil (S3) in PEM containg double chamber microbial fuel cell (Apendix M2)

4.6.2B Effect Of Resistance On Voltage Output

Resistance effect was checked after enrichment of electroactive bacteria by varying the resistors from 10Ω to 100kΩ (10Ω, 47Ω, 57Ω, 68Ω, 100Ω, 170Ω, 3kΩ and 100kΩ). Voltage was increased with increase in resistance. It has been noticed that at 10Ω, voltage of 0.5mV was produced. At 100Ω, 30.7mv was produced. When the circuit was closed by connecting through100kΩ resistor on 6th day of working of cell, the voltage increased from 332mV to 530mV as shown in graph Fig: 4.6.2(b).The maximum voltage measured after switching to 100kΩ from 3kΩ was 179.9mV at 1st stage of enrichment while during 2nd stage of enrichment, it was increased up to 530mV from 332mV. Subsequently, the minimum voltage was noticed at 10Ω resistance during 1st and 2nd stage of enrichment of about 0.2mV and 0.5mV respectively.

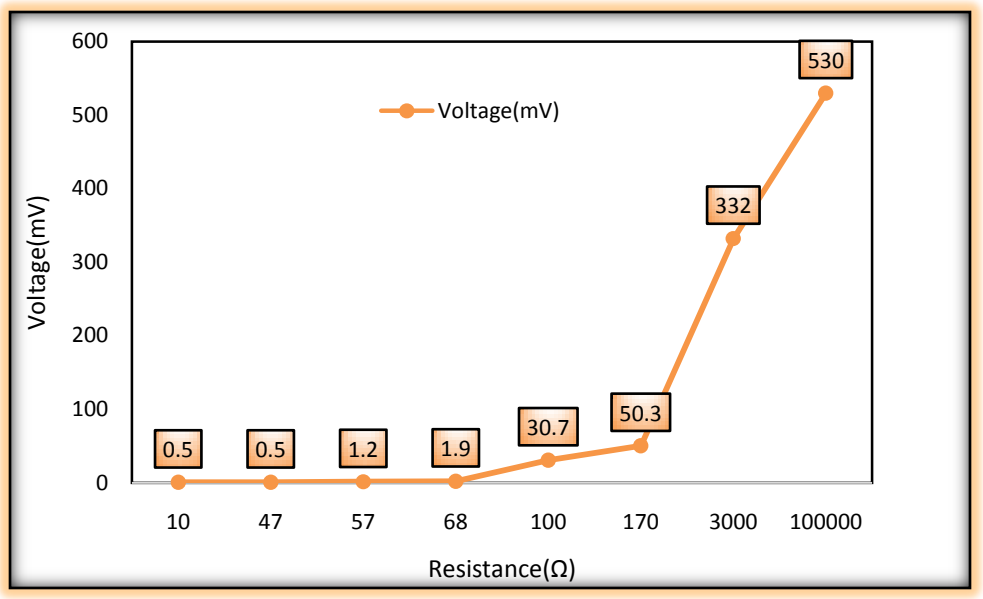
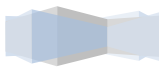


Fig4.6.2(b). Voltage across various resistors at enrichment stage 2 of electrogenic bacteria from soil (S3) in PEM containing H-shape microbial fuel cell (Apendix N2)

4.6.3B Polarization Curve

Current (mA) was also measured along with voltage (mV) at varying resistances (Ω) (10Ω, 47Ω, 57Ω, 68Ω, 100Ω, 170Ω, 3kΩ and 100kΩ). Current generation decreased with increase in resistance. But at the start of experiment an unusual behavior was observed. Initially current production increased with increase in resistance along with increase in voltage as well. At 10Ω, current of about 0.05mA was recorded at a minimum voltage of 0.5mV. By switching to 47Ω from 10Ω, current was decreased from 0.05mA to 0.01mA. Further increase in resistance would led to the increase in current along with increase in voltage. As at 57Ω, the current was increased from 0.01mA to 0.021mA. While at 100Ω, maximum current of 0.3mA was recorded.

It was noticed that after further increase in resistance from 100Ω to onwards, current generation was decreased but voltage increased gradually. At 100kΩ the current production was further decreased to 0.0053mA which was the minimum current recorded against variable resistance.



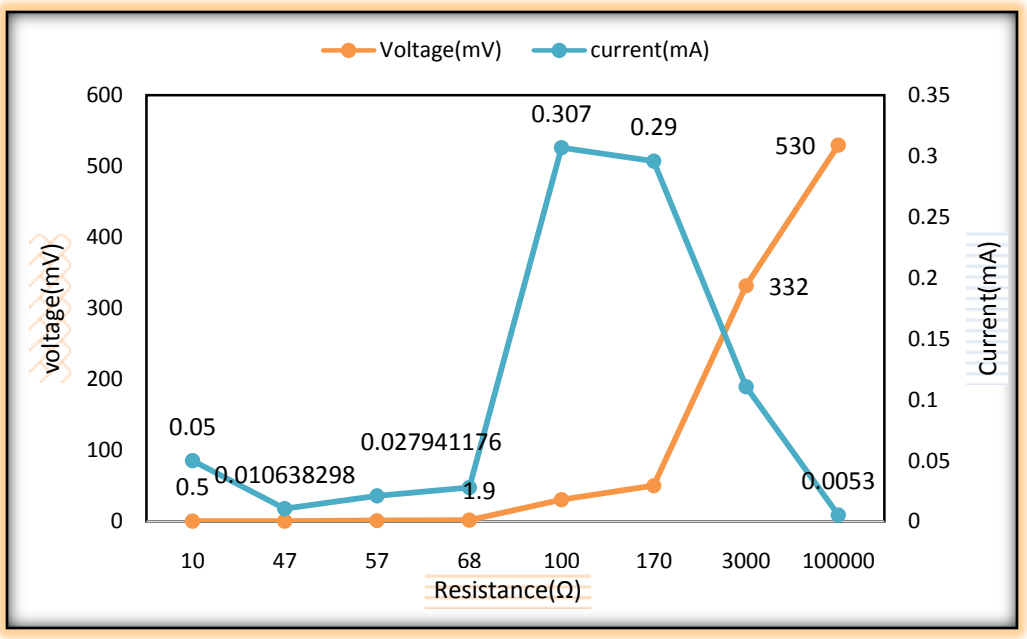


Fig 4.6.3(b).Polarization curve of current Vs voltage atenrichment stage 2 of electrogenic bacteria from soil (S3) in membrane double chamber microbial fuel cell(appendix P2)

4.6.4B Voltage And Current Relationship At Constant Resistance

It has been observed that current is directly proportional to voltage at constant resistant. As with increase in voltage, current was also increased. Resistance was kept constant at 3kΩ. Initially the voltage was low but it started to increase gradually. Primarily, the current was also low which started to increase steadily along with increase in voltage. At the voltage of 0.1mV on very 1st day of working of fuel cell, current was very low of about 3.3x10^{-5}, which was the minimum voltage and current generated. After getting stabilized the voltage and current started to increase reaching their maximum values that the bacteria were capable to produce. On 6th day, maximum voltage of 332mV was recorded, similarly maximum current of 0.110mA was generated at a maximum voltage output. Then a long decline phase was analyzed at the end of experiment.

The voltage and current dropped to 61mV and 0.020mA respectively on the last day of working of fuel cell.

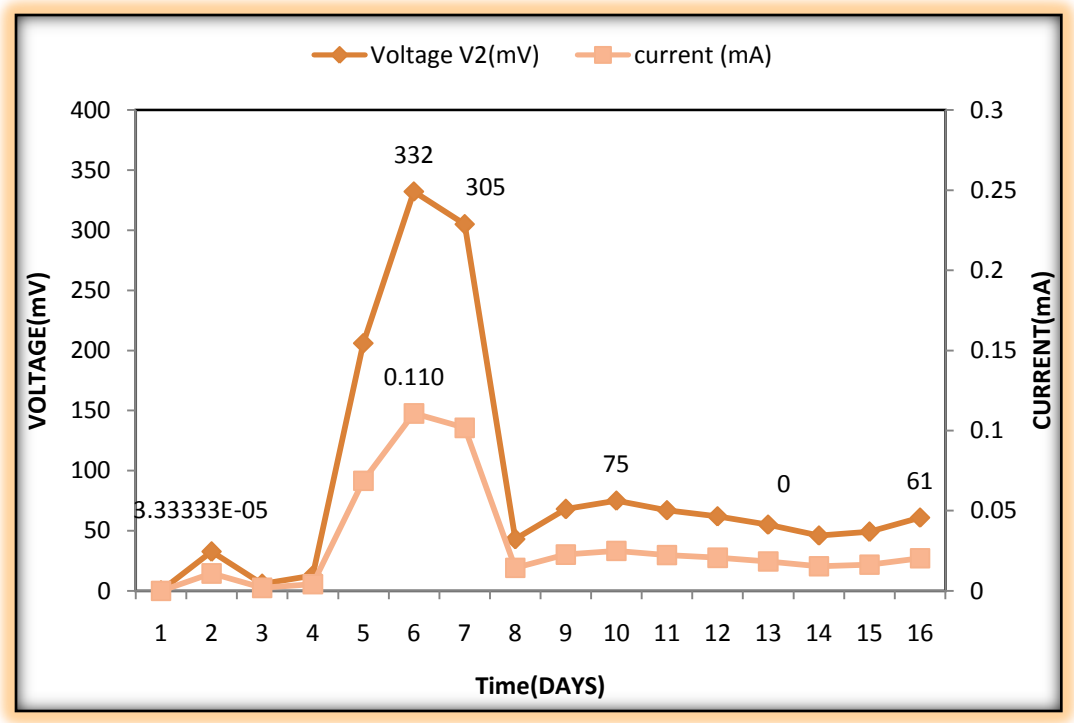


Fig 3.6.4(b). Voltage (mV) Vs Current (mA) at enrichment stage 2 of electrogenic bacteria from soil(S3) in membrane H-shaped microbial fuel cell (Apendix P2)

3.6.5B Effect Of Voltage Output On Power Generation

Voltage and power are directly proportional to each other. With increase in voltage, power output was also increased. During 2nd stage of enrichment, a dramatic change was observed. The maximum power generation was increased from 3.7µW to 36.7µW about 12 time increase was observed during 2nd stage of enrichment. At the start, the voltage and power output was low, it started to increase gradually after 3rd day of working. At 205.9mV, 14.131µW of power was generated. The power was increased from 14.13µW to 36.74µW. after reaching its maximum potential, the power started to decrease. Second maximum value of power recorded was about 31.008µW.

Minimum and maximum power output recorded was about $3.3x10^{-06}$ µW and 36.74µW after enrichment of fuel cell.

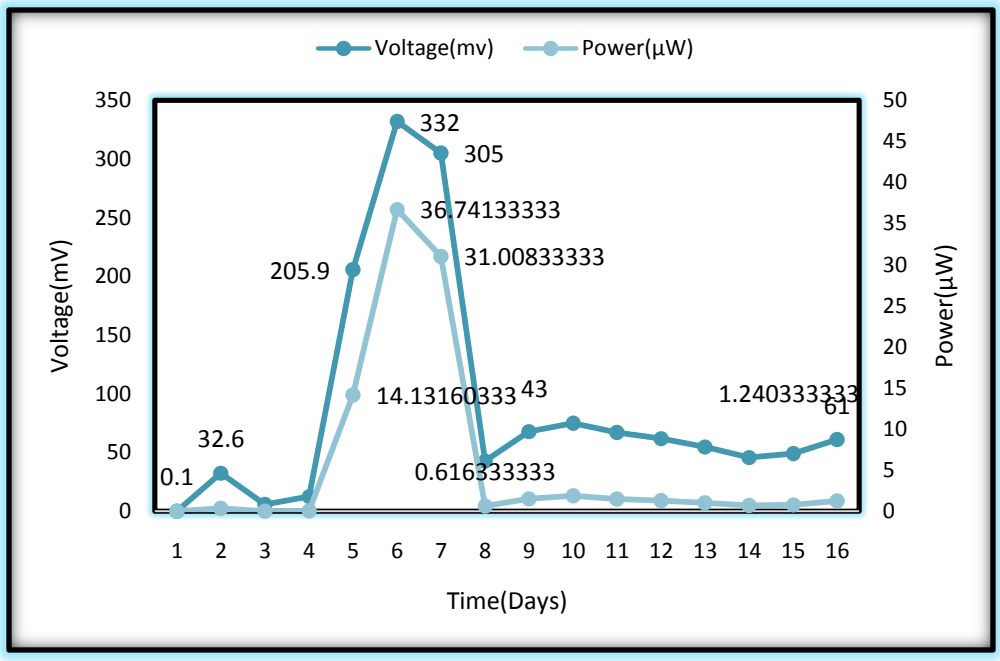


Fig 4.6.5(b). Effect of voltage on power output at enrichment stage 2 of electrogenic bacteria from soil(S3) in PEM-MFC (Apendix Q2)

4.6.6B Current(mA) Vs Power density(µW/m^2) relationship

Increase in Power density (µW/m2) was observed with increase in current generation at a constant resistance. Current, voltage, power and power density are directly proportional to each other. With increase in one variable other variable automatically increased. Maximum Power density of 0.0073µW/m2 was recorded at maximum current 0.11mA after enrichment of soil (S3) in membrane double chamber MFC.

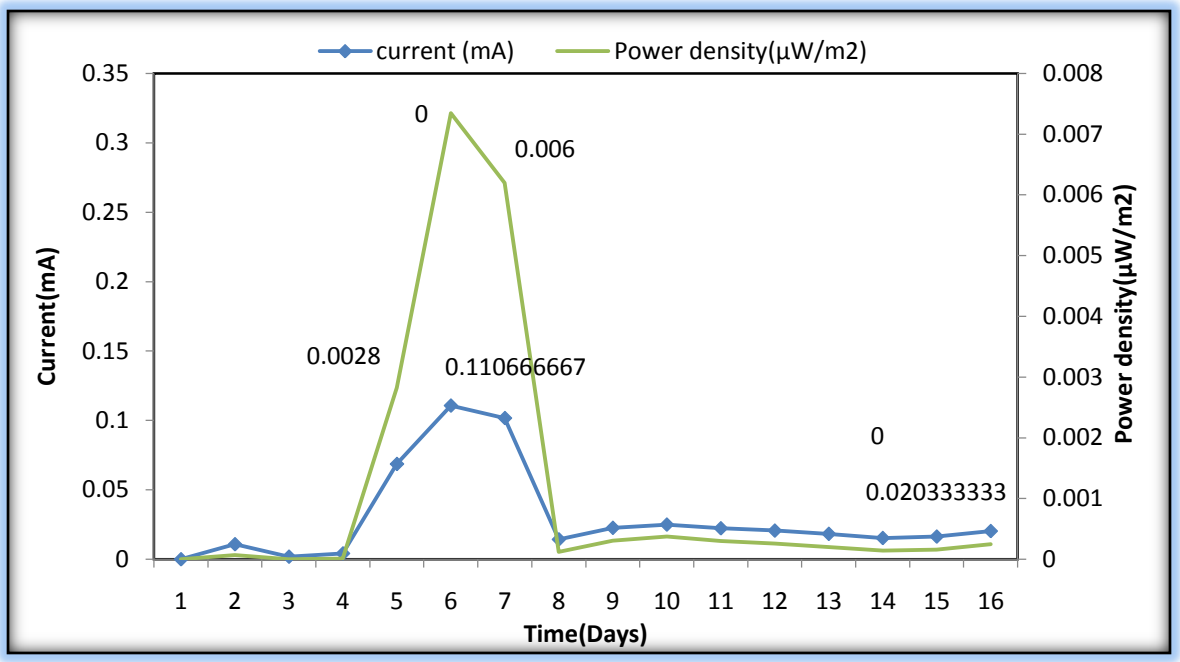


Fig 4.6.6(b). Current Vs Power density relationship at enrichment stage 2 of electrogenic bacteria from soil(S3) in double chamber PEM-microbial fuel cell (Apendix R2)

4.6.7 Comparison Of Voltage Generation At 1st and 2nd Stage Of Enrichment Of Electrogenic Bacteria From Soil(S3) In PEM-Double Chamber Microbial Fuel Cell

It has been observed that maximum voltage generated before enrichment was comparatively less than that generated after enrichment. As it has been recorded that before enrichment maximum voltage generated across 3kΩ resistor was 105.7mV while after enrichment it was 332mV. When the resistor changed from 3kΩ to 100kΩ, the voltage was increased 179.9mV before enrichment to 530mV after enrichment.

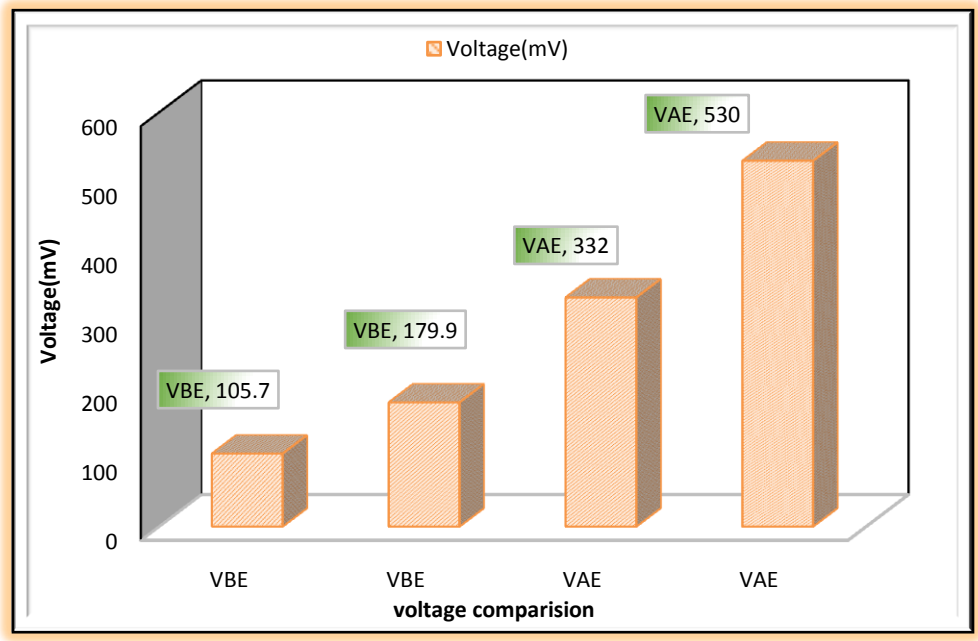


Fig 4.6.6(b). Comparision of at 1st and 2nd stage of enrichment of electrogenic bacteria from soil (S3) across 3kΩ and 100kΩ resistance in PEM- double chamber MFC(Apendix R2A)

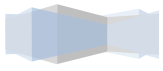
4.7: Enrichment Of Soil(S1) In Proton Exchange Membrane Containing Double Chamber Microbial Fuel Cell

The cell was operated for one month but it did not show any electrogenic activity with no power output.No voltage output was observed.

4.8 Biofilm Formation Assay

A total of 170 bacterial isolates were isolated from anode surfaces (adherent bacteria) as well as from anodic solution (suspended bacteria). Suspended and adherent bacteria were isolated during the operation of fuel cells. All of the isolates were subjected to biofilm formation screening in microtiter biofilm formation assay. Optical density measurements would allow us to divide bacterial isolates into four categories non-adherent (NA), weakly-adherent (WA), moderately adherent (MA) and strongly-adherent (SA).

It has been found that out of total 170 isolates, 26 were non biofilm formers means showing no adherence, 123 isolates were weakly adherent, 12 were moderately adherent while 4 were strong biofilm formers showing strong adherence. It has been deduced from the assay results that 75% were contributed by weak biofilm formers, 2% by strong biofilm formers, 7% by moderate biofilm formers and 7% by non- biofilm formers bacterial isolates in voltage and power output in all experiments.



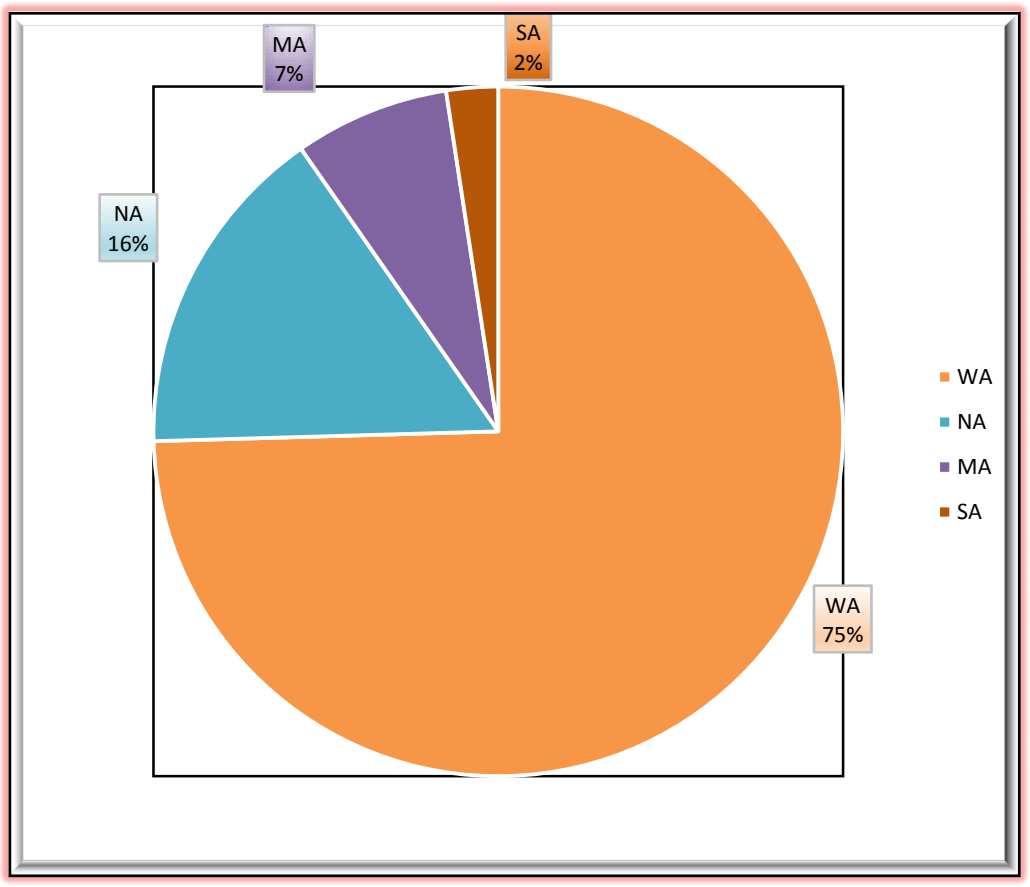


Fig 4.8.1. %age contribution of biofilm forming capability of bacterial isolates isolated during the operation of MFC 1, 2, 3 at enrichment stage 1 and 2. WA represents weakly-adherent, NA (non-adherent), MA (moderetly-adherent) while SA is Strongly-adherent (Apendix S)

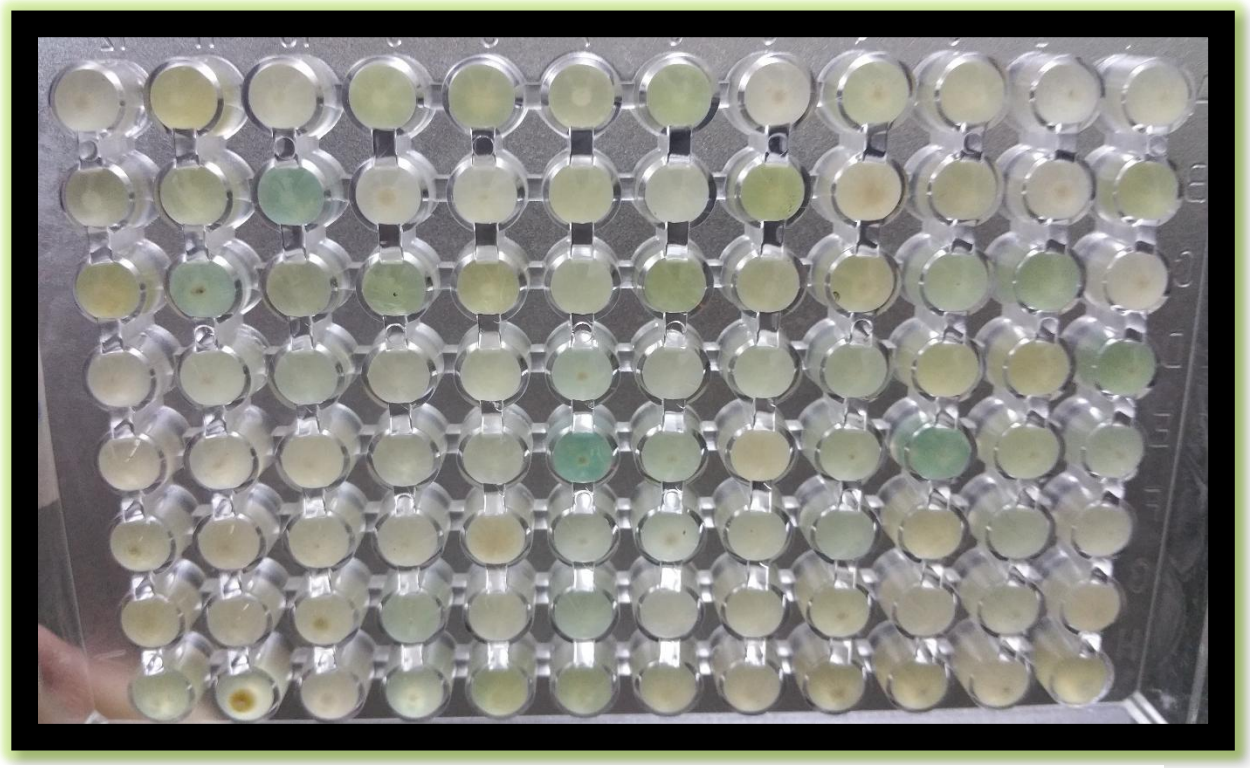


Fig 4.8.2(a). Microtiter plate containing isolates before staining

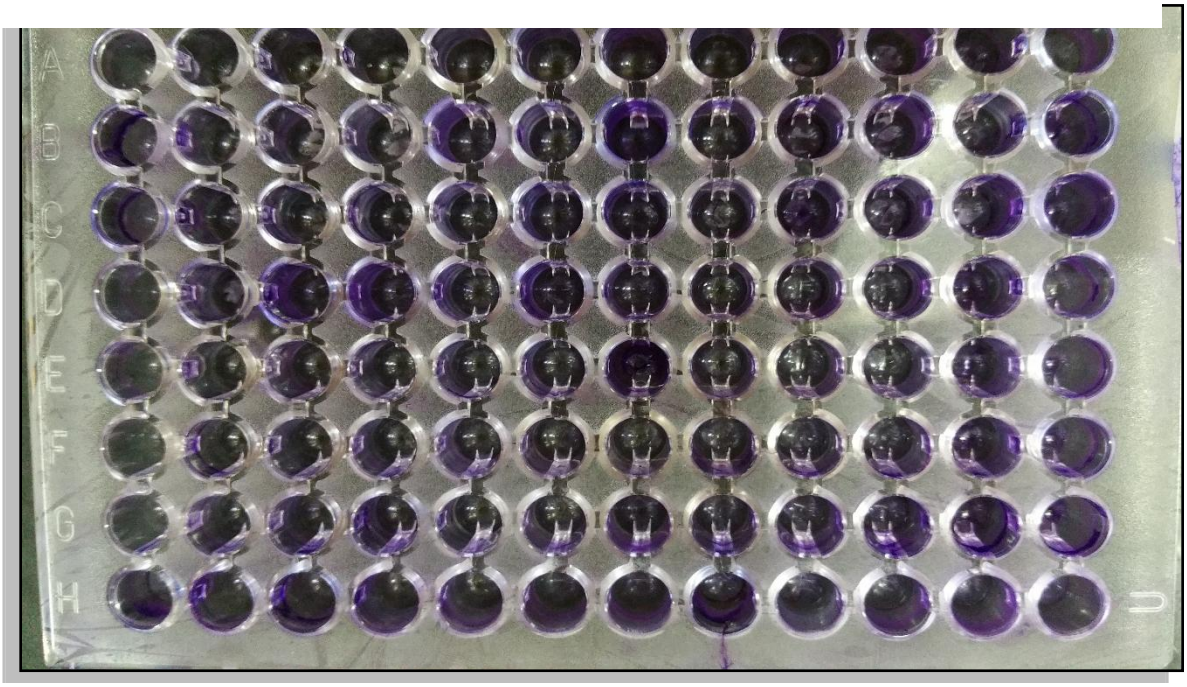


Fig 4.8.2(b). Screening of biofilm formers with crystal violet stain in 96-well microtiter plate

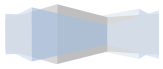
4.9Bacterial Community Structure Of Electrogenic Bacteria From Activated Sludge Using Pyrosequencing

To analyze the bacterial community associated with anode surface and their percentage contribution in current generation, 454 pyrosequencing was done. Blue primers (530R and 104F) were used targeting the V2 and V3 region of 16S rRNA bacterial genes. It has been found that samples went through enrichment step were greatly enriched for a particular group of bacteria. A comparision was made with the original environmental inoculum source and the biofilm bacterial communities developed after enrichment.

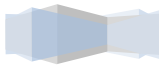
In the initial sludge inoculum four kingdoms were present bacteria accounts for 99% while archea, viridiplantae and eukaryote all contributes to 1% in the sample community. It has been revealed that after enrichment kingdoms were reduce to bacteria contributing 99.3% and archaea 0.007% while rest of the two kingdoms were absent.

Table. 4.9.1. %age relative abundance at kingdom level in activated sludge and anodic biofilm on carbon cloth from 2nd stage of enrichment in acetate fed double chamber microbial fuel cell

Kingdoms	%age relative abundance in activated sludge	%age relative abundance in Anodic Biofilm
Bacteria	99.37095	99.99267
Archaea	0.235895	0.007333
Viridiplantae	0.235895	0
Eukaryota	0.157264	0



On the phylum level distribution, it has been observed that original inoculum of sludge contains *Spirochaetes* which contributes about 50.22% followed by *Actinobacteria* (12.64%), *Bacillariophyta* (7.03%), *Armatimonadetes* (5.5%), *Ignavibacteriae* (4.99%), *Chrysiogenetes* (2.96%), *Chlorophyte* (2.06%), *Proteobacteria* (1.92%), *Acidobacteria* (0.314%) and *Fermicutes* (0.07%) and many more. While at 2nd stage of enrichment, only 13 phylums left, which contribute to power output. In those phyla, *Proteobacteria* were the major contributor of approx.96.44% in anodic community, while rest of the phylum include *Bacteriodes* (0.96%), *Actinobacteria* (0.77%), *Cyanobacteria* (0.41%),*Acidobacteria* (0.05%), *Firmicutes* (0.06%) and others having the low contribution.



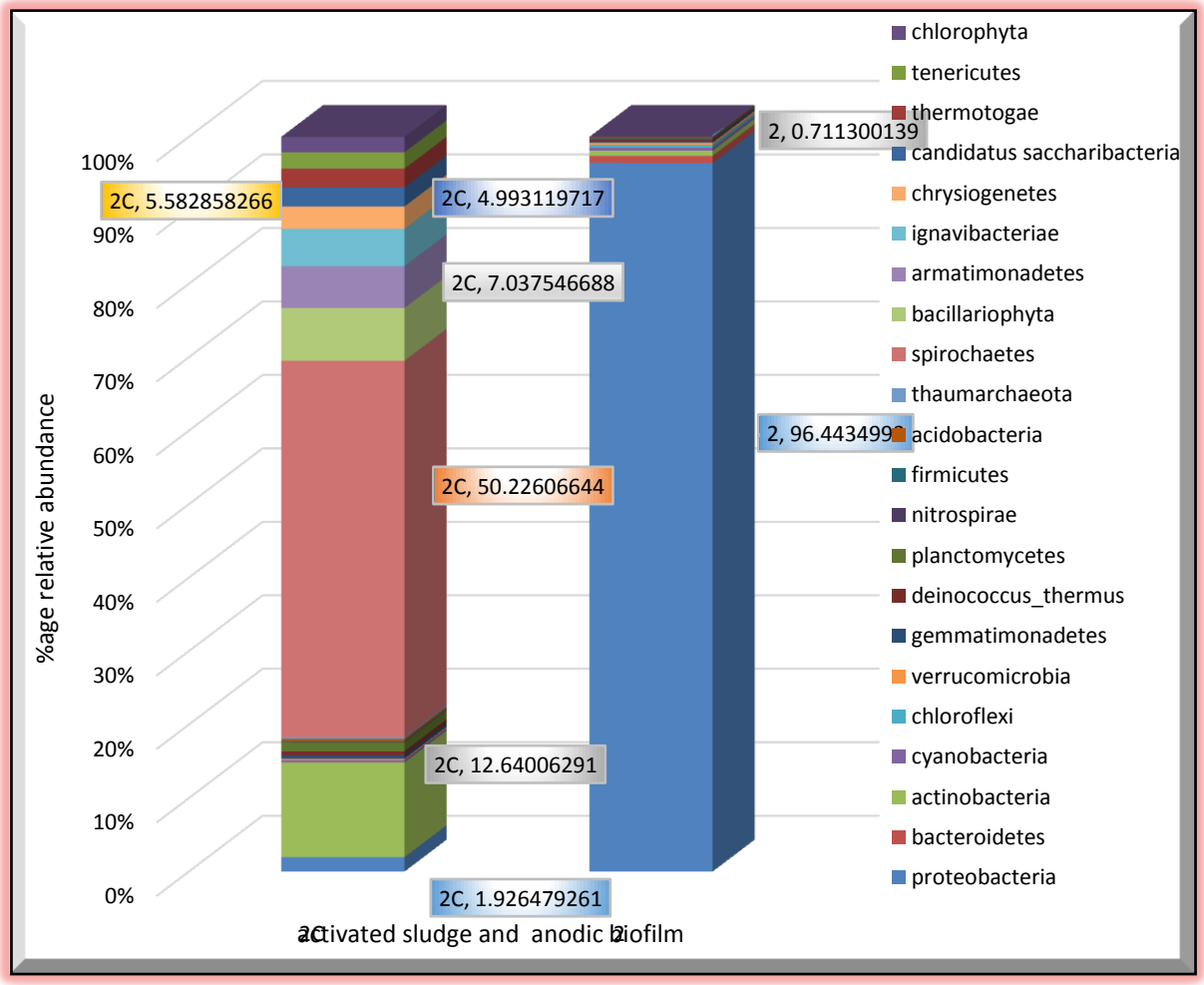
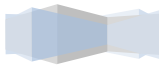


Fig. 4.9.1. %age Relative abundance at phylum level. 2C represents : activated sludge, 2 represents: anodic biofilm formed by bacteria in MFC at enrichment stage 2 (Appendix T)

On the class level distribution, in the initial activated sludge sample major %age relativeabundance was found to be of *Beta-proteobacteria* contributing 19.26% followed by *Gemmatimonadetes* (12.72%), *Gamma-proteobacteria* (11.35%), *Alpha-proteobacteria* (9.9%), *Delta-proteobacteria* (8.34%) *Actinobacteria* (5.02%), *Cllostridia* 3.67%), *Spirochaetia* (2.98%), *Erysipelotrichia* (2.59%), *Nitrospiria* (2.571%), *Sphingobacteriia* (2.53%), *Acidobacteriia* (1.46%) and many other classes which have %age relative abundance one or less than one. Subsequently, at 2nd stage of enrichment, %age relative abundance of *alpha-proteobacteria* increased to 48.51% approximately 5 times increased from initial inoculum, while *Beta-proteobacteria* (31.48%) about one times increased, *Gamma-proteobacteria* (16.16%) third largest abundant class of bacteria, *Sphingobacteriia* (0.616%) about two times decreased while rest of the classes have less than 1 percent relative abundance as shown in fig 4.9.2. Two classes of archaea werepredominant.*Thaumarchaeota* (66.6%) and *Archaeoglobi* (33.3%)while 100% was contributed by class archaeoglobi at 2nd stage of enrichment.



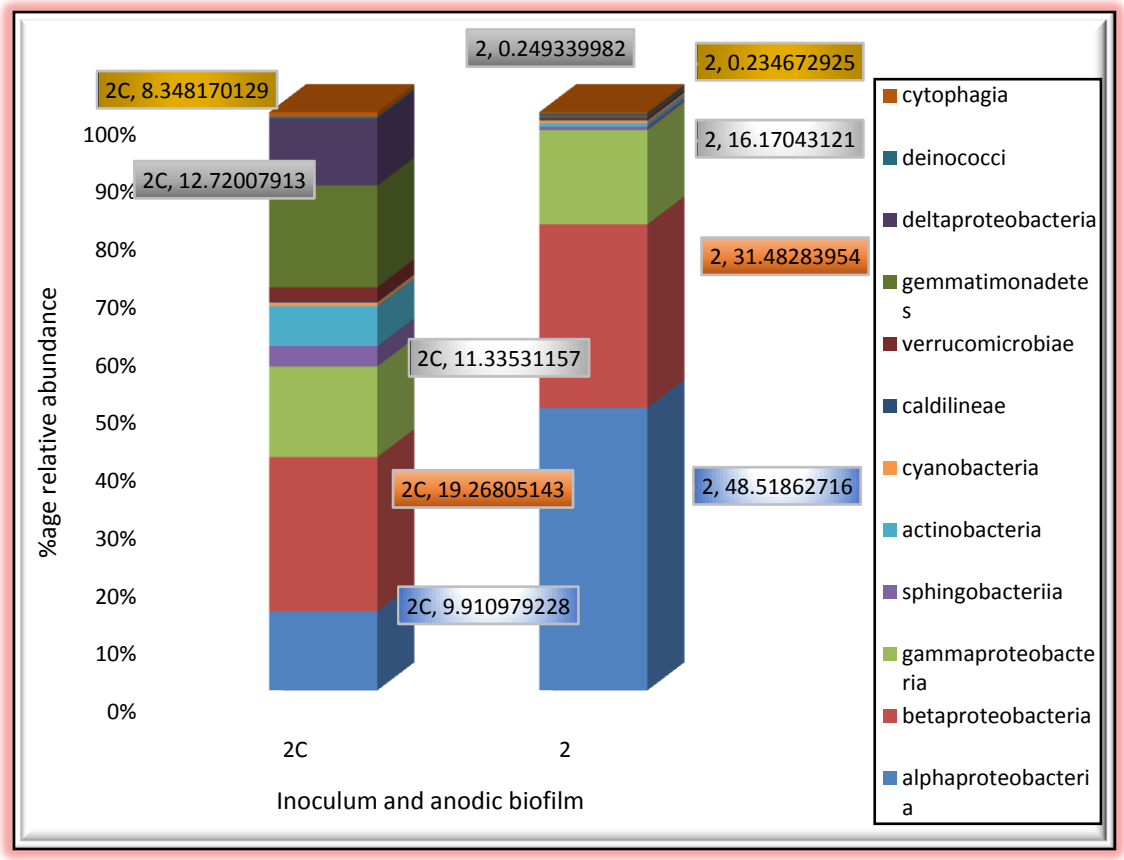


Fig 4.9.2. %age relative abundance at class level in MFC. 2C represents: sludge inoculum as control while 2 represents: anodic biofilm at enrichment stage 2 in MFC (Apendix U)

Total of 24.83% was found to becontributed by bacterium *Brevundimonas diminuta* belong to class alpha-proteobacteria,*Massilia timonae* have about 9.06% relative abundance, *Pseudomonas stutzeri* (5.918%), *Pseudoxanthomonas Mexicana* (5.148%), *Paracoccus aestuarii* (5.038%) and other species as shown in table 4.8.2.

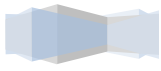
Table. 4.9.2. Bacterial species identified at enrichment stage 2 on anodic biofilm through pyrosequencing 454 (Apendix V)

Species identified	Classes	Morphological characteristics	%age Relative Abundance
Brevundimonas diminuta	*Alpha-proteobacteria*	Gram-negative, single polar flagellum, rod shaped, non-lactose fermenter(210).	24.83%
Massilia timonae	*Beta-proteobacteria*	Gram-negative, rod shaped, Non-fermentative(211).	9.064241713
Pseudomonas stutzeri	*Gamma-proteobacteria*	Gram-negative, single polar flagellum, rod shaped, motile(212).	5.918157818
Pseudoxanthomonas Mexicana	*Gamma-proteobacteria*	Gram-negative, single polar flagellum, rod shaped, motile,mesophilic(213)	5.148137284
Paracoccus aestuarii	*Alpha-proteobacteria*	Gram-negative, rods, non-motile, orange colored colonies(214)	5.03813435
Duganella spp.	*Beta-proteobacteria*	Purple pigment producing Violacein, mesophilic(215, 216)	3.886770314
Nitrosomonas europaea	*Beta-proteobacteria*	Gram-negative, obligate chemolithoautotroph, single polar flagellum(217)	3.087415664
Bosea thiooxidans	*Alpha-proteobacteria*	Gram negative, chemolithtrophic, oxiders of sodium thiosulfate,(218)	2.940745087
Limnobacter spp.	*Beta-proteobacteria*	Anaerobic, Gram-negative, non spore-former(219)	2.691405104
Alcaligenes sp.	*Beta-proteobacteria*	Gram-negative, aerobic, peritichous flagellum, rod shaped bacterium(220)	2.038721033
Paracoccus marcusii	*Alpha-proteobacteria*	Orange, Gram negative coccus(221)	1.730712819
Methyloversatilis universalis	*Beta-proteobacteria*	Gram-negative, curved, slightly rods, non-motile(222)	1.628043414
Achromobacter xylosoxidans	*Beta-proteobacteria*	Gram negative, peritritious flagellum, aerobic(223).	1.554708126
Mycoplana spp.	*Alpha-proteobacteria*	Gram-negative, chemoorganotrophic (224).	1.488706366

4.10 Bacterial Community Structure Of Electrogenic Bacteria From Soil(S3) Using Pyrosequencing

In the initial submerged soil sample it has been investigated that major %age relative abundance of kingdom bacteria comprises of 99.96% while archaea presence was less to about 0.0395%. After 2nd enrichment step in double chamber microbial fuel cell, the community associated with anodic surface were comprised of kingdom bacteria (100%). While Archaeal community vanished completely after 2nd enrichment.

On the phylum level classification it has been observed that in soil sample about 22 phylums were present, out of which Proteobacteria have relatively higher abundance approx. 63.94% followed by *Acidobacteria* (10.78%), *Chloroflexi* (7.86%), *Firmicuties* (3.75), *Bacteroidetes* (2.58%),*Gemmatimonadetes* **(**1.65%),*Verrucomicrobia* (1.65%), *Planctomycetes* (1.38%), *Nitrospirae* (1.05%) and rest of the phylum have less than 1% relative abundance. After 2nd stage of enrichment, anodic biofilm bacterial community accounts for electrochemically active bacteria. It has been observed that after 2nd stage of enrichment anodic bacteria community comprised of *Proteobacteria* having a highest relative abundance (94.42%), about half times increased after enrichment while *Verrucomicrobia* increased about 2 times than before (3.20%), Firmicutes reduced about three times (0.82), phylum *Bacteroidetes* was also reduced about 2 times (0.60%). A major shift was observed in Phylum *Acidobacteria* which have % relative abundance about 0.45%, it means it decreases about 10 times after 2nd stage of enrichment. Phylum *Actinobacteria* having 0.15% and *Nitrospirae* 0.12% relative abundance, while rest of the phylums have less than 0.01% percentage relative abundance.



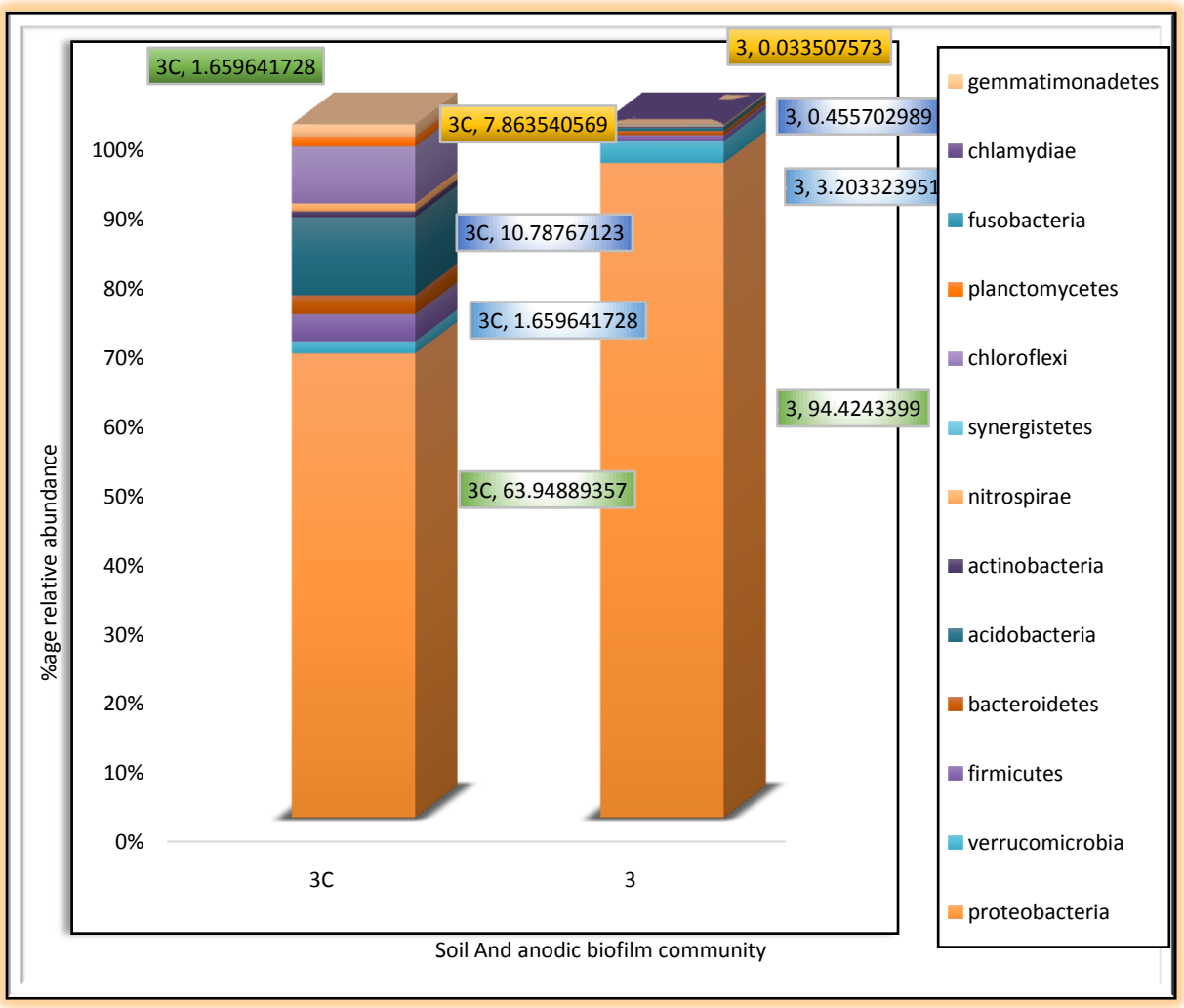
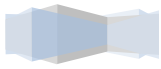


Fig 4.10.1. %age relative abundance at phylum level in soil inoculum and anodic biofilm community at enrichment stage 2 in MFC. 3C represents: Submerged soil (S3), while 3 represents biofilm formed on anode surface at enrichment stage 2(Apendix W)

It has been observed that soil community is more diverse than sludge community. On the class level distribution it has been investigated that about 41 different classes were present in soil sample. Highest abundance was found to be of *Alpha-Proteobacteria* accounts for 18.479%, followed by *Gamma-Proteobacteria* (16.74%),

beta-Proteobacteria (16.39%), *delta-Proteobacteria* (12.05), *Acidobacteriia* (8.32%), (3.33%) *Anaerolineae*, *Clostridia* having %age abundance of about 2.41%, *Holophagae* (2.305%), *Caldilineae* (2.05%), *Gemmatimonadetes* (1.65%), *Sphingobacteriia* (1.40%), *Chloroflexia* (1.40%) *Verrucomicrobiae*(1.27%), *Nitrospira* (1.053%), *Planctomycetia* (1.04%) while rest of the classes have less than 1% relative abundance. After 2nd stage of enrichment, anodic biofilm bacterial community classes were reduced to 20. It has been observed from the pyrosequencing data analysis that anodic bacterial community comprised of major class *gamma-Proteobacteria* which have relative abundance of about 68.301% which indicates that after enrichment γ-*Proteobacteria* increased about 4 times than before. Rest of the classes and their abundance includes *β-Proteobacteria* (23.30%) about half times increased after enrichment, Opitutae (3.18%), *alpha-Proteobacteria* decreased from 18,4% to 2.69%, *Bacilli* and *Sphingobacteriia* having an abundance of about 0.77% and 0.583% while rest of the classes have less than 0.5% relative abundance.



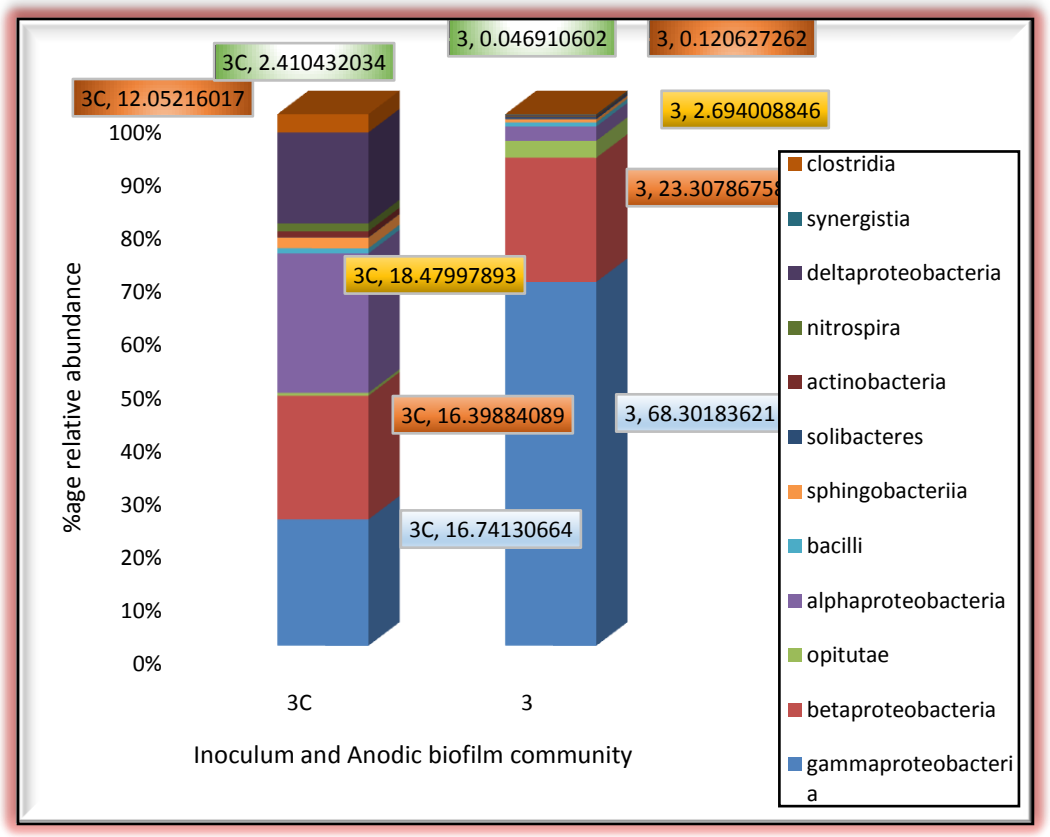


Fig 4.9.2. %age relative abundance at class level in soil inoculum and anodic biofilm bacterial community at enrichmentstage 2 in PEM-MFC. 3C represents: Soil(S3) as control and 3 represents: Anodic biofilm bacterial community at enrichment stage 2 (Apendix X)

442 species were identified from soil sample while after 2nd stage of enrichment in biofilm bacterial community the number of species were reduced to 142. Here only the bacterial species identified on anodic surfaces were presented. Highest relative abundance on the anodic surface was found to be of*Pseudomonas spp.* of about 35.73% followed by *Methyloversatilis universalis* (16.237%),

Pseudomonas plecoglossicida (7.16%), *Pseudoxanthomonas Mexicana* (5.589%), *Pseudomonas monteilii* (5.52%), *Pseudomonas oryzihabitans* (4.73%),

Pseudomonas putida (3.67%), *Opitutus sp.* Having a relative abundave of 2.57% etc. all of these bacteria and few others, their description to which class they belong morphological characteristics and their %age relative abundance were presented in table 4.10.1.

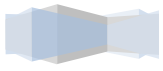
Table.4.10.1. Name of species identified on Anodic surface at 2nd stage of enrichment and their classification on class level, morphological description and %age relative abundance.(Apendix Y)

Name of Species	Classes	Morphological Characteristics	%age relative abundance
Pseudomonas spp.	*Gamma-Proteobacteria*	Gram negative, one or more polar flagella, Aerobic, yellow-green siderophores(225).	35.73247554
Methyloversatilis universalis	*Beta-proteobacteria*	Gram-negative, curved, slightly rods, non-motile(222).	16.23776974
Pseudomonas plecoglossicida	*Gamma-Proteobacteria*	Gram-negative, rod shaped, motile, non-flourescent(226).	7.163919046
Pseudoxanthomonas mexicana	*Gamma-Proteobacteria*	Mesophilic, gram-negative, motile,polar flagellum, rod shaped(227).	5.589063128
pseudomonas monteilii	*Gamma-Proteobacteria*	Gram-negative, motile, rod shaped(228).	5.528749497
Pseudomonas oryzihabitans	*Gamma-Proteobacteria*	Yellow-pigmented,ros shaped, Gram-negative	4.731269267
Pseudomonas putida	*Gamma-Proteobacteria*	Gram-negative, rod shaped, saprotropic soil bacterium(228)	3.672429969
Opitutus sp.	*Chlamydiae*	Gram negative, motile, saprotropic soil bacterium,un pigmented(229)	2.573381584
Methyloversatilis spp.	*Beta-proteobacteria*	Gram nehative, curved slightly rod shaped, nonmotile.	2.466157352
Pseudomonas taiwanensis	*Gamma-Proteobacteria*	Noval, gram-negative, rod shaped, motile ,nonspore forming(230).	1.434124112

Acidovorax facilis	*Beta-proteobacteria*	Aerobic, chemoorganotrophic, rod shaped, gram-negative, single polat flagellum	1.38721351
Pseudomonas sp.	*Gamma-Proteobacteria*	Gram negative, rod shaped, Pigmented, motile(231).	1.025331725
Pseudomonas stutzeri	*Gamma-Proteobacteria*	Denitifing bacteria, Gramotile by single polar flagellum-negative,rod shaped(232)	1.011928696
Pseudomonas vancouverensis	*Gamma-Proteobacteria*	Gram-negative soil bacterium.	0.898002949
Bacillus spp.	*Bacilli*	Gram-positive, facultative anaerobe, rod shaped(233)	0.777375687
Balneola spp.	*Sphingobacteria*	Aerobic, Gram-negative bacterium(234).	0.562927222

4.11Scanning Electron Microscopy Of Carbon Cloth Containing Biofilm Developed At 2nd stage of Enrchment Using ActivatedSludge

Scanning electron microscopy has revealed that complex bacterial consortia was forming biofilm on anode surface after enrichment on carbon cloth using K acetate as carbon souce. Different cocci and rod- shaped bacteria were present.Both rod-shaped bacteria and cocci were shown to interact with each other, forming consortium.



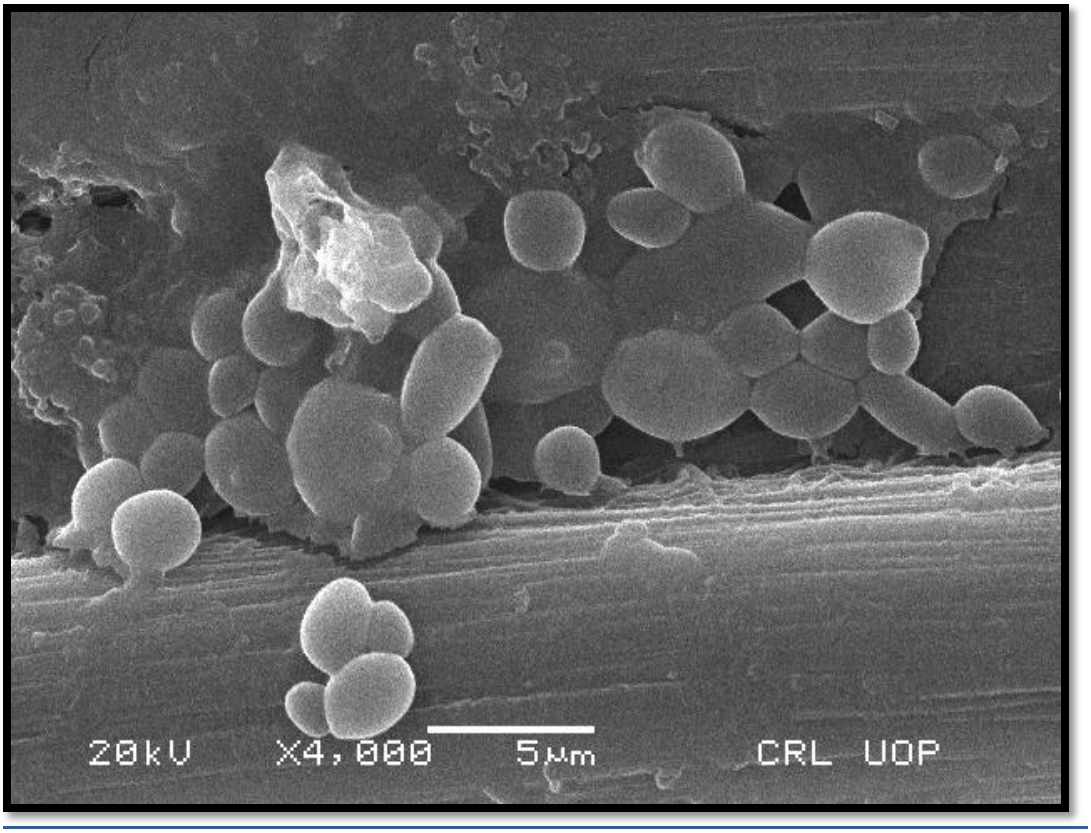


Fig 4.11.1. SEM image of anodic biofilm growth on carbon cloth. Clusters of bacteria were shown to be present on fiber of carbon cloth

At slightly higher resolution another image of SEM has been shown in fig 4.10.2. As shown in fig 4.10.2, a mesh of bacteria were present on carbon cloth.

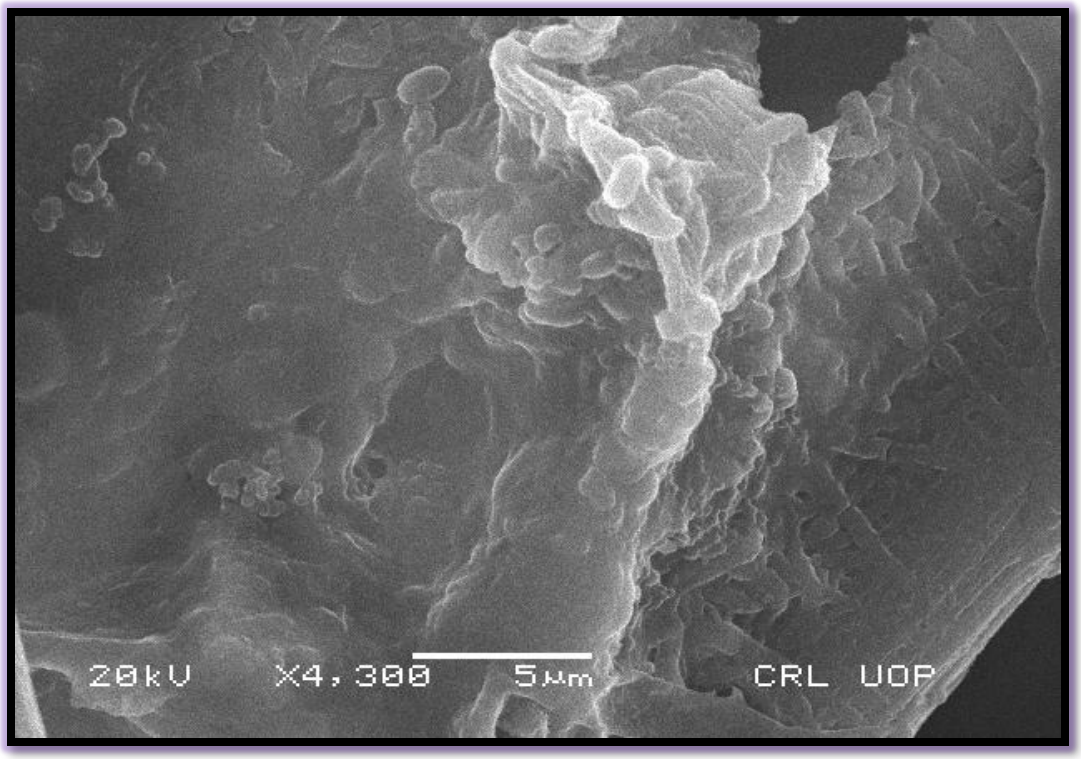


Fig 4.11.2. SEM imageof anodic biofilm community at 4,300 resolution

4.12 Scanning Electron Microscopy Of Carbon Cloth Containing Biofilm Developed At Enrchment Stage 2 Of electrogenic Bacteria Using Submerged Soil(S3)

SEM image of anodic biofilm with soil sample(S3) has revealed that majority of rod-shaped bacteria were present on the carbon cloth fiber, interacting with each other. Clusters of rod- shaped bacteria forming network through out the biofilm. Mostly, these rod shaped bacteria may belong to γ- and β-*Proteobacteria* as shown by the diversity analysis of soil biofilm community.

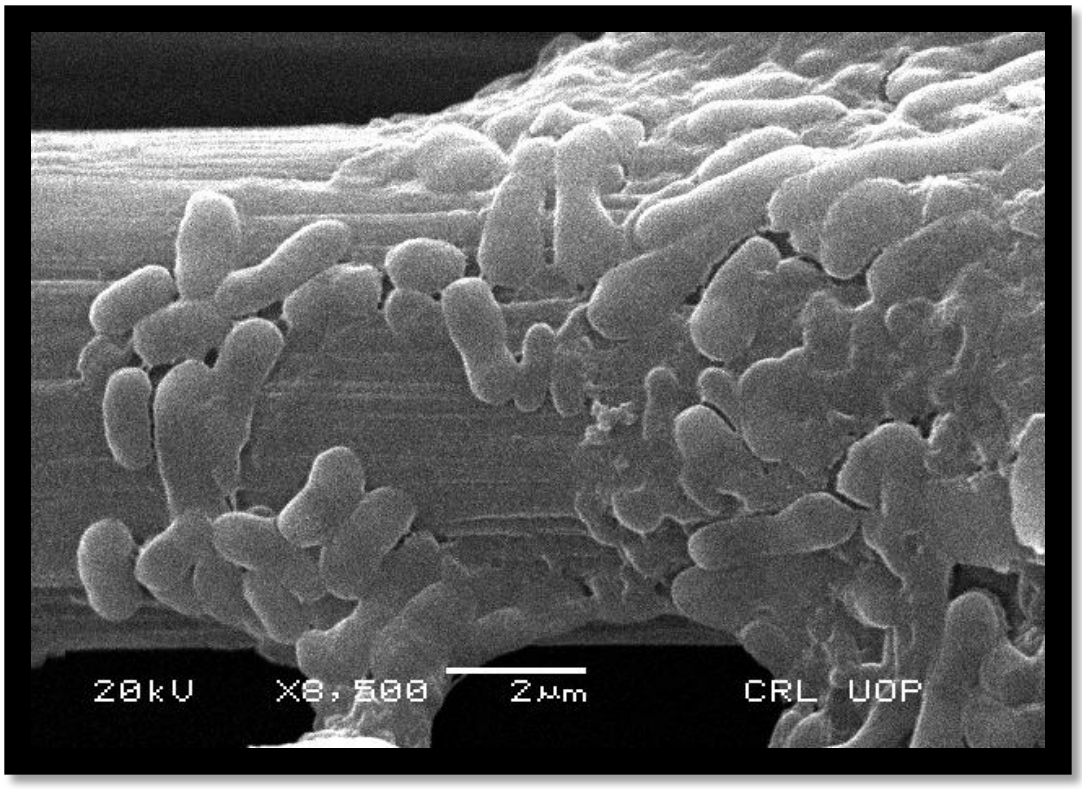


Fig 4.12.1. SEM image of anodic biofilm community from soil (S3)

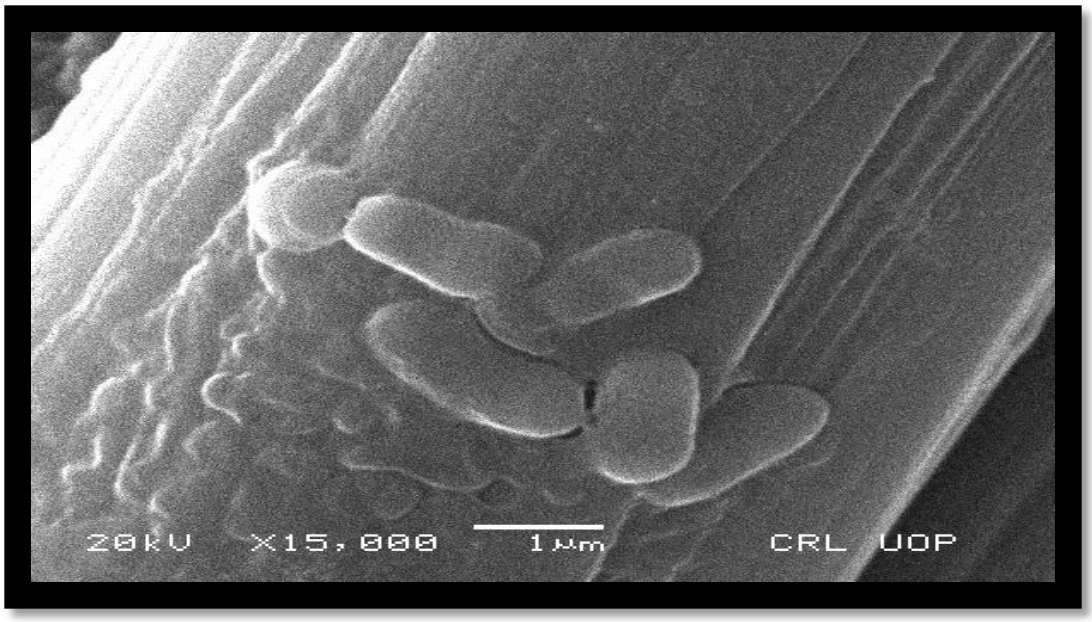


Fig 4.12.2. SEM image of anodic biofilm enriched at stage 2 from soil (S3) at 15000 resolution

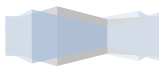
4.13 Biochemical Characterisation Of Bacteria Isolated From Anodic Biofilm

Microorganisms isolated from anode chamber include suspended microorganisms along with attached microbes on anode surface. These microorganisms were further streaked on Nutrient agar, Blood agar and MacConkey agar and different morphological charateristics and gram staining results were noted and shown in table:

Table 4.13.1. Biochemical identification tests for the characterization of bacteria iolated from anodic biofilm

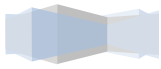
Isolate Name	Biofilm Forming Capbitity	Catalase	oxidase	Motility	indole	H2S	TSI	G staining	Possible Identification
WB4	MA	+	+	+	-	-	R/Y	G- Rods	*Pseudomona aeruginosa*
WBF6/7	SA	+	-	+	-	+	R/Y+H2S	G- Rods in chains	*Salmonella spp.*
WBF9/1	MA	+	+	+	-	-	R/Y	G- Rod	*Proteus mirabilis*
WE1	MA	+	-	+	-	-	R/Y	G- Rods	*E.coli*
WE8	MA	+	+	+	-	+	Y/Y	G- rod	*Citrobacter frundii*
WE3	MA	-	W+	+	-	+	R/Y	G- Diplobacilli	*Vibrio metschnikovii*
WEF1	MA	+	+	+	-	-	R/Y	G- Rods	*Proteus mirabilus*
WEF2	MA	+	+	+	-	-	R/Y	G- bacilli	*E.coli*
WEFA1/2	MA	+	+	+	-	-	R/Y	G- Rods	*Pseudomona aeruginosa*
S3B4	MA	+	+	+	-	-	R/Y	G- rods in	*Pseudomona*

								chains	*aeruginosa*
S3BF1/2	MA	+	+	+	-	-	R/Y	G- Rods	*Serratia marcescens*
S3B9/10	SA	+	+	+	-	-	R/Y	G- Diplobacilli	*Pseudomona aeruginosa*



CHAPTER#5

DISCUSSION



Discussion

Global energy demand is increasing rapidly with the development of science and technology(17, 202). Energy reserves are being depleting faster than new alternative energy generation discoveries made by researchers(29). Developed as well as developing countries are facing serious energy shortage issues(235). It has been estimated that energy demand would be projected to more than 50% by 2025(6, 7). In addition, conventional waste water treatment plants required high energy input(9). So, there is need to develop some effective treatment technique which will be cost effective and operate at low energy input(17, 202). In the current scenario when the energy problem is on hype, the researchers are trying to explore new alternate non-fossil fuel energy resources(202). One common consensus among scientific community is to adopt multi-faced approach to alleviate current energy crises(202). Geothermal(236, 237), wind(238), nuclear(239), solar(240) and bioenergy all play an important role(241, 242). Microbial fuel cell could be alternative solution to this dilemma (29, 30, 243, 244). Microbial fuel cells (MFCs) is a new approach which electrochemically convert organic substrate containing chemical energy directly into electric current(29, 30). The distinct advantage of this technology is to exploit the low-grade organic contents even waste water which otherwise not being used(42). So, the microbial power is being utilized in MFC to simultaneously generate electricity and treat waste water (36, 202, 245, 246). Consequently, the high energy content of waste water is no more a waste, but seen as valuable energy reserve.

Numerous research studies has been done to investigate the reactor configuration, design, electrodes being used and operational parameters(36). So, the energy output has been improved but the main element of MFC "Microorganisms" have not been well investigated to maximize the power output(64). Recently, the knowledge of these electrochemically active bacteria has been expanded greatly, known as electricigens or anode respiring bacteria(64) like *Rhodoferax ferrireducens*(42), Geobacter sulfurreducens(4, 162, 247),

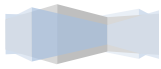
Pseudomonas aeruginosa(57, 59),*Aeromonas Hydrophila*(69),*Escherichia coli*(17, 116, 146),*Shewanella putrefaciens*(17),Enterococcus faecium(31, 248, 249)(64). Different reactor configurations have been used in many research studies. Typically a MFC consist of two chambers named as anode and cathode. In between the two chambers proton conducting material is present connecting the two chambers(12). The basic Principle used in this technology is very simple. Organic contents are metabolized by bacteria in anodic chamber, in results protons and electrons are produced (13, 14, 16, 129). Electrons are transported to the cathode through external circuit while protons transferred through proton conducting material for electricity generation(250).

In the current research study, double chamber microbial fuel cell has been constructed with two different proton conducting material. In one type of MFC agar salt bridge was used while in second type Nafion membrane 115 was used for proton conduction in between the two chambers. This type of architecture was inexpensive and widely used on lab-scale studies although it was complex to handle and pose problems when need to be scaled up. Two types of bacterial inoculums (Activated Sludge and Submerged Soils) were used as bacterial source for simultaneous break down of complex substrate and current generation. Salt bridged MFC were operated with activated sludge with two different carbon sources (Sucrose and Potassium acetate) while submerged soils named as S2 and S3 were operated in nafion membrane containing MFC under agitation at 50rpm. It has been observed that fuel cell operated with sucrose has better efficiency than Potassium acetate. In sucrose fed cell, maximum voltage and power output was 347Ω and 40.136µW when the circuit was connected through 3kΩ. While after enrichment the voltage and power output was dropped to 74.9mV and 1.87µW. the low voltage generation may be due to the reason of electrochemically activity of the bacterial community was maybe formed by the activity of two or more electrogenic bacterial isolates, which work in consortium by cooperating with each other in terms of power output (251). So, may be after enrichment consortium of such electricigens braked because may be after enrichment only few bacteria were capable to grow and power output was reduced overall.

Similarly, cell operated with K acetate maximum voltage and power produced were of 28.8mV and 0.27 µW. after enrichment the voltage and power dropped to about 24mV and 0.192 µW. the decreased may be due to the same reason may be only dilutions of electricigens and breakage of consortium that are actively playing role in power output(199, 251). It has been observed that electrochemically active bacteria are better adopted in sucrose fed cell compared to k Acetate while in literatue it has be reported that electrogenic bacteria were better adopted in Acetate fed medium(252). Membrane containing fuel cell was operated with Soil sample under agitation at 50rpm. It has pbserved that soil took a long time to get stabilized and produce stable power output. It has been observed that voltage and poer output was increased after enrichment to about 332mV and 3674.3µW. this may be due to agitation factor and nafion membrane as proton conducting material. As in current generation along with other rate limiting steps diffusion is also one factor. Proximity of the bacteria to electrode surface and eventually the controlled electron diffusion to electrode surface is the function of diffusion. Under static conditions self mixing do occurs due to the production of CO_2. In soil inoculum containing fuel cell agitation may eleminates this diffusion-limited step(12, 45).

Resistance have a negative effect on current and power production while positive on voltage output(253, 254). In all experiments shown in fig:4.1.2a, 4.2.2b, 4.3.2a, 4.4.2b, 4.5.2a, 4.6.2b somewhat unusual behavior was observed at the start of experiments. Current increases with increase in resistance this may be because of lower electron utilization rate at cathode than rate of electron transfer through outer circuit. May be due to less proton transfer or limited oxygen supply(9, 255). Lower current production means by some other mechanisms electrons are being consumed(28, 250).

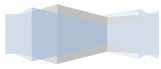
A few studies have been done to isolate pure cultures from wild multi-species biofilms(256, 257). Bacterial strains were always preceded by a phase of enrichment that promotes the growth of a given type of bacteria selected according to the nutritional and physicochemical conditions of the medium.



Techniques that allow access to individual strains introduce a partiality inherent in culture-dependent methods in two ways. The growth of some species, genera and families is favoured depending on the culture conditions and the medium used. Furthermore, only a very small proportion of the microorganisms contained in environmental samples is cultivable. It has been estimated that only 0.001–0.1 % of the bacterial isolates were contained in seawater that can be cultivated using conventional microbiological techniques(258). In freshwater sediments the ratio is around 0.25% and up to 15% to activated sludge. It has been studied bacterial classes identified in plain granular graphite microbial fuel cell operated with anoxic sludge for 40 days across 30Ω were *proteobacteria* accounts for 50% followed by 21.6% of *bacteroides*, 9.5% of *alpha-proteobacteria*, 8.1% *chlorobi*, *4.1% delta-proteobacteria*,4.1% *actinobacteria* and 2.6% of *gamma-proteobacteria(259).* At 250Ω, in untreated glassy carbon microbial fuel cell run with activated sludge for 10 months, it has been fount that *alpha-proteobateria, firmicutes, beta-proteobacteria, gamma-proteobacteria, bacteroidetes*were predominant(260). In plain granular graphite MFC operated for 400 days across 30Ωwith anaerobic/anoxic sludge predominated by *proteobacteria* followed by*bacteroidetes, actinobacteria, planctomycetes, firmicutes* and uncultured bacteria(261).In aerobic sludge inoculum operated graphite granules MFC for 180 days, it has been found that predominant bacteria class were delta-proteobacteria(262)while in graphite felt MFCoperated for 48 days with mixture of environmental samples from river-rusted metal were predominated by*alpha-proteobacteria, bacteroidetes, gamma-proteobacteria(256).*Is their any significant effect of enrichment on the bacterial growth as compared to original inoculum?It has been found in previous reports that after enrichment the bacterial community attaced to anosic surface was significantly different from the original inoculum sources. The dominant sequences in attached bacterial population were found be affiliated with δ-*Proteobacteria*which showed high similarity with other bacterial communities isolated from different MFCs. In the current research studies, it has been detected that yes there is signifianct difference between initial inoculum and biofilm bacterial community after enrichment as shown in fig 4.9.2. it has

been fount that in activated sludge inoculum at enrichment 2nd stage, the biofilm community appreared on anode were predominantly belonged to *α-Proteobacteria*(48.5%) followed by *β-Proteobacteria* (31.48%),

γ-Proteobacteria(16.17%), *sphinogobacteria, actinobacteria* and other classes whing have less relative contribution.*α-Proteriobacteria* were commonly found in sludge and soil samples. It has been observed that soil community is more diverse than sludge community. On the class level distribution it has been investigated that about 41 different classes were present in soil sample. Highest abundance was found to be of *Alpha-Proteobacteria* accounts for 18.479%, followed by *Gamma-Proteobacteria* (16.74%) while rest of the classes have less relative abundance. After 2nd stage of enrichment, anodic biofilm bacterial community classes were reduced to 20. It has been observed from the pyrosequencing data analysis that anodic bacterial community comprised of major class *gamma-Proteobacteria* which have relative abundance of about 68.301% which indicates that after enrichment γ-*Proteobacteria* increased about 4 times than before. Rest of the classes and their abundance includes *β-Proteobacteria* (23.30%) about half times increased after enrichment, Opitutae (3.18%), *alpha-Proteobacteria* decreased from 18,4% to 2.69%, *Bacilli* and *Sphingobacteriia* having an abundance of about 0.77% and 0.583% while rest of the classes have less than 0.5% relative abundance.



Conclusion

It is concluded from the present study that both the samples; soil (S3) and activated sludge have a considerable amount of power generation capability containing electrochemically active bacteria

In salt bridge Microbial fuel cell fed with two different carbon sources, it is determined that electrochemically active bacteria were better capable of utilizing sucrose as carbon source instead of Potassium Acetate. Better efficiency is achieved in fuel cell operated with mixed flora of activated sludge with sucrose as carbon source without the addition of exogenous electron mediators than Potassium acetate.

It is deduced that Power output efficiency is better in Membrane containing fuel cell than salt bridge MFC.

It is concluded that soil(S3) contain more diverse bacterial flora having (approximately 442 bacterial species) than activated sludge (268 species detected).

The power efficiency (36.74µW) is better achieved with soil sample than activated sludge(1.87µW).

It is concluded that agitation has a positive effect on current production (0.116mA) than under static conditions (0.024mA) after enrichment.

The findings of the current study shows resistance had a negative effect on current production while positive effect on voltage generation.

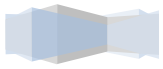
From the diversity analysis of soil, activated sludge and the biofilm formed on anodic surfaces by the bacterial communities from these samples after enrichment, it is concluded that enrichment has a great effect on the selection of electrochemically active bacteria.

From the biofilm formation analysis, it has been deduced that both samples contain weak, moderate and strong biofilm formers but majority of weak biofilm formers were present.

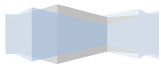
Future Prospects

Microbial fuel cell is still a new discipline a lot of efforts needed for its gromming and achieving highest possible results.

- It in the need of time to scale up MFC architecture and optimization of process to attain high power output.
- Further improvementare needed to be made in MFC configurations to improve energy recovery or to increase voltages by linking MFCs in series, resulting in new technologiesthat will make electricity generation using MFCs a practical method of wastewater treatment.
- Energy losses through overpotentials and other ways needed to be reduce by applying effective anode or cathode catalyst, cultivating and genetically modifying the electrochemically active bacterial consortia, and increasing the surface area of electrodes for microbial attachment and redox reactions may reduce this overpotential.
- Enrichment strategies needed to be improved that consider the effect of system engineering design and operational factors.
- One of the most critical step in the MFC process is the bacterial transfer of electrons to the anodic surface. Thus the mechanisms associated with anodic electron transfer and the metabolic pathways governing the conversion of substrate to electricity in MFCs needed to be investigated.
- An understanding of the prevailing side reactions in the anodic compartment (e.g., methanogenesis and aerobic oxidation) that bypass anodic electron transfer and designing the strategies for their proper control and inhibition are required. These strategies should lead to improvement in substrate oxidation and coulombic efficiency of MFCs
- Currenty, knowledge is scarce on interspecies associations among bacteria and



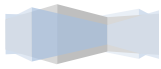
their interactions with various design and operational factors in microbial fuel cells. There are yet many questions remained to be unanswered at the interface of biology and electrochemistry in MFCs; 1) How do bacterial populationdevelop to respire with the anode as an electron acceptor and maintain that function?, 2) What are the underlying principles of microbial enrichment in MFCs?, 3) What bacterialstructure and functional group are optimal for electricity production?, 4) What operational conditions promotes the formation of the desired community with its desired function?, 5) Are the conditions and outcomes reproducible,predictable and controllable?, and, 6) What specific methods and procedures are required to control the conditions?



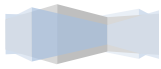
Refrences

1. **Li J.** 2013. An Experimental Study of Microbial Fuel Cells for Electricity Generating: Performance Characterization and Capacity Improvement. Journal of Sustainable Bioenergy Systems **3:**171.
2. **Khan MR, Karim M, Amin M.** 2012. Generation of Bio-electricity by Microbial Fuel Cells. International Journal of Engineering & Technology **1:**231-237.
3. **Rasool G, Ehsan F, Shahbaz M.** 2015. A systematic literature review on electricity management systems. Renewable and Sustainable Energy Reviews **49:**975-989.
4. **Bond DR, Lovley DR.** 2003. Electricity production by Geobacter sulfurreducens attached to electrodes. Applied and environmental microbiology **69:**1548-1555.
5. **Catal T, Li K, Bermek H, Liu H.** 2008. Electricity production from twelve monosaccharides using microbial fuel cells. Journal of Power Sources **175:**196-200.
6. **Deval A, Dikshit AK.** 2013. Construction, working and standardization of microbial fuel cell. APCBEE Procedia **5:**59-63.
7. **Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Frederick WJ, Hallett JP, Leak DJ, Liotta CL.** 2006. The path forward for biofuels and biomaterials. science **311:**484-489.
8. **Zhou M, Wang H, Hassett DJ, Gu T.** 2013. Recent advances in microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) for wastewater treatment, bioenergy and bioproducts. Journal of Chemical Technology and Biotechnology **88:**508-518.
9. **Ghangrekar M, Shinde V.** Microbial fuel cell: a new approach of wastewater treatment with power generation, p 20-21. *In* (ed),
10. **Octave S, Thomas D.** 2009. Biorefinery: toward an industrial metabolism. Biochimie **91:**659-664.

11. **Lovley DR.** 2008. The microbe electric: conversion of organic matter to electricity. Current opinion in Biotechnology **19:**564-571.
12. **S Mathuriya A, Sharma V.** 2010. Bioelectricity production from various wastewaters through microbial fuel cell technology. Journal of Biochemical Technology **2:**133-137.
13. **S Mathuriya A, Sharma V.** 2009. Bioelectricity production from paper industry waste using a microbial fuel cell by Clostridium species. Journal of Biochemical Technology **1:**49-52.
14. **Allen RM, Bennetto HP.** 1993. Microbial fuel-cells. Applied biochemistry and biotechnology **39:**27-40.
15. **Kim BH, Ikeda T, Park HS, Kim HJ, Hyun MS, Kano K, Takagi K, Tatsumi H.** 1999. Electrochemical activity of an Fe (III)-reducing bacterium, Shewanella putrefaciens IR-1, in the presence of alternative electron acceptors. Biotechnology Techniques **13:**475-478.
16. **Rosenbaum M, Zhao F, Quaas M, Wulff H, Schröder U, Scholz F.** 2007. Evaluation of catalytic properties of tungsten carbide for the anode of microbial fuel cells. Applied Catalysis B: Environmental **74:**261-269.
17. **Liu H, Ramnarayanan R, Logan BE.** 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environmental science & technology **38:**2281-2285.
18. **Min B, Logan BE.** 2004. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environmental science & technology **38:**5809-5814.
19. **Dentel S, Strogen B, Chiu P.** 2004. Direct generation of electricity from sludges and other liquid wastes. Water Science & Technology **50:**161-168.
20. **Tiehm A, Nickel K, Zellhorn M, Neis U.** 2001. Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. Water Research **35:**2003-2009.



21. **Ra C, Lo K, Shin J, Oh J, Hong B.** 2000. Biological nutrient removal with an internal organic carbon source in piggery wastewater treatment. Water Research **34:**965-973.
22. **Sevrin-Reyssac J.** 1998. Biotreatment of swine manure by production of aquatic valuable biomasses. Agriculture, ecosystems & environment **68:**177-186.
23. **Van Ginkel SW, Oh S-E, Logan BE.** 2005. Biohydrogen gas production from food processing and domestic wastewaters. International Journal of Hydrogen Energy **30:**1535-1542.
24. **Maekawa T, Liao C-M, Feng X-D.** 1995. Nitrogen and phosphorus removal for swine wastewater using intermittent aeration batch reactor followed by ammonium crystallization process. Water Research **29:**2643-2650.
25. **Reimers CE, Tender LM, Fertig S, Wang W.** 2001. Harvesting energy from the marine sediment-water interface. Environmental science & technology **35:**192-195.
26. **Wingard LB, Shaw CH, Castner JF.** 1982. Bioelectrochemical fuel cells. Enzyme and Microbial Technology **4:**137-142.
27. **Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH.** 2002. A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. Enzyme and Microbial Technology **30:**145-152.
28. **Gil G-C, Chang I-S, Kim BH, Kim M, Jang J-K, Park HS, Kim HJ.** 2003. Operational parameters affecting the performannce of a mediator-less microbial fuel cell. Biosensors and Bioelectronics **18:**327-334.
29. **Du Z, Li H, Gu T.** 2007. A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnology advances **25:**464-482.
30. **Logan BE, Regan JM.** 2006. Microbial fuel cells-challenges and applications. Environmental science & technology **40:**5172-5180.



31. **Rabaey K, Boon N, Höfte M, Verstraete W.** 2005. Microbial phenazine production enhances electron transfer in biofuel cells. Environmental science & technology **39:**3401-3408.
32. **Rao J, Richter G, Von Sturm F, Weidlich E.** 1976. The performance of glucose electrodes and the characteristics of different biofuel cell constructions. Bioelectrochemistry and Bioenergetics **3:**139-150.
33. **Pant D, Van Bogaert G, Diels L, Vanbroekhoven K.** 2010. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresource technology **101:**1533-1543.
34. **Rabaey K, Boon N, Siciliano SD, Verhaege M, Verstraete W.** 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. Applied and environmental microbiology **70:**5373-5382.
35. **Lin CQ.** 2013. Simultaneous power generation and waste water treatment using microbial fuel cell.
36. **Hernández-Fernández F, de los Ríos AP, Salar-García M, Ortiz-Martínez V, Lozano-Blanco L, Godínez C, Tomás-Alonso F, Quesada-Medina J.** 2015. Recent progress and perspectives in microbial fuel cells for bioenergy generation and wastewater treatment. Fuel Processing Technology **138:**284-297.
37. **Davis F, Higson SP.** 2007. Biofuel cells—recent advances and applications. Biosensors and Bioelectronics **22:**1224-1235.
38. **Park DH, Zeikus JG.** 2000. Electricity generation in microbial fuel cells using neutral red as an electronophore. Applied and environmental microbiology **66:**1292-1297.
39. **Tokuji I, Kenji K.** 2003. Vioelectrocatalyses-based application of quinoproteins and quinprotein-containing bacterial cells in biosensors and biofuel cells. Biochim Biophys Acta **1647:**121-126.
40. **Vega CA, Fernández I.** 1987. Mediating effect of ferric chelate compounds in microbial fuel cells with Lactobacillus plantarum, Streptococcus lactis, and Erwinia dissolvens. Bioelectrochemistry and Bioenergetics **17:**217-222.

41. **Kim B-H, Kim H-J, Hyun M-S, Park D-H.** 1999. Direct electrode reaction of Fe (III)-reducing bacterium, Shewanella putrefaciens. Journal of Microbiology and Biotechnology **9:**127-131.

42. **Chaudhuri SK, Lovley DR.** 2003. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nature biotechnology **21:**1229-1232.

43. **Schröder U, Nießen J, Scholz F.** 2003. A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude. Angewandte Chemie International Edition **42:**2880-2883.

44. **Min B, Cheng S, Logan BE.** 2005. Electricity generation using membrane and salt bridge microbial fuel cells. Water research **39:**1675-1686.

45. **Ieropoulos IA, Greenman J, Melhuish C, Hart J.** 2005. Comparative study of three types of microbial fuel cell. Enzyme and microbial technology **37:**238-245.

46. **Debabov V.** 2008. Electricity from microorganisms. Microbiology **77:**123-131.

47. **Qian F, Morse DE.** 2011. Miniaturizing microbial fuel cells. Trends in biotechnology **29:**62-69.

48. **Qiao Y, Bao S-J, Li CM.** 2010. Electrocatalysis in microbial fuel cells—from electrode material to direct electrochemistry. Energy & Environmental Science **3:**544-553.

49. **Malvankar NS, Lovley DR.** 2012. Microbial nanowires: a new paradigm for biological electron transfer and bioelectronics. ChemSusChem **5:**1039-1046.

50. **Marsili E, Baron DB, Shikhare ID, Coursolle D, Gralnick JA, Bond DR.** 2008. Shewanella secretes flavins that mediate extracellular electron transfer. Proceedings of the National Academy of Sciences **105:**3968-3973.

51. **Myers CR, Nealson KH.** 1988. Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. Science **240**.

52. **Ringeisen BR, Henderson E, Wu PK, Pietron J, Ray R, Little B, Biffinger JC, Jones-Meehan JM.** 2006. High power density from a miniature microbial fuel cell using Shewanella oneidensis DSP10. Environmental science & technology **40:**2629-2634.

53. **Xing D, Zuo Y, Cheng S, Regan JM, Logan BE.** 2008. Electricity generation by Rhodopseudomonas palustris DX-1. Environmental science & technology **42:**4146-4151.

54. **Kim BH, Chang IS, Gadd GM.** 2007. Challenges in microbial fuel cell development and operation. Applied Microbiology and Biotechnology **76:**485-494.

55. **Logan BE.** 2008. Microbial fuel cells. John Wiley & Sons.

56. **Shewa WA, Chaganti SR, Lalman JA.** Electricity Generation and Biofilm Formation in Microbial Fuel Cells Using Plate Anodes Constructed from Various Grades of Graphite. Journal of Green Engineering **4:**13-32.

57. **Rabaey K, Verstraete W.** 2005. Microbial fuel cells: novel biotechnology for energy generation. TRENDS in Biotechnology **23:**291-298.

58. **Shukla A, Suresh P, Berchmans S, Rajendran A.** 2004. Biological fuel cells and their applications. Current Science **87:**455-468.

59. **Logan BE.** 2009. Exoelectrogenic bacteria that power microbial fuel cells. Nature Reviews Microbiology **7:**375-381.

60. **Li W-W, Yu H-Q, He Z.** 2014. Towards sustainable wastewater treatment by using microbial fuel cells-centered technologies. Energy & Environmental Science **7:**911-924.

61. **Borah D, More S, Yadav R.** 2013. Construction of Double Chambered Microbial Fuel Cell (MFC) Using Household Materials and Bacillus megaterium Isolate from Tea Garden Soil. Advances in Biological Research **7:**136-140.

62. **Rittmann BE, Krajmalnik-Brown R, Halden RU.** 2008. Pre-genomic, genomic and post-genomic study of microbial communities involved in bioenergy. Nature Reviews Microbiology **6:**604-612.

63. **Logan BE, Hamelers B, Rozendal R, Schröder U, Keller J, Freguia S, Aelterman P, Verstraete W, Rabaey K.** 2006. Microbial fuel cells: methodology and technology. Environmental science & technology **40:**5181-5192.

64. **Zhi W, Ge Z, He Z, Zhang H.** 2014. Methods for understanding microbial community structures and functions in microbial fuel cells: a review. Bioresource technology **171:**461-468.

65. **Nevin KP, Woodard TL, Franks AE, Summers ZM, Lovley DR.** 2010. Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. MBio **1:**e00103-00110.

66. **Rosenbaum MA, Franks AE.** 2014. Microbial catalysis in bioelectrochemical technologies: status quo, challenges and perspectives. Applied microbiology and biotechnology **98:**509-518.

67. **Patil SA, Harnisch F, Koch C, Hübschmann T, Fetzer I, Carmona-Martínez AA, Müller S, Schröder U.** 2011. Electroactive mixed culture derived biofilms in microbial bioelectrochemical systems: the role of pH on biofilm formation, performance and composition. Bioresource technology **102:**9683-9690.

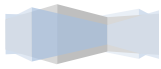
68. **Lovley DR.** 2006. Bug juice: harvesting electricity with microorganisms. Nature Reviews Microbiology **4:**497-508.

69. **Logan BE, Regan JM.** 2006. Electricity-producing bacterial communities in microbial fuel cells. TRENDS in Microbiology **14:**512-518.

70. **Wrighton KC, Agbo P, Warnecke F, Weber KA, Brodie EL, DeSantis TZ, Hugenholtz P, Andersen GL, Coates JD.** 2008. A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells. The ISME journal **2:**1146-1156.

71. **Ieropoulos IA, Ledezma P, Stinchcombe A, Papaharalabos G, Melhuish C, Greenman J.** 2013. Waste to real energy: the first MFC powered mobile phone. Physical Chemistry Chemical Physics **15:**15312-15316.

72. **Jadhav G, Ghangrekar M.** 2009. Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. Bioresource Technology **100:**717-723.



73. **Liu H, Cheng S, Logan BE.** 2005. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environmental science & technology **39:**658-662.

74. **Pham CA, Jung SJ, Phung NT, Lee J, Chang IS, Kim BH, Yi H, Chun J.** 2003. A novel electrochemically active and Fe (III)-reducing bacterium phylogenetically related to Aeromonas hydrophila, isolated from a microbial fuel cell. FEMS Microbiology Letters **223:**129-134.

75. **Kim N, Choi Y, Jung S, Kim S.** Development of microbial fuel cells using Proteus vulgaris.

76. **Kim G, Hyun M, Chang I, Kim H, Park H, Kim B, Kim S, Wimpenny J, Weightman AJ.** 2005. Dissimilatory Fe (III) reduction by an electrochemically active lactic acid bacterium phylogenetically related to Enterococcus gallinarum isolated from submerged soil. Journal of applied microbiology **99:**978-987.

77. **Vasyliv OM, Maslovska OD, Ferensovych YP, Bilyy OI, Hnatush SO.** Interconnection between tricarboxylic acid cycle and energy generation in microbial fuel cell performed by desulfuromonas acetoxidans IMV B-7384, p 94930J-94930J-94937. *In* (ed), International Society for Optics and Photonics,

78. **Foley JM, Rozendal RA, Hertle CK, Lant PA, Rabaey K.** 2010. Life cycle assessment of high-rate anaerobic treatment, microbial fuel cells, and microbial electrolysis cells. Environmental science & technology **44:**3629-3637.

79. **Liu Z, Du Z, Lian J, Zhu X, Li S, Li H.** 2007. Improving energy accumulation of microbial fuel cells by metabolism regulation using Rhodoferax ferrireducens as biocatalyst. Letters in applied microbiology **44:**393-398.

80. **Chae K-J, Choi M-J, Lee J-W, Kim K-Y, Kim IS.** 2009. Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. Bioresource Technology **100:**3518-3525.

81. **Freguia S, Rabaey K, Yuan Z, Keller Jr.** 2008. Syntrophic processes drive the conversion of glucose in microbial fuel cell anodes. Environmental science & technology **42:**7937-7943.

82. **He Z, Kan J, Mansfeld F, Angenent LT, Nealson KH.** 2009. Self-sustained phototrophic microbial fuel cells based on the synergistic cooperation between photosynthetic microorganisms and heterotrophic bacteria. Environmental science & technology **43:**1648-1654.

83. **Parameswaran P, Zhang H, Torres CI, Rittmann BE, Krajmalnik-Brown R.** 2010. Microbial community structure in a biofilm anode fed with a fermentable substrate: the significance of hydrogen scavengers. Biotechnology and bioengineering **105:**69-78.

84. **Ren Z, Ward TE, Regan JM.** 2007. Electricity production from cellulose in a microbial fuel cell using a defined binary culture. Environmental science & technology **41:**4781-4786.

85. **Cheng KY, Ho G, Cord-Ruwisch R.** 2010. Novel methanogenic rotatable bioelectrochemical system operated with polarity inversion. Environmental science & technology **45:**796-802.

86. **Khan A, Nawaz K, Usama M, Ashraf Z.** 2015. Feasibility Study of Municipal Plastic Waste for Power Generation in Lahore City. Pakistan J Chem Eng Process Technol **6:**229.

87. **Liu J, Lowy D, Baumann R, Tender L.** 2007. Influence of anode pretreatment on its microbial colonization. Journal of applied microbiology **102:**177-183.

88. **Jung S, Regan JM.** 2007. Comparison of anode bacterial communities and performance in microbial fuel cells with different electron donors. Applied microbiology and biotechnology **77:**393-402.

89. **Lee H-S, Parameswaran P, Kato-Marcus A, Torres CI, Rittmann BE.** 2008. Evaluation of energy-conversion efficiencies in microbial fuel cells (MFCs) utilizing fermentable and non-fermentable substrates. Water Research **42:**1501-1510.

90. **Ishii Si, Shimoyama T, Hotta Y, Watanabe K.** 2008. Characterization of a filamentous biofilm community established in a cellulose-fed microbial fuel cell. Bmc Microbiology **8:**6.

91. **Kim JR, Jung SH, Regan JM, Logan BE.** 2007. Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. Bioresource technology **98:**2568-2577.

92. **Kim G, Webster G, Wimpenny J, Kim B, Kim H, Weightman AJ.** 2006. Bacterial community structure, compartmentalization and activity in a microbial fuel cell. Journal of applied microbiology **101:**698-710.

93. **Rismani-Yazdi H, Christy AD, Dehority BA, Morrison M, Yu Z, Tuovinen OH.** 2007. Electricity generation from cellulose by rumen microorganisms in microbial fuel cells. Biotechnology and bioengineering **97:**1398-1407.

94. **Liang P, Wu W, Wei J, Yuan L, Xia X, Huang X.** 2011. Alternate charging and discharging of capacitor to enhance the electron production of bioelectrochemical systems. Environmental science & technology **45:**6647-6653.

95. **Esteve-Núñez A, Sosnik J, Visconti P, Lovley DR.** 2008. Fluorescent properties of c-type cytochromes reveal their potential role as an extracytoplasmic electron sink in Geobacter sulfurreducens. Environmental microbiology **10:**497-505.

96. **Dewan A, Beyenal H, Lewandowski Z.** 2009. Intermittent energy harvesting improves the performance of microbial fuel cells. Environmental science & technology **43:**4600-4605.

97. **Katuri KP, Scott K, Head IM, Picioreanu C, Curtis TP.** 2011. Microbial fuel cells meet with external resistance. Bioresource technology **102:**2758-2766.

98. **Schrott GD, Bonanni PS, Robuschi L, Esteve-Nuñez A, Busalmen JP.** 2011. Electrochemical insight into the mechanism of electron transport in biofilms of Geobacter sulfurreducens. Electrochimica Acta **56:**10791-10795.

99. **Harris HW, El-Naggar MY, Bretschger O, Ward MJ, Romine MF, Obraztsova A, Nealson KH.** 2010. Electrokinesis is a microbial behavior that requires extracellular electron transport. Proceedings of the National Academy of Sciences **107:**326-331.

100. **Morishima K, Yoshida M, Furuya A, Moriuchi T, Ota M, Furukawa Y.** 2007. Improving the performance of a direct photosynthetic/metabolic bio-fuel cell

(DPBFC) using gene manipulated bacteria. Journal of Micromechanics and Microengineering **17:**S274.

101. **Muyzer G, Smalla K.** 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van Leeuwenhoek **73:**127-141.
102. **Schröder U.** 2007. Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency. Physical Chemistry Chemical Physics **9:**2619-2629.
103. **Bennetto P.** 1987. Microbes come to power. New Scientist **114:**36-39.
104. **Park D, Zeikus J.** 2002. Impact of electrode composition on electricity generation in a single-compartment fuel cell using Shewanella putrefaciens. Applied microbiology and biotechnology **59:**58-61.
105. **Boon N, Aelterman P, Clauwaert P, De Schamphelaire L, Vanhaecke L, De Maeyer K, Höfte M, Verstraete W, Rabaey K.** 2008. Metabolites produced by Pseudomonas sp. enable a Gram-positive bacterium to achieve extracellular electron transfer. Applied Microbiology and Biotechnology **77:**1119-1129.
106. **Qiao Y, Li CM, Bao S-J, Lu Z, Hong Y.** 2008. Direct electrochemistry and electrocatalytic mechanism of evolved Escherichia coli cells in microbial fuel cells. Chemical Communications**:**1290-1292.
107. **Shi L, Richardson DJ, Wang Z, Kerisit SN, Rosso KM, Zachara JM, Fredrickson JK.** 2009. The roles of outer membrane cytochromes of Shewanella and Geobacter in extracellular electron transfer. Environmental Microbiology Reports **1:**220-227.
108. **Von Canstein H, Ogawa J, Shimizu S, Lloyd JR.** 2008. Secretion of flavins by Shewanella species and their role in extracellular electron transfer. Applied and Environmental Microbiology **74:**615-623.
109. **Bretschger O, Obraztsova A, Sturm CA, Chang IS, Gorby YA, Reed SB, Culley DE, Reardon CL, Barua S, Romine MF.** 2007. Current production and metal oxide reduction by Shewanella oneidensis MR-1 wild type and mutants. Applied and Environmental Microbiology **73:**7003-7012.

110. **Kim B-C, Postier BL, DiDonato RJ, Chaudhuri SK, Nevin KP, Lovley DR.** 2008. Insights into genes involved in electricity generation in Geobacter sulfurreducens via whole genome microarray analysis of the OmcF-deficient mutant. Bioelectrochemistry **73:**70-75.

111. **Reguera G, McCarthy KD, Mehta T, Nicoll JS, Tuominen MT, Lovley DR.** 2005. Extracellular electron transfer via microbial nanowires. Nature **435:**1098-1101.

112. **Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim KS.** 2006. Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms. Proceedings of the National Academy of Sciences **103:**11358-11363.

113. **Bergel A, Féron D, Mollica A.** 2005. Catalysis of oxygen reduction in PEM fuel cell by seawater biofilm. Electrochemistry Communications **7:**900-904.

114. **Bond DR, Holmes DE, Tender LM, Lovley DR.** 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. Science **295:**483-485.

115. **Oh S, Logan BE.** 2005. Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. Water research **39:**4673-4682.

116. **Oh S, Min B, Logan BE.** 2004. Cathode performance as a factor in electricity generation in microbial fuel cells. Environmental science & technology **38:**4900-4904.

117. **Liu H, Logan BE.** 2004. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environmental science & technology **38:**4040-4046.

118. **Rabaey K, Clauwaert P, Aelterman P, Verstraete W.** 2005. Tubular microbial fuel cells for efficient electricity generation. Environmental science & technology **39:**8077-8082.

119. **He Z, Minteer SD, Angenent LT.** 2005. Electricity generation from artificial wastewater using an upflow microbial fuel cell. Environmental science & technology **39:**5262-5267.

120. **Wei J, Liang P, Huang X.** 2011. Recent progress in electrodes for microbial fuel cells. Bioresource technology **102:**9335-9344.

121. **Rabaey K.** 2010. Bioelectrochemical systems: from extracellular electron transfer to biotechnological application. IWA publishing.

122. **Oh ST, Kim JR, Premier GC, Lee TH, Kim C, Sloan WT.** 2010. Sustainable wastewater treatment: How might microbial fuel cells contribute. Biotechnology Advances **28:**871-881.

123. **Rittmann BE.** 2006. Microbial ecology to manage processes in environmental biotechnology. TRENDS in Biotechnology **24:**261-266.

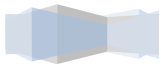
124. **Curtis TP, Head IM, Graham DW.** 2003. Peer reviewed: theoretical ecology for engineering biology. Environmental science & technology **37:**64A-70A.

125. **Holmes DE, Nicoll JS, Bond DR, Lovley DR.** 2004. Potential role of a novel psychrotolerant member of the family Geobacteraceae, Geopsychrobacter electrodiphilus gen. nov., sp. nov., in electricity production by a marine sediment fuel cell. Applied and environmental microbiology **70:**6023-6030.

126. **Phung NT, Lee J, Kang KH, Chang IS, Gadd GM, Kim BH.** 2004. Analysis of microbial diversity in oligotrophic microbial fuel cells using 16S rDNA sequences. FEMS microbiology letters **233:**77-82.

127. **Choo YF, Lee J, Chang IS, Kim BH.** 2006. Bacterial communities in microbial fuel cells enriched with high concentrations of glucose and glutamate. Journal of microbiology and biotechnology **16:**1481.

128. **Lee J, Phung NT, Chang IS, Kim BH, Sung HC.** 2003. Use of acetate for enrichment of electrochemically active microorganisms and their 16S rDNA analyses. FEMS microbiology letters **223:**185-191.



129. **Kim B, Park H, Kim H, Kim G, Chang I, Lee J, Phung N.** 2004. Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. Applied Microbiology and Biotechnology **63:**672-681.

130. **Borole AP, Hamilton CY, Vishnivetskaya TA, Leak D, Andras C, Morrell-Falvey J, Keller M, Davison B.** 2009. Integrating engineering design improvements with exoelectrogen enrichment process to increase power output from microbial fuel cells. Journal of Power Sources **191:**520-527.

131. **Cha J, Choi S, Yu H, Kim H, Kim C.** 2010. Directly applicable microbial fuel cells in aeration tank for wastewater treatment. Bioelectrochemistry **78:**72-79.

132. **Chung K, Okabe S.** 2009. Continuous power generation and microbial community structure of the anode biofilms in a three-stage microbial fuel cell system. Applied microbiology and biotechnology **83:**965-977.

133. **Sharma V, Kundu P.** 2010. Biocatalysts in microbial fuel cells. Enzyme and Microbial Technology **47:**179-188.

134. **Habermann W, Pommer E.** 1991. Biological fuel cells with sulphide storage capacity. Applied microbiology and biotechnology **35:**128-133.

135. **Liu H, Grot S, Logan BE.** 2005. Electrochemically assisted microbial production of hydrogen from acetate. Environmental Science & Technology **39:**4317-4320.

136. **Cheng S, Liu H, Logan BE.** 2006. Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. Environmental science & technology **40:**364-369.

137. **Liu H, Cheng S, Huang L, Logan BE.** 2008. Scale-up of membrane-free single-chamber microbial fuel cells. Journal of Power Sources **179:**274-279.

138. **Fan Y, Hu H, Liu H.** 2007. Sustainable power generation in microbial fuel cells using bicarbonate buffer and proton transfer mechanisms. Environmental science & technology **41:**8154-8158.

139. **Mohan SV, Mohanakrishna G, Reddy BP, Saravanan R, Sarma P.** 2008. Bioelectricity generation from chemical wastewater treatment in mediatorless (anode) microbial fuel cell (MFC) using selectively enriched hydrogen producing

mixed culture under acidophilic microenvironment. Biochemical Engineering Journal **39:**121-130.

140. **Mohan SV, Raghavulu SV, Sarma P.** 2008. Influence of anodic biofilm growth on bioelectricity production in single chambered mediatorless microbial fuel cell using mixed anaerobic consortia. Biosensors and Bioelectronics **24:**41-47.
141. **Huang L, Zeng RJ, Angelidaki I.** 2008. Electricity production from xylose using a mediator-less microbial fuel cell. Bioresource technology **99:**4178-4184.
142. **Logan B, Cheng S, Watson V, Estadt G.** 2007. Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. Environmental science & technology **41:**3341-3346.
143. **Ieropoulos I, Greenman J, Melhuish C.** Imitating metabolism: Energy autonomy in biologically inspired robots, p 191-194. *In* (ed),
144. **Aelterman P, Rabaey K, Pham HT, Boon N, Verstraete W.** 2006. Continuous electricity generation at high voltages and currents using stacked microbial fuel cells. Environmental science & technology **40:**3388-3394.
145. **Han Y, Yu C, Liu H.** 2010. A microbial fuel cell as power supply for implantable medical devices. Biosensors and Bioelectronics **25:**2156-2160.
146. **Logan BE, Murano C, Scott K, Gray ND, Head IM.** 2005. Electricity generation from cysteine in a microbial fuel cell. Water Research **39:**942-952.
147. **Read ST, Dutta P, Bond PL, Keller J, Rabaey K.** 2010. Initial development and structure of biofilms on microbial fuel cell anodes. BMC microbiology **10:**98.
148. **Marshall CW, May HD.** 2009. Electrochemical evidence of direct electrode reduction by a thermophilic Gram-positive bacterium, Thermincola ferriacetica. Energy & Environmental Science **2:**699-705.
149. **Thauer R, Zinkhan D, Spormann A.** 1989. Biochemistry of acetate catabolism in anaerobic chemotrophic bacteria. Annual Reviews in Microbiology **43:**43-67.
150. **Cord-Ruwisch R, Lovley DR, Schink B.** 1998. Growth of Geobacter sulfurreducens with acetate in syntrophic cooperation with hydrogen-oxidizing anaerobic partners. Applied and environmental microbiology **64:**2232-2236.

151. **Franks AE, Nevin KP.** 2010. Microbial fuel cells, a current review. Energies **3:**899-919.

152. **Park HS, Kim BH, Kim HS, Kim HJ, Kim GT, Kim M, Chang IS, Park YK, Chang HI.** 2001. A novel electrochemically active and Fe (III)-reducing bacterium phylogenetically related to Clostridium butyricum isolated from a microbial fuel cell. Anaerobe **7:**297-306.

153. **Zhang T, Cui C, Chen S, Yang H, Shen P.** 2008. The direct electrocatalysis of Escherichia coli through electroactivated excretion in microbial fuel cell. Electrochemistry communications **10:**293-297.

154. **Zuo Y, Cheng S, Call D, Logan BE.** 2007. Tubular membrane cathodes for scalable power generation in microbial fuel cells. Environmental science & technology **41:**3347-3353.

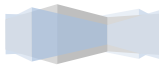
155. **Zhao F, Rahunen N, Varcoe JR, Chandra A, Avignone-Rossa C, Thumser AE, Slade RC.** 2008. Activated carbon cloth as anode for sulfate removal in a microbial fuel cell. Environmental science & technology **42:**4971-4976.

156. **Borole AP, O'Neill H, Tsouris C, Cesar S.** 2008. A microbial fuel cell operating at low pH using the acidophile Acidiphilium cryptum. Biotechnology letters **30:**1367-1372.

157. **Prasad D, Arun S, Murugesan M, Padmanaban S, Satyanarayanan R, Berchmans S, Yegnaraman V.** 2007. Direct electron transfer with yeast cells and construction of a mediatorless microbial fuel cell. Biosensors and Bioelectronics **22:**2604-2610.

158. **Walker AL, Walker CW.** 2006. Biological fuel cell and an application as a reserve power source. Journal of Power Sources **160:**123-129.

159. **Bond DR, Lovley DR.** 2005. Evidence for involvement of an electron shuttle in electricity generation by Geothrix fermentans. Applied and environmental microbiology **71:**2186-2189.



160. **Torres CI, Marcus AK, Rittmann BE.** 2007. Kinetics of consumption of fermentation products by anode-respiring bacteria. Applied microbiology and biotechnology **77:**689-697.

161. **Lanthier M, Gregory KB, Lovley DR.** 2008. Growth with high planktonic biomass in Shewanella oneidensis fuel cells. FEMS microbiology letters **278:**29-35.

162. **Nevin KP, Richter H, Covalla S, Johnson J, Woodard T, Orloff A, Jia H, Zhang M, Lovley D.** 2008. Power output and columbic efficiencies from biofilms of Geobacter sulfurreducens comparable to mixed community microbial fuel cells. Environmental microbiology **10:**2505-2514.

163. **Rabaey K, Lissens G, Siciliano SD, Verstraete W.** 2003. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnology letters **25:**1531-1535.

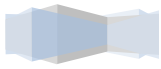
164. **Chang IS, Moon H, Bretschger O, Jang JK, Park HI, Nealson KH, Kim BH.** 2006. Electrochemically active bacteria (EAB) and mediator-less microbial fuel cells. J Microbiol Biotechnol **16:**163-177.

165. **Kang KH, Jang JK, Moon H, Chang IS, Kim BH.** 2003. A microbial fuel cell with improved cathode reaction as a low biochemical oxygen demand sensor. Biotechnology Letters **25:**1357-1361.

166. **Mathuriya AS, Sharma V.** 2010. Electricity Generation by Saccharomyces cerevisae and Clostridium acetobutylicum via Microbial Fuel Cell Technology. A Comparative Study. Advances in biological research **4:**217-223.

167. **Samrot AV, Senthilkumar P, Pavankumar K, Akilandeswari G, Rajalakshmi N, Dhathathreyan K.** 2010. RETRACTED: Electricity generation by Enterobacter cloacae SU-1 in mediator less microbial fuel cell. International Journal of Hydrogen Energy **35:**7723-7729.

168. **Bennetto H.** 1990. Electricity generation by microorganisms. Biotechnology education **1:**163-168.



169. **Niessen J, Schröder U, Scholz F.** 2004. Exploiting complex carbohydrates for microbial electricity generation–a bacterial fuel cell operating on starch. Electrochemistry Communications **6:**955-958.

170. **Xia X, Cao X-x, Liang P, Huang X, Yang S-p, Zhao G-g.** 2010. Electricity generation from glucose by a Klebsiella sp. in microbial fuel cells. Applied microbiology and biotechnology **87:**383-390.

171. **Freguia S, Masuda M, Tsujimura S, Kano K.** 2009. Lactococcus lactis catalyses electricity generation at microbial fuel cell anodes via excretion of a soluble quinone. Bioelectrochemistry **76:**14-18.

172. **Reed G, Nagodawithana TW.** 1990. Brewer's yeast, p 89-149, Yeast Technology. Springer.

173. **Ramanathan G, Birthous RS, Abirami D, Durai H.** 2011. Efficacy of Marine Microalgae as Exoelectrogen in Microbial Fuel Cell System for Bioelectricity Generation. World **3:**79-87.

174. **Biffinger JC, Fitzgerald LA, Ray R, Little BJ, Lizewski SE, Petersen ER, Ringeisen BR, Sanders WC, Sheehan PE, Pietron JJ.** 2011. The utility of Shewanella japonica for microbial fuel cells. Bioresource technology **102:**290-297.

175. **Liu M, Yuan Y, Zhang L-x, Zhuang L, Zhou S-g, Ni J-r.** 2010. Bioelectricity generation by a Gram-positive Corynebacterium sp. strain MFC03 under alkaline condition in microbial fuel cells. Bioresource technology **101:**1807-1811.

176. **Kiely PD, Cusick R, Call DF, Selembo PA, Regan JM, Logan BE.** 2011. Anode microbial communities produced by changing from microbial fuel cell to microbial electrolysis cell operation using two different wastewaters. Bioresource technology **102:**388-394.

177. **ter Heijne A, Hamelers HV, Saakes M, Buisman CJ.** 2008. Performance of non-porous graphite and titanium-based anodes in microbial fuel cells. Electrochimica Acta **53:**5697-5703.

178. **Pocaznoi D, Erable B, Delia M-L, Bergel A.** 2012. Ultra microelectrodes increase the current density provided by electroactive biofilms by improving their electron transport ability. Energy & Environmental Science **5:**5287-5296.

179. **Pocaznoi D, Calmet A, Etcheverry L, Erable B, Bergel A.** 2012. Stainless steel is a promising electrode material for anodes of microbial fuel cells. Energy & Environmental Science **5:**9645-9652.

180. **Chen S, He G, Liu Q, Harnisch F, Zhou Y, Chen Y, Hanif M, Wang S, Peng X, Hou H.** 2012. Layered corrugated electrode macrostructures boost microbial bioelectrocatalysis. Energy & Environmental Science **5:**9769-9772.

181. **Rousseau R, Dominguez-Benetton X, Délia M-L, Bergel A.** 2013. Microbial bioanodes with high salinity tolerance for microbial fuel cells and microbial electrolysis cells. Electrochemistry Communications **33:**1-4.

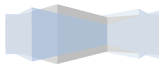
182. **Pocaznoi D, Erable B, Etcheverry L, Delia M-L, Bergel A.** 2012. Forming microbial anodes under delayed polarisation modifies the electron transfer network and decreases the polarisation time required. Bioresource technology **114:**334-341.

183. **Logan BE, Rabaey K.** 2012. Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies. Science **337:**686-690.

184. **Fan Y, Sharbrough E, Liu H.** 2008. Quantification of the internal resistance distribution of microbial fuel cells. Environmental science & technology **42:**8101-8107.

185. **Aracic S, Semenec L, Franks AE.** 2014. Investigating microbial activities of electrode-associated microorganisms in real-time. Frontiers in microbiology **5**.

186. **Kan J, Hsu L, Cheung AC, Pirbazari M, Nealson KH.** 2010. Current production by bacterial communities in microbial fuel cells enriched from wastewater sludge with different electron donors. Environmental science & technology **45:**1139-1146.



187. **Kiely PD, Regan JM, Logan BE.** 2011. The electric picnic: synergistic requirements for exoelectrogenic microbial communities. Current opinion in biotechnology **22:**378-385.

188. **Stratford JP, Beecroft NJ, Slade RC, Grüning A, Avignone-Rossa C.** 2014. Anodic microbial community diversity as a predictor of the power output of microbial fuel cells. Bioresource technology **156:**84-91.

189. **Zhou J, Liu W, Deng Y, Jiang Y-H, Xue K, He Z, Van Nostrand JD, Wu L, Yang Y, Wang A.** 2013. Stochastic assembly leads to alternative communities with distinct functions in a bioreactor microbial community. Mbio **4:**e00584-00512.

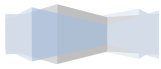
190. **Lu L, Xing D, Ren N.** 2012. Pyrosequencing reveals highly diverse microbial communities in microbial electrolysis cells involved in enhanced H 2 production from waste activated sludge. water research **46:**2425-2434.

191. **Möller B, Oßmer R, Howard BH, Gottschalk G, Hippe H.** 1984. Sporomusa, a new genus of gram-negative anaerobic bacteria including Sporomusa sphaeroides spec. nov. and Sporomusa ovata spec. nov. Archives of microbiology **139:**388-396.

192. **Yamamuro A, Kouzuma A, Abe T, Watanabe K.** 2014. Metagenomic analyses reveal the involvement of syntrophic consortia in methanol/electricity conversion in microbial fuel cells.

193. **Puig S, Serra M, Coma M, Cabré M, Balaguer MD, Colprim J.** 2011. Microbial fuel cell application in landfill leachate treatment. Journal of Hazardous Materials **185:**763-767.

194. **Pham HT, Boon N, Aelterman P, Clauwaert P, De Schamphelaire L, Van Oostveldt P, Verbeken K, Rabaey K, Verstraete W.** 2008. High shear enrichment improves the performance of the anodophilic microbial consortium in a microbial fuel cell. Microbial biotechnology **1:**487-496.



195. **Yates MD, Kiely PD, Call DF, Rismani-Yazdi H, Bibby K, Peccia J, Regan JM, Logan BE.** 2012. Convergent development of anodic bacterial communities in microbial fuel cells. The ISME journal **6:**2002-2013.

196. **An D, Parsek MR.** 2007. The promise and peril of transcriptional profiling in biofilm communities. Current opinion in microbiology **10:**292-296.

197. **Ishii Si, Suzuki S, Norden-Krichmar TM, Nealson KH, Sekiguchi Y, Gorby YA, Bretschger O.** 2012. Functionally stable and phylogenetically diverse microbial enrichments from microbial fuel cells during wastewater treatment. PLoS One **7:**e30495.

198. **Cheng S, Liu H, Logan BE.** 2006. Increased performance of single-chamber microbial fuel cells using an improved cathode structure. Electrochemistry Communications **8:**489-494.

199. **Miceli III JF, Parameswaran P, Kang D-W, Krajmalnik-Brown R, Torres CsI.** 2012. Enrichment and analysis of anode-respiring bacteria from diverse anaerobic inocula. Environmental science & technology **46:**10349-10355.

200. **Ghangrekar M, Shinde V.** 2007. Performance of membrane-less microbial fuel cell treating wastewater and effect of electrode distance and area on electricity production. Bioresource Technology **98:**2879-2885.

201. **Watson VJ, Logan BE.** 2011. Analysis of polarization methods for elimination of power overshoot in microbial fuel cells. Electrochemistry Communications **13:**54-56.

202. **Min B, Kim J, Oh S, Regan JM, Logan BE.** 2005. Electricity generation from swine wastewater using microbial fuel cells. Water research **39:**4961-4968.

203. **Cheng S, Liu H, Logan BE.** 2006. Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing. Environmental science & technology **40:**2426-2432.

204. **Gao Y, Zhou P, Mao L, Zhi Y, Zhang C, Shi W.** 2010. Effects of plant species coexistence on soil enzyme activities and soil microbial community structure

under Cd and Pb combined pollution. Journal of Environmental Sciences **22:**1040-1048.

205. **Kumari S, Mangwani N, Das S.** 2015. Low-voltage producing microbial fuel cell constructs using biofilm-forming marine bacteria. CURRENT SCIENCE **108:**925.

206. **George A.** 2011. Microtiter dish biofilm formation assay. Journal of Visualized Experiments.

207. **GHELLAI L, HASSAINE H, KLOUCHE N, KHADIR A, AISSAOUI N, NAS F, ZINGG W.** 2014. Detection of biofilm formation of a collection of fifty strains of Staphylococcus aureus isolated in Algeria at the University Hospital of Tlemcen. Journal of Bacteriology Research **6:**1-6.

208. **Christensen GD, Simpson W, Younger J, Baddour L, Barrett F, Melton D, Beachey E.** 1985. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. Journal of clinical microbiology **22:**996-1006.

209. **Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M.** 2011. Evaluation of different detection methods of biofilm formation in the clinical isolates. Brazilian Journal of Infectious Diseases **15:**305-311.

210. **Segers P, Vancanneyt M, Pot B, Torck U, Hoste B, Dewettinck D, Falsen E, Kersters K, De Vos P.** 1994. Classification of Pseudomonas diminuta Leifson and Hugh 1954 and Pseudomonas vesicularis Büsing, Döll, and Freytag 1953 in Brevundimonas gen. nov. as Brevundimonas diminuta comb. nov. and Brevundimonas vesicularis comb. nov., respectively. International journal of systematic bacteriology **44:**499-510.

211. **Lindquist D, Murrill D, Burran WP, Winans G, Janda JM, Probert W.** 2003. Characteristics of Massilia timonae and Massilia timonae-like isolates from human patients, with an emended description of the species. Journal of clinical microbiology **41:**192-196.

212. **Lalucat J, Bennasar A, Bosch R, García-Valdés E, Palleroni NJ.** 2006. Biology of Pseudomonas stutzeri. Microbiology and Molecular Biology Reviews **70:**510-547.

213. **Thierry S, Macarie H, Iizuka T, Geißdörfer W, Assih EA, Spanevello M, Verhe F, Thomas P, Fudou R, Monroy O.** 2004. Pseudoxanthomonas mexicana sp. nov. and Pseudoxanthomonas japonensis sp. nov., isolated from diverse environments, and emended descriptions of the genus Pseudoxanthomonas Finkmann et al. 2000 and of its type species. International journal of systematic and evolutionary microbiology **54:**2245-2255.

214. **Roh SW, Nam Y-D, Chang H-W, Kim K-H, Kim M-S, Shin K-S, Yoon J-H, Oh H-M, Bae J-W.** 2009. Paracoccus aestuarii sp. nov., isolated from tidal flat sediment. International journal of systematic and evolutionary microbiology **59:**790-794.

215. **Aranda S, Montes-Borrego M, Landa BB.** 2011. Purple-pigmented violacein-producing Duganella spp. inhabit the rhizosphere of wild and cultivated olives in southern Spain. Microbial ecology **62:**446-459.

216. **Li W-J, Zhang Y-Q, Park D-J, Li C-T, Xu L-H, Kim C-J, Jiang C-L.** 2004. Duganella violaceinigra sp. nov., a novel mesophilic bacterium isolated from forest soil. International journal of systematic and evolutionary microbiology **54:**1811-1814.

217. **Engel M.** 1961. Morphology of Nitrosomonas europaea and classification of the nitrifying bacteria. Journal of bacteriology **81:**833.

218. **Das SK, Mishra AK, Tindall BJ, Rainey FA, Stackebrandt E.** 1996. Oxidation of Thiosulfate by a New Bacterium, Bosea thiooxidans.(strain BI-42) gen. nov., sp. nov.: Analysis of Phylogeny Based on Chemotaxonomy and 16S Ribosomal DNA Sequencing. International journal of systematic bacteriology **46:**981-987.

219. **Vedler E, Heinaru E, Jutkina J, Viggor S, Koressaar T, Remm M, Heinaru A.** 2013. Limnobacter spp. as newly detected phenol-degraders among Baltic Sea

surface water bacteria characterised by comparative analysis of catabolic genes. Systematic and applied microbiology **36:**525-532.

220. **Coenye T, Falsen E, Vancanneyt M, Hoste B, Govan JR, Kersters K, Vandamme P.** 1999. Classification of Alcaligenes faecalis-like isolates from the environment and human clinical samples as Ralstonia gilardii sp. nov. International Journal of Systematic Bacteriology **49:**405-413.

221. **Harker M, Hirschberg J, Oren A.** 1998. Paracoccus marcusii sp. nov., an orange gram-negative coccus. International journal of systematic bacteriology **48:**543-548.

222. **Doronina NV, Kaparullina EN, Trotsenko YA.** 2014. Methyloversatilis thermotolerans sp. nov., a novel thermotolerant facultative methylotroph isolated from a hot spring. International journal of systematic and evolutionary microbiology **64:**158-164.

223. **Weitkamp J-H, Tang Y-W, Haas DW, Midha NK, Crowe JE.** 2000. Recurrent Achromobacter xylosoxidans bacteremia associated with persistent lymph node infection in a patient with hyper-immunoglobulin M syndrome. Clinical infectious diseases **31:**1183-1187.

224. **Jumas-Bilak E, Michaux-Charachon S, Bourg G, Ramuz M, Allardet-Servent A.** 1998. Unconventional genomic organization in the alpha subgroup of the Proteobacteria. Journal of bacteriology **180:**2749-2755.

225. **Tryfinopoulou P, Tsakalidou E, Nychas G-J.** 2002. Characterization of Pseudomonas spp. associated with spoilage of gilt-head sea bream stored under various conditions. Applied and environmental microbiology **68:**65-72.

226. **Li-feng G, Jian-dong J, Xiao-hui L, Ali SW, Shun-peng L.** 2007. Biodegradation of ethametsulfuron-methyl by Pseudomonas sp. SW4 isolated from contaminated soil. Current microbiology **55:**420-426.

227. **Kolton M, Harel YM, Pasternak Z, Graber ER, Elad Y, Cytryn E.** 2011. Impact of biochar application to soil on the root-associated bacterial community

structure of fully developed greenhouse pepper plants. Applied and environmental microbiology **77:**4924-4930.

228. **Friman H, Schechter A, Nitzan Y, Cahan R.** 2012. Effect of external voltage on Pseudomonas putida F1 in a bio electrochemical cell using toluene as sole carbon and energy source. Microbiology **158:**414-423.

229. **Ueno Y, Kitajima Y.** 2014. Suppression of methane gas emissions and analysis of the electrode microbial community in a sediment-based bio-electrochemical system. Advances in Microbiology **2014**.

230. **Schmitz S, Nies S, Wierckx N, Blank LM, Rosenbaum MA.** 2015. Engineering mediator-based electroactivity in the obligate aerobic bacterium Pseudomonas putida KT2440. Frontiers in microbiology **6**.

231. **Sun Y, Wei J, Liang P, Huang X.** 2011. Electricity generation and microbial community changes in microbial fuel cells packed with different anodic materials. Bioresource technology **102:**10886-10891.

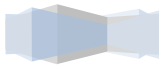
232. **Morris JM, Jin S, Crimi B, Pruden A.** 2009. Microbial fuel cell in enhancing anaerobic biodegradation of diesel. Chemical Engineering Journal **146:**161-167.

233. **Ismail ZZ, Jaeel AJ.** 2013. Sustainable Power Generation in Continuous Flow Microbial Fuel Cell Treating Actual Wastewater: Influence of Biocatalyst Type on Electricity Production. The Scientific World Journal **2013**.

234. **Cole JK, Hutchison JR, Renslow RS, Kim Y-M, Chrisler WB, Engelmann HE, Dohnalkova AC, Hu D, Metz TO, Fredrickson JK.** 2014. Phototrophic biofilm assembly in microbial-mat-derived unicyanobacterial consortia: model systems for the study of autotroph-heterotroph interactions. Frontiers in microbiology **5**.

235. **Kothari R, Tyagi V, Pathak A.** 2010. Waste-to-energy: A way from renewable energy sources to sustainable development. Renewable and Sustainable Energy Reviews **14:**3164-3170.

236. **Boyle G.** 2004. Renewable energy. OXFORD university press.



237. **Lund JW, Freeston DH.** 2001. World-wide direct uses of geothermal energy 2000. Geothermics **30:**29-68.

238. **Association EWE.** 2009. The economics of wind energy. EWEA.

239. **Bowman C, Arthur E, Lisowski P, Lawrence G, Jensen R, Anderson J, Blind B, Cappiello M, Davidson J, England T.** 1992. Nuclear energy generation and waste transmutation using an accelerator-driven intense thermal neutron source. Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment **320:**336-367.

240. **Liu BY, Jordan RC.** 1963. The long-term average performance of flat-plate solar-energy collectors: with design data for the US, its outlying possessions and Canada. Solar Energy **7:**53-74.

241. **Johnstone N, Haščič I, Popp D.** 2010. Renewable energy policies and technological innovation: evidence based on patent counts. Environmental and Resource Economics **45:**133-155.

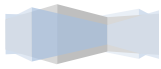
242. **Panwar N, Kaushik S, Kothari S.** 2011. Role of renewable energy sources in environmental protection: a review. Renewable and Sustainable Energy Reviews **15:**1513-1524.

243. **Dresselhaus M, Thomas I.** 2001. Alternative energy technologies. Nature **414:**332-337.

244. **Demain AL.** 2009. Biosolutions to the energy problem. Journal of industrial microbiology & biotechnology **36:**319-332.

245. **Logan BE.** 2005. Simultaneous wastewater treatment and biological electricity generation. Water Science & Technology **52:**31-37.

246. **Patil SA, Surakasi VP, Koul S, Ijmulwar S, Vivek A, Shouche Y, Kapadnis B.** 2009. Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber. Bioresource technology **100:**5132-5139.



247. **Reguera G, Nevin KP, Nicoll JS, Covalla SF, Woodard TL, Lovley DR.** 2006. Biofilm and nanowire production leads to increased current in Geobacter sulfurreducens fuel cells. Applied and environmental microbiology **72:**7345-7348.

248. **Pham T, Rabaey K, Aelterman P, Clauwaert P, De Schamphelaire L, Boon N, Verstraete W.** 2006. Microbial fuel cells in relation to conventional anaerobic digestion technology. Engineering in Life Sciences **6:**285-292.

249. **Sun M, Tong Z-H, Sheng G-P, Chen Y-Z, Zhang F, Mu Z-X, Wang H-L, Zeng RJ, Liu X-W, Yu H-Q.** 2010. Microbial communities involved in electricity generation from sulfide oxidation in a microbial fuel cell. Biosensors and Bioelectronics **26:**470-476.

250. **Jang JK, Pham TH, Chang IS, Kang KH, Moon H, Cho KS, Kim BH.** 2004. Construction and operation of a novel mediator-and membrane-less microbial fuel cell. Process Biochemistry **39:**1007-1012.

251. **Feng Y, Lee H, Wang X, Liu Y.** Electricity generation in microbial fuel cells at different temperature and isolation of electrogenic bacteria, p 1-5. *In* (ed), IEEE,

252. **Ghoreyshi A, Jafary T, Najafpour G, Haghparast F.** Effect of type and concentration of substrate on power generation in a dual chambered microbial fuel cell, p. *In* (ed),

253. **Khan MR, Bhattacharjee R, Amin M.** 2012. Performance of the salt bridge based microbial fuel cell. International Journal of Engineering & Technology **1:**115-123.

254. **Mohan SV, Saravanan R, Raghavulu SV, Mohanakrishna G, Sarma P.** 2008. Bioelectricity production from wastewater treatment in dual chambered microbial fuel cell (MFC) using selectively enriched mixed microflora: effect of catholyte. Bioresource Technology **99:**596-603.

255. **Chonde SG.** 2014. Microbial Fuel cell: A New Approach of Wastewater Treatment with Power Generation. Pharmaceutical Research **5:**8-12.

256. **Rabaey K, Read ST, Clauwaert P, Freguia S, Bond PL, Blackall LL, Keller J.** 2008. Cathodic oxygen reduction catalyzed by bacteria in microbial fuel cells. The ISME journal **2:**519-527.

257. **Erable B, Vandecandelaere I, Faimali M, Delia M-L, Etcheverry L, Vandamme P, Bergel A.** 2010. Marine aerobic biofilm as biocathode catalyst. Bioelectrochemistry **78:**51-56.

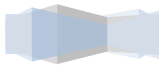
258. **Amann RI, Ludwig W, Schleifer K-H.** 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiological reviews **59:**143-169.

259. **Chen G-W, Choi S-J, Lee T-H, Lee G-Y, Cha J-H, Kim C-W.** 2008. Application of biocathode in microbial fuel cells: cell performance and microbial community. Applied microbiology and biotechnology **79:**379-388.

260. **Aldrovandi A, Marsili E, Stante L, Paganin P, Tabacchioni S, Giordano A.** 2009. Sustainable power production in a membrane-less and mediator-less synthetic wastewater microbial fuel cell. Bioresource technology **100:**3252-3260.

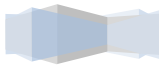
261. **Chen G-W, Cha J-H, Choi S-J, Lee T-H, Kim C-W.** 2010. Characterization of an open biocathode microbial fuel cell for electricity generation and effluent polish. Korean Journal of Chemical Engineering **27:**828-835.

262. **Erable B, Féron D, Bergel A.** 2012. Microbial catalysis of the oxygen reduction reaction for microbial fuel cells: a review. ChemSusChem **5:**975-987.



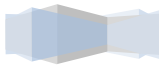
Appendix A

Time(DAYS)	Voltage(mV)
1	**54.5**
2	**109**
3	**88.3**
4	**63.8**
5	**37.3**
6	**28.6**
7	**7.8**
8	**15.2**
9	**10.8**
10	**21.5**
11	**347**
12	**210**
13	**174**
14	**33.3**
15	**19.8**
16	**18.6**
17	**274**
18	**18.1**



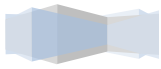
Appendix B

Resistance(Ω)	Voltage(mV)
10	**0.5**
47	**9.2**
57	**10.8**
68	**12.2**
100	**17.2**
170	**32.4**
3000	**347**
100000	**597**



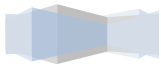
Appendix C

Resistance(Ω)	Voltage(mV)	Current(mA)
10	0.5	0.05
47	9.2	0.195745
57	10.8	0.189474
68	12.2	0.179412
100	17.2	0.172
170	32.4	0.190588
3000	347	0.115667
100000	597	0.00597



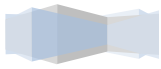
Appendix D

Time(DAYS)	Voltage(mV)	Current(mA)
1	54.5	0.018167
2	109	0.036333
3	88.3	0.029433
4	63.8	0.021267
5	37.3	0.012433
6	28.6	0.009533
7	7.8	0.0026
8	15.2	0.005067
9	10.8	0.0036
10	21.5	0.007167
11	347	0.115667
12	210	0.07
13	174	0.058
14	33.3	0.0111
15	19.8	0.0066
16	18.6	0.0062
17	274	0.091333
18	18.1	0.006033



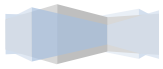
Appendix E

Time(DAYS)	Voltage(mV)	Power(µW)
1	**54.5**	0.990083
2	**109**	3.960333
3	**88.3**	2.598963
4	**63.8**	1.356813
5	**37.3**	0.463763
6	**28.6**	0.272653
7	**7.8**	0.02028
8	**15.2**	0.077013
9	**10.8**	0.03888
10	**21.5**	0.154083
11	**347**	40.13633
12	**210**	14.7
13	**174**	10.092
14	**33.3**	0.36963
15	**19.8**	0.13068
16	**18.6**	0.11532
17	**274**	25.02533
18	**18.1**	0.109203



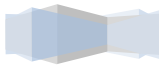
Appendix F

Time(DAYS)	Current(mA)	Power density(µW/m2)
1	0.018167	0.000198
2	0.036333	0.000792
3	0.029433	0.00052
4	0.021267	0.000271
5	0.012433	9.28E-05
6	0.009533	5.45E-05
7	0.0026	4.06E-06
8	0.005067	1.54E-05
9	0.0036	7.78E-06
10	0.007167	3.08E-05
11	0.115667	0.008027
12	0.07	0.00294
13	0.058	0.002018
14	0.0111	7.39E-05
15	0.0066	2.61E-05
16	0.0062	2.31E-05
17	0.091333	0.005005
18	0.006033	2.18E-05



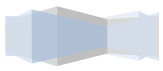
Appendix A1

Time(DAYS)	Voltage(mV)
1	44.3
2	50.5
3	52.3
4	74.9
5	69.4
6	15.6
7	14.7
8	10.9
9	9.8
10	8.5
11	6.2
12	6.1
13	4.3
14	11.5
15	11.7
16	10.2
17	8.4
18	5.7



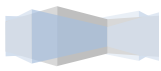
Appendix B2

Resistance(Ω)	Voltage(mV)
10	0.1
47	1.1
57	1.7
68	1.8
100	2.5
170	3.9
3000	74.9
100000	345.7



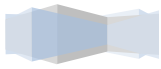
Appendix C2

Resistance(Ω)	Voltage(mV)	Current(mA)
10	0.1	0.01
47	1.1	0.023404
57	1.7	0.029825
68	1.8	0.026471
100	2.5	0.025
170	3.9	0.022941
3000	74.9	0.024967
100000	345.7	0.003457



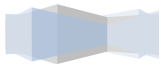
Appendix D2

Time(DAYS)	Voltage(mV)	Current(mA)
1	44.3	0.014767
2	50.5	0.016833
3	52.3	0.017433
4	74.9	0.024967
5	69.4	0.023133
6	15.6	0.0052
7	14.7	0.0049
8	10.9	0.003633
9	9.8	0.003267
10	8.5	0.002833
11	6.2	0.002067
12	6.1	0.002033
13	4.3	0.001433
14	11.5	0.003833
15	11.7	0.0039
16	10.2	0.0034
17	8.4	0.0028
18	5.7	0.0019



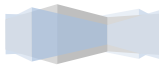
Apendix E2

Time(Days)	Voltage(mV)	Power(µW)
1	44.3	0.654163
2	50.5	0.850083
3	52.3	0.911763
4	74.9	1.870003
5	69.4	1.605453
6	15.6	0.08112
7	14.7	0.07203
8	10.9	0.039603
9	9.8	0.032013
10	8.5	0.024083
11	6.2	0.012813
12	6.1	0.012403
13	4.3	0.006163
14	11.5	0.044083
15	11.7	0.04563
16	10.2	0.03468
17	8.4	0.02352
18	5.7	0.01083



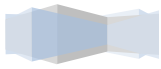
Appendix F2

Time(DAYS)	Voltage(mV)	Power density(µW/m2)
1	44.3	0.000131
2	50.5	0.00017
3	52.3	0.000182
4	74.9	0.000374
5	69.4	0.000321
6	15.6	1.62E-05
7	14.7	1.44E-05
8	10.9	7.92E-06
9	9.8	6.4E-06
10	8.5	4.82E-06
11	6.2	2.56E-06
12	6.1	2.48E-06
13	4.3	1.23E-06
14	11.5	8.82E-06
15	11.7	9.13E-06
16	10.2	6.94E-06
17	8.4	4.7E-06
18	5.7	2.17E-06



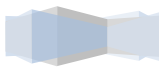
Apendix G

Time(Days)	Voltage(mV)
1	19.8
2	22.5
3	28.8
4	15.2
5	2.6
6	8.7
7	5.1
8	0.3
9	0.1
10	0.9
11	0
12	0.5
13	0.8
14	9.6
15	18.9
16	21.2



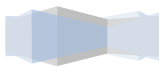
Apendix H

Resistance(Ω)	Voltage(mV)
3000	28.8
100000	136.2
100	0.6
68	0.3
57	0.3
47	0.2
170	1.2
10	0.1



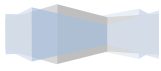
Apendix I

Resistance(Ω)	Voltage(mV)	Current(mA)
3000	28.8	0.0096
100000	136.2	0.001362
100	0.6	0.006
68	0.3	0.004412
57	0.3	0.005263
47	0.2	0.004255
170	1.2	0.007059
10	0.1	0.01



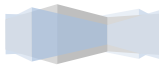
Apendix J

Time(Days)	Voltage(mV)	Current(mA)
1	19.8	0.0066
2	22.5	0.0075
3	28.8	0.0096
4	15.2	0.005067
5	2.6	0.000867
6	8.7	0.0029
7	5.1	0.0017
8	0.3	0.0001
9	0.1	3.33E-05
10	0.9	0.0003
11	0	0
12	0.5	0.000167
13	0.8	0.000267
14	9.6	0.0032
15	18.9	0.0063
16	21.2	0.007067



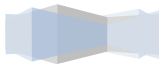
Apendix K

Time(Days)	Voltage(mV)	Power(µW)
1	19.8	0.13068
2	22.5	0.16875
3	28.8	0.27648
4	15.2	0.077013
5	2.6	0.002253
6	8.7	0.02523
7	5.1	0.00867
8	0.3	0.00003
9	0.1	3.33E-06
10	0.9	0.00027
11	0	0
12	0.5	8.33E-05
13	0.8	0.000213
14	9.6	0.03072
15	18.9	0.11907
16	21.2	0.149813



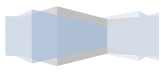
Apendix L

Time(Days)	Current(mA)	Power density(µW/m2)
1	0.0066	0.000026136
2	0.0075	0.00003375
3	0.0096	0.000055296
4	0.005067	1.54027E-05
5	0.000867	4.50667E-07
6	0.0029	0.000005046
7	0.0017	0.000001734
8	0.0001	0.000000006
9	3.33E-05	6.66667E-10
10	0.0003	0.000000054
11	0	0
12	0.000167	1.66667E-08
13	0.000267	4.26667E-08
14	0.0032	0.000006144
15	0.0063	0.000023814
16	0.007067	2.99627E-05



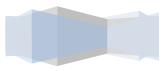
Apendix G2

Times(Days)	Voltage(mV)
1	12.1
2	13
3	11
4	7.6
5	5.5
6	20
7	24
8	15
9	13
10	18
11	10
12	8
13	11
14	9
15	7
16	6



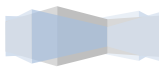
Apendix H2

Resisance(Ω)	Voltage(mV)
10	0
47	0.6
57	0.8
68	1.7
100	2
170	2.6
3000	24
100000	76



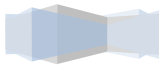
Apendix I2

Resisance(Ω)	Voltage(mV)	Current(mA)
10	0	0
47	0.6	0.012766
57	0.8	0.014035
68	1.7	0.025
100	2	0.02
170	2.6	0.015294
3000	24	0.008
100000	76	0.00076



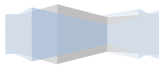
Apendix J2

Times(Days)	Voltage(mV)	Current(mA)
1	12.1	0.004033
2	13	0.004333
3	11	0.003667
4	7.6	0.002533
5	5.5	0.001833
6	20	0.006667
7	24	0.008
8	15	0.005
9	13	0.004333
10	18	0.006
11	10	0.003333
12	8	0.002667
13	11	0.003667
14	9	0.003
15	7	0.002333
16	6	0.002



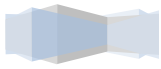
Apendix K2

Times(Days)	Voltage(mV)	Power(µW)
1	12.1	0.048803
2	13	0.056333
3	11	0.040333
4	7.6	0.019253
5	5.5	0.010083
6	20	0.133333
7	24	0.192
8	15	0.075
9	13	0.056333
10	18	0.108
11	10	0.033333
12	8	0.021333
13	11	0.040333
14	9	0.027
15	7	0.016333
16	6	0.012



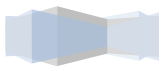
Apendix L2

Times(Days)	Current(mA)	Power density(µW/m2)
1	0.004033	9.76067E-06
2	0.004333	1.12667E-05
3	0.003667	8.06667E-06
4	0.002533	3.85067E-06
5	0.001833	2.01667E-06
6	0.006667	2.66667E-05
7	0.008	0.0000384
8	0.005	0.000015
9	0.004333	1.12667E-05
10	0.006	0.0000216
11	0.003333	6.66667E-06
12	0.002667	4.26667E-06
13	0.003667	8.06667E-06
14	0.003	0.0000054
15	0.002333	3.26667E-06
16	0.002	0.0000024



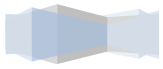
Appendix L2A

Cell Type	Voltage(mV)
MFC1(BE)	**347**
MFC1(AE)	74.9
MFC2(BE)	28.8
MFC2(AE)	24



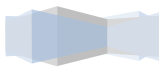
Apendix M

Time(Days)	Voltage(mV)
1	105
2	8.9
3	13.3
4	1
5	0.8
6	0.3
7	1.4
8	2.3
9	12.2
10	15.5
11	10.7
12	8.8
13	78.1
14	81.6
15	100.5
16	105.7



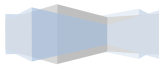
Apendix N

Resistance(Ω)	Voltage(mV)
3000	105.7
100000	179.9
100	3.8
68	0.9
57	0.5
47	0.4
170	16.4
10	0.2



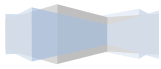
Apendix O

Resistance(Ω)	Voltage(mV)	Current(mA)
3000	105.7	0.035233
100000	179.9	0.001799
100	3.8	0.038
68	0.9	0.013235
57	0.5	0.008772
47	0.4	0.008511
170	16.4	0.096471
10	0.2	0.02



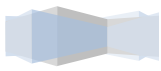
Apendix P

Time(Days)	Voltage(mV)	Current(mA)
1	105	0.035
2	8.9	0.002967
3	13.3	0.004433
4	1	0.000333
5	0.8	0.000267
6	0.3	0.0001
7	1.4	0.000467
8	2.3	0.000767
9	12.2	0.004067
10	15.5	0.005167
11	10.7	0.003567
12	8.8	0.002933
13	78.1	0.026033
14	81.6	0.0272
15	100.5	0.0335
16	105.7	0.035233



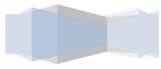
Apendix Q

Time(Days)	Voltage(mV)	Power(µW)
1	105	3.675
2	8.9	0.026403
3	13.3	0.058963
4	1	0.000333
5	0.8	0.000213
6	0.3	0.00003
7	1.4	0.000653
8	2.3	0.001763
9	12.2	0.049613
10	15.5	0.080083
11	10.7	0.038163
12	8.8	0.025813
13	78.1	2.033203
14	81.6	2.21952
15	100.5	3.36675
16	105.7	3.724163



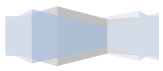
Appendix R

Time(Days)	Current(mA)	Power density(µW/m2)
1	0.035	0.000735
2	0.002967	5.28E-06
3	0.004433	1.18E-05
4	0.000333	6.67E-08
5	0.000267	4.27E-08
6	0.0001	6E-09
7	0.000467	1.31E-07
8	0.000767	3.53E-07
9	0.004067	9.92E-06
10	0.005167	1.6E-05
11	0.003567	7.63E-06
12	0.002933	5.16E-06
13	0.026033	0.000407
14	0.0272	0.000444
15	0.0335	0.000673
16	0.035233	0.000745



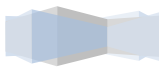
Apendix M2

Time(Days)	Voltage(mv)
1	0.1
2	32.6
3	5.7
4	12.7
5	205.9
6	332
7	305
8	43
9	68
10	75
11	67
12	62
13	55
14	46
15	49.1
16	61



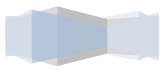
Apendix N2

Resistance(Ω)	Voltage(mV)
10	0.5
47	0.5
57	1.2
68	1.9
100	30.7
170	50.3
3000	332
100000	530



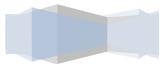
Apendix O2

Resistance(Ω)	Voltage(mV)	Current(mA)
10	0.5	0.05
47	0.5	0.010638
57	1.2	0.021053
68	1.9	0.027941
100	30.7	0.307
170	50.3	0.295882
3000	332	0.110667
100000	530	0.0053



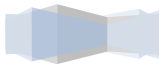
Apendix P2

Time(Days)	Voltage(mV)	Current(mA)
1	0.1	3.33333E-05
2	32.6	0.010866667
3	5.7	0.0019
4	12.7	0.004233333
5	205.9	0.068633333
6	332	0.110666667
7	305	0.101666667
8	43	0.014333333
9	68	0.022666667
10	75	0.025
11	67	0.022333333
12	62	0.020666667
13	55	0.018333333
14	46	0.015333333
15	49.1	0.016366667
16	61	0.020333333



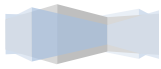
Apendix Q2

Time(Days)	Voltage(mV)	Power(µW)
1	0.1	3.33333E-06
2	32.6	0.354253333
3	5.7	0.01083
4	12.7	0.053763333
5	205.9	14.13160333
6	332	36.74133333
7	305	31.00833333
8	43	0.616333333
9	68	1.541333333
10	75	1.875
11	67	1.496333333
12	62	1.281333333
13	55	1.008333333
14	46	0.705333333
15	49.1	0.803603333
16	61	1.240333333



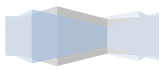
Apendix R2

Time(Days)	Current(mA)	Power density(µW/m2)
1	3.33333E-05	6.66667E-10
2	0.010866667	7.08507E-05
3	0.0019	0.000002166
4	0.004233333	1.07527E-05
5	0.068633333	0.002826321
6	0.110666667	0.007348267
7	0.101666667	0.006201667
8	0.014333333	0.000123267
9	0.022666667	0.000308267
10	0.025	0.000375
11	0.022333333	0.000299267
12	0.020666667	0.000256267
13	0.018333333	0.000201667
14	0.015333333	0.000141067
15	0.016366667	0.000160721
16	0.020333333	0.000248067



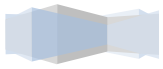
Apendix R2A

Cell Type	Voltage(mV)
VBE	105.7
VBE	179.9
VAE	332
VAE	530

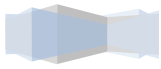


Apendix S

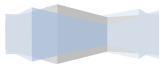
Name of isolates	Mean(OD)	Odc	2ODc	4ODc	ADHERANCE CAPABILITY	AVERAGE
WB5	0.437666667	0.3	0.6	1.2	WA	72.35294118
WB4	0.590666667				MA	15.29411765
WB3	0.444666667				WA	7.058823529
WB2/4	0.291				NA	2.352941176
WB2/3	0.252666667				WA	
WB2/2	0.467				WA	
WB2/1	0.466333333				WA	
WB1	0.324				WA	
WBF2	0.277				NA	
WBF3	0.467333333				WA	
WBF4	0.365				WA	
WBF5	0.374				WA	
WBF 6/7	1.88				SA	
WBF7	0.496				WA	
WBF8	0.485333333				WA	
WBF9/1	0.622666667				MA	
WBF9/2	0.375				WA	
WBF10	0.277333333				NA	
WE1	0.896666667				MA	
WE3	0.338666667				WA	
WE3	0.485333333				WA	
WE4	0.447666667				WA	
WE5	0.416666667				WA	
WE6	1.314				SA	
WE7	1.738666667				SA	
WE8	0.574333333				MA	
WE9	0.409666667				WA	
WE10	0.473666667				WA	
WE11	0.318666667				WA	
WE12	0.447666667				WA	
WE10(R)	0.39				WA	
WE3(R)	0.459333333				WA	
WE3(R)	0.574				MA	
WE4(R)	0.55				MA	
WEF1	0.768				MA	
WEF2	0.699333333				MA	
WEF3	0.262				NA	
WEF4	0.376				WA	
WEF5	0.412				WA	



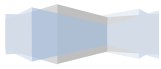
WEF6/7	0.478666667	WA
WEF5/6/7/8	0.267333333	NA
WEF8	0.5	WA
WEF10	0.347333333	WA
WBA1	0.318	WA
WBA2	0.348333333	WA
WBA3	0.441	WA
WBA4	0.478	WA
WBA5	0.67	MA
WBA6	0.359	WA
WBA7	0.502	WA
WBA8	0.330333333	WA
WBA9	0.301	WA
WBA10	0.297333333	WA
WBA11	0.355333333	WA
WEFA1/2	0.981	MA
WEFA3	0.390333333	WA
WEFA4	0.308	WA
WEFA5/6/7/8	0.238666667	NA
WBFA1	0.355	WA
WBFA2	0.378	WA
WBF(ACE)4	0.207666667	NA
WBFA6	0.298	WA
WBFA8	0.229	NA
WEFA5/6/7/8(R)	0.273	NA
S3BF1	0.399	WA
S3BF1	0.518	WA
S3BF2	0.454	WA
S3BF2	0.487333333	WA
S3BF3	0.445666667	WA
S3BF4	0.417	WA
S3BF4	0.554	WA
S3BF5	0.358666667	WA
S3BF6	0.453333333	WA
S3BF6	0.307333333	WA
S3BF7	0.408333333	WA
S3BF8	0.364333333	WA
S3EF1/2	0.489333333	WA
S3EF1	0.514333333	WA
S3EF4	0.346	WA
S3EF	0.338	WA



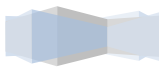
S3EF5	0.385	WA
S3EF5	0.388	WA
S3EF6	0.293	NA
S3EF7/8	0.288	NA
S3E1	0.307	WA
S3E8	0.274666667	NA
S3E9	0.468	WA
S3E10	0.41	WA
S3B1/2	0.382	WA
S3B11/12/13	0.325666667	WA
ANAEROBIC SEB2	0.325	WA
S3B1/3	0.294333333	WA
S3B4	0.555	MA
S3B5	0.254	WA
S3B4/5	0.316333333	WA
S3B6	0.314	WA
S3B6	0.357	WA
SEB7	0.504666667	WA
S3E1	0.460666667	WA
S3E7	0.285	WA
S3BF10	0.414	WA
S3E2/3/4	0.326	WA
S3E5/6/8	0.335333333	WA
S3B1	0.305333333	WA
S3B2	0.376	WA
WE1	0.288333333	WA
WE1/2/3	0.280666667	WA
WE2	0.305333333	WA
WE2	0.551333333	MA
WE4	0.304666667	WA
WE5	0.371666667	WA
WB1	0.342666667	WA
WB2	0.480333333	WA
WB3	0.312333333	WA
WB4	0.327	WA
WEF1	0.483333333	WA
WEF2	0.272666667	WA
WEF3	0.318	WA
WEF3	0.382333333	WA
WEF4	0.284	WA



WEF6	0.442	WA
WEF7	0.329333333	WA
WEF8	0.373	WA
WBF6	0.299333333	WA
W1	0.372	WA
W2	0.237333333	NA
W4	0.360666667	WA
W5	0.248333333	NA
W6	0.352	WA
W7	0.351	WA
W8	0.4	WA
WB(MIX)	0.334666667	WA
S3BF7	0.439	WA
S3BF8	0.428333333	WA
S3BF9	0.470666667	WA
S3BF1	0.453666667	WA
S3BF12	0.673666667	MA
WBFA3	0.507666667	WA
WEF1	0.326666667	WA
WEF2	0.257333333	NA
WEF5	0.208	NA
WEF6	0.258666667	WA
WEF7	0.406666667	WA
WEF8	0.188	NA
WE6	0.127666667	NA
WE7	0.135666667	NA
WEF4	0.199333333	NA
WBF3	0.317	WA
S3B3	0.283666667	WA
S3B4	0.205	NA
S3B5	0.201333333	NA
S3B5	0.277333333	WA
S3B6	0.343	WA
S3B7	0.297	WA
S3B9/10	1.337666667	SA
S3E	0.607	MA
S3E5	0.299	WA
S3E6	0.263	WA
S3E7	0.416	WA
WB6	0.245	NA

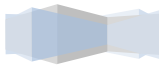


WB7	0.208	NA
WB8	0.249666667	NA
WB9	0.201	NA
WB10/11	0.274333333	WA
WB12	0.21	NA
WBFA2	0.355666667	WA
WBFA6	0.492666667	WA
WBFA7	0.521333333	WA
WBFA8	0.364333333	WA

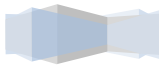


Appendix T

Phylum	2C	2
Proteobacteria	1.926479261	96.4434993
Bacteroidetes	0	0.960621838
Actinobacteria	12.64006291	0.711300139
Cyanobacteria	0.334185178	0.410647503
Chloroflexi	0	0.38864853
Verrucomicrobia	0.157263613	0.278653663
Gemmatimonadetes	0.471790839	0.249321698
deinococcus_thermus	0.530764694	0.205323752
Planctomycetes	1.218793002	0.109994867
Nitrospirae	0.058973855	0.109994867
Firmicutes	0.078631807	0.06599692
Acidobacteria	0.314527226	0.058663929
Thaumarchaeota	0.23589542	0.007332991
Spirochaetes	50.22606644	0
Bacillariophyta	7.037546688	0
Armatimonadetes	5.582858266	0
Ignavibacteriae	4.993119717	0
Chrysiogenetes	2.968350698	0
candidatus saccharibacteria	2.555533713	0
Thermotogae	2.496559858	0
Tenericutes	2.182032632	0
Chlorophyta	2.064084922	0
Chlamydiae	0.432474936	0

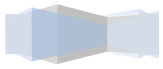


Fusobacteria	0.314527226	0
Thermodesulfobacteria	0.294869275	0
Fibrobacteres	0.255553371	0
Synergistetes	0.157263613	0
Bacteria	0.157263613	0
Chlorobi	0.11794771	0
Dictyoglomi	0.078631807	0
Eukaryote	0.058973855	0
Caldiserica	0.058973855	0

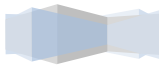


Apendix U

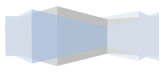
Classes	2C	2
alphaproteobacteria	9.910979228	48.51862716
betaproteobacteria	19.26805143	31.48283954
gammaproteobacteria	11.33531157	16.17043121
sphingobacteriia	2.53214639	0.616016427
actinobacteria	5.024727992	0.608682898
cyanobacteria	0.435212661	0.410677618
caldilineae	0.178041543	0.308008214
verrucomicrobiae	1.740850643	0.278674098
gemmatimonadetes	12.72007913	0.249339982
deltaproteobacteria	8.348170129	0.234672925
deinococci	0.257171118	0.205338809
cytophagia	0.573689416	0.183338222
nitrospira	2.571711177	0.110002933
planctomycetia	0.929772502	0.110002933
thermoleophilia	0	0.102669405
flavobacteriia	1.483679525	0.080668818
bacteroidia	0.791295747	0.080668818
anaerolineae	0.771513353	0.06600176
acidobacteriia	1.463897132	0.058668231
Bacilli	0.138476756	0.051334702
epsilonproteobacteria	1.681503462	0.044001173



clostridia	3.679525223	0.014667058
chloroflexia	0.257171118	0.014667058
spirochaetia	2.987141444	0
erysipelotrichia	2.591493571	0
fibrobacteria	1.721068249	0
dehalococcoidia	0.989119683	0
opitutae	0.771513353	0
negativicutes	0.672601385	0
armatimonadetes	0.474777448	0
holophagae	0.415430267	0
lentisphaeria	0.395647873	0
candidatus saccharibacteria	0.336300692	0
bacteria	0.316518299	0
dictyoglomia	0.316518299	0
ignavibacteria	0.296735905	0
phycisphaerae	0.296735905	0
bacteroidetes	0.237388724	0
chitinivibrionia	0.21760633	0
cloacimonetes	0.158259149	0
oligosphaeria	0.138476756	0
solibacteres	0.138476756	0
fusobacteriia	0.118694362	0
thermotogae	0.079129575	0

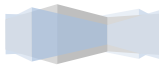


caldisericia	0.059347181	0
mollicutes	0.059347181	0
chlamydiia	0.059347181	0
acidobacteria	0.059347181	0

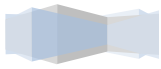


Apendix V

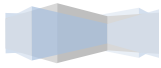
Species	2
brevundimonas diminuta	24.83132884
massilia timonae	9.064241713
pseudomonas stutzeri	5.918157818
pseudoxanthomonas mexicana	5.148137284
paracoccus aestuarii	5.03813435
duganella spp.	3.886770314
nitrosomonas europaea	3.087415664
bosea thiooxidans	2.940745087
limnobacter spp.	2.691405104
alcaligenes sp.	2.038721033
paracoccus marcusii	1.730712819
methyloversatilis universalis	1.628043414
achromobacter xylosoxidans	1.554708126
mycoplana spp.	1.488706366
nordella oligomobilis	1.474039308
sphingomonas sp.	1.474039308
ochrobactrum pseudogrignonense	1.283367556
brevundimonas sp.	0.814021707
stenotrophomonas acidaminiphila	0.777354063
alicycliphilus denitrificans	0.762687005



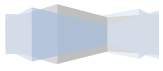
afipia sp.	0.748019947
diaphorobacter sp.	0.740686418
massilia sp.	0.740686418
sphingopyxis witflariensis	0.718685832
achromobacter spp.	0.696685245
achromobacter piechaudii	0.630683485
brevundimonas spp.	0.59401584
rhizobium giardinii	0.579348783
pseudomonas putida	0.520680551
acinetobacter johnsonii	0.498679965
methylobacterium aminovorans	0.484012907
bordetella hinzii	0.476679378
rhodomicrobium spp.	0.46201232
pseudomonas aeruginosa	0.403344089
sphingopyxis chilensis	0.388677031
pseudomonas pseudoalcaligenes	0.388677031
methylobacterium spp.	0.374009974
sphingopyxis sp.	0.366676445
pseudomonas spp.	0.366676445
pseudomonas sp.	0.359342916
terrimonas spp.	0.344675858
caldilinea spp.	0.308008214
microcystis sp.	0.300674685



methyloversatilis spp.	0.293341156
acidovorax spp.	0.286007627
schlegelella spp.	0.26400704
prosthecobacter spp.	0.249339982
gemmatimonas spp.	0.249339982
derxia sp.	0.242006454
methylobacterium suomiense	0.234672925
bradyrhizobium spp.	0.234672925
blastococcus aggregatus	0.227339396
pseudomonas resinovorans	0.220005867
nitrosomonas nitrosa	0.205338809
thermus spp.	0.205338809
acinetobacter spp.	0.19800528
pandoraea pulmonicola	0.190671751
phenylobacterium sp.	0.161337636
erythrobacter spp.	0.161337636
propionibacterium acnes	0.146670578
algoriphagus aquatilis	0.139337049
bdellovibrio spp.	0.139337049
thiobacter spp.	0.13200352
curvibacter delicates	0.13200352
acinetobacter junii	0.13200352
stenotrophomonas maltophilia	0.13200352

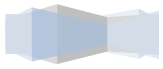


methylocystis sp.	0.124669991
leptothrix sp.	0.124669991
sphingobacterium faecium	0.124669991
burkholderia spp.	0.124669991
sphingobacterium multivorum	0.124669991
sphingomonas spp.	0.117336462
phaeospirillum fulvum	0.110002933
candidatus kuenenia stuttgartiensis	0.110002933
inquilinus spp.	0.110002933
marinobacter spp.	0.110002933
nitrospira spp.	0.110002933
anabaena spp.	0.110002933
hyphomicrobium aestuarii	0.102669405
rhizobium spp.	0.102669405
thermoleophilum spp.	0.102669405



Apendix W

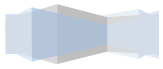
Phylums	3C	3
Proteobacteria	63.94889	94.42434
Verrucomicrobia	1.659642	3.203324
Firmicutes	3.753952	0.824286
Bacteroidetes	2.581665	0.609838
Acidobacteria	10.78767	0.455703
Actinobacteria	0.803477	0.154135
Nitrospirae	1.053741	0.127329
Synergistetes	0	0.093821
Chloroflexi	7.863541	0.033508
Planctomycetes	1.383035	0.026806
Fusobacteria	0	0.026806
Chlamydiae	0	0.020105
gemmatimonadetes	1.659642	0
Dictyoglomi	0.961538	0
candidatus saccharibacteria	0.922023	0
Fibrobacteres	0.698103	0
Bacteria	0.592729	0
Ignavibacteriae	0.474183	0
Cyanobacteria	0.461012	0
Tenericutes	0.131718	0
Lentisphaerae	0.105374	0
thermodesulfobacteria	0.079031	0
deinococcus_thermus	0.039515	0
Chrysiogenetes	0.039515	0



Apendix X

Classes	3C	3
gammaproteobacteria	16.74131	68.30184
Betaproteobacteria	16.39884	23.30787
Opitutae	0.381981	3.183219
Alphaproteobacteria	18.47998	2.694009
Bacilli	0.684932	0.777376
Sphingobacteriia	1.409378	0.583032
Solibacteres	0.039515	0.395389
Actinobacteria	0.803477	0.154135
Nitrospira	1.053741	0.127329
Deltaproteobacteria	12.05216	0.120627
Synergistia	0	0.093821
Clostridia	2.410432	0.046911
Anaerolineae	3.332455	0.033508
Holophagae	2.305058	0.033508
Acidobacteriia	8.324552	0.026806
Planctomycetia	1.040569	0.026806
Bacteroidia	0.105374	0.026806
Fusobacteriia	0	0.026806
Verrucomicrobiae	1.277661	0.020105
Chlamydiia	0	0.020105
Caldilineae	2.054795	0
Gemmatimonadetes	1.659642	0

Chloroflexia	1.409378	0
Dictyoglomia	0.961538	0
Dehalococcoidia	0.922023	0
candidatus saccharibacteria	0.922023	0
Fibrobacteria	0.698103	0
Negativicutes	0.619073	0
Cytophagia	0.619073	0
Bacteria	0.592729	0
Ignavibacteria	0.474183	0
Cyanobacteria	0.461012	0
Flavobacteriia	0.44784	0
Phycisphaerae	0.342466	0
epsilonproteobacteria	0.276607	0
Thermomicrobia	0.144889	0
Mollicutes	0.131718	0
Acidobacteria	0.118546	0
Lentisphaeria	0.105374	0
thermodesulfobacteria	0.079031	0
Deinococci	0.039515	0
Chrysiogenetes	0.039515	0
Thermolithobacteria	0.039515	0



Apendix Y

Species	3
pseudomonas spp.	35.73247554
methyloversatilis universalis	16.23776974
pseudomonas plecoglossicida	7.163919046
pseudoxanthomonas mexicana	5.589063128
pseudomonas monteilii	5.528749497
pseudomonas oryzihabitans	4.731269267
pseudomonas putida	3.672429969
opitutus sp.	2.573381584
methyloversatilis spp.	2.466157352
pseudomonas taiwanensis	1.434124112
acidovorax facilis	1.38721351
pseudomonas sp.	1.025331725
pseudomonas stutzeri	1.011928696
pseudomonas vancouverensis	0.898002949
bacillus spp.	0.777375687
opitutus spp.	0.609837823
balneola spp.	0.562927222
massilia timonae	0.475807533
dokdonella koreensis	0.469106018
brevundimonas diminuta	0.402090873
candidatus solibacter uncultured solibacter sp.	0.395389358
nitrosomonas europaea	0.361881785
pseudomonas pseudoalcaligenes	0.281463611
mycoplana spp.	0.274762096

hydrogenophaga pseudoflava	0.261359067
acidovorax spp.	0.227851494
dechloromonas spp.	0.180940893
hydrogenophaga spp.	0.180940893
sphingopyxis macrogoltabida	0.180940893
leptothrix sp.	0.174239378
paracoccus aestuarii	0.167537864
parvibaculum spp.	0.160836349
hyphomicrobium spp.	0.14743332
thauera sp.	0.134030291
achromobacter xylosoxidans	0.134030291
nitrobacter winogradskyi	0.134030291
pseudomonas nitroreducens	0.127328776
rhodococcus sp.	0.120627262
thauera spp.	0.113925747
pseudomonas alcaligenes	0.113925747
dokdonella spp.	0.113925747
hydrogenophaga sp.	0.113925747
paracoccus marcusii	0.107224233
bosea thiooxidans	0.100522718
stenotrophomonas maltophilia	0.100522718