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Remodeling in Microbial Fuel Cell (MFC) design and parameters for the sustained production of electricity

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Abstract— *In the recent years Microbial fuel cells (MFCs) have gained much attention as an alternate source of sustainable power production. In MFCs the bacteria at anode are used as catalyst for extraction of electrons from biodegradable substrate. In the present study bacteria having electricity production potential were isolated from pond's sludge and were identified using different microscopic, staining techniques and with the help of different biochemical tests. Double chambered MFCs were constructed to check the ability of those bacteria for current generation. Initially double chambered MFC was constructed using 250 ml of sludge as a source and maximum current produced was 119 mV. This experiment was repeated using 500 ml of mixed culture and the maximum current production of 169 mV was recorded. Glucose, peptone and yeast extract were used as a substrate for the growth and current production by bacteria. Different parameters such as chemical mediators, different electrode types and sizes and salt-bridge concentrations were utilized for production and amplification of current generated in MFC. Methylene blue indicator dye was found suitable for enhancing current in MFCs for short time. Two modes of feeding were used to increase the lifetime of cell i.e., and from the results of current study, it was concluded that fed batch mode was more effective as compared to non-fed batch culture.*

Keywords— ***Double chambered microbial fuel cells, Electricity production, fed batch mode, Microbial fuel cell (MFC's), Salt-bridge.***

I. INTRODUCTION

Microbial fuel cell (MFC) is a device which uses bacteria as the catalysts to oxidize organic and inorganic matter for the generation of electric current. When these bacteria produce electron from these substrates, then they are transferred to the anode i.e., negative terminal and then it streams to the cathode i.e., positive terminal which is connected through a conductive material and that is composed of a salt bridge [1]. So MFC essentially converts the energy which is provided in bio-convertible substrate into the electric current reliably [2].

From the 1960s till very lately, it was believed that the exogenous mediators are required to be added into the microbial fuel cell to produce sensible amounts of power. However, Kim and his co-workers verified that power could be produced by a naturally existing association of bacteria

in the absenteeism of exogenous mediators. Other workers then disclosed that by simply placing produced to power subsurface devices Now it appears that the current can be produced from any material that is biodegradable, extending from pure compounds for instance acetate, bovine serum albumin, ethanol, glucose and cysteine to complex mixtures of organic matter that involves domestic (of human), animal, food-processing and meat-packing wastewaters. The bacteria which are electrochemically active in MFCs are considered to be the iron-reducing bacteria such as *Shewanella* and *Geobacter* species, but the examination of such communities shows a diversity of bacteria much greater than these model iron reducers persevering in the biofilm community. The maximum power densities are limited due to the high amount of internal resistance. And the comparisons made with different systems by using pure cultures or mixed cultures cannot establish which microbial community is accomplished by the highest power density [3].

1.1.1 APPLICATIONS

MFC has many uses but we will discuss some of its dominant uses for instance as environmental sensors, production of hydrogen gas in MFCs, for the production of electricity, biosensors, as a pollutant degrader, as removers of fermentation inhibitors and for the empowerment of implanted medical devices etc.

1.1.2 AS ENVIRONMENTAL SENSORS

In order to understand and indicate the response of ecosystem the information about normal atmosphere can be helpful. The sensors that are dispersed in the natural atmosphere want power. MFC can be used to give power to these devices, mostly in areas such as deep-water and river environments where it is difficult to habitually approach the structure to change the batteries. Sedimental fuel cells are being recognized to screen the environmental systems for instance streams, brooks and the ocean. The organic matter in the residues function as power for these devices.

1.1.3. PRODUCTION OF HYDROGEN GAS BY MFCS

Hydrogen can be considered as a significant constituent of an energy structure that decreases CO2 emissions if hydrogen is produced from non-fossil fuel sources and then it is used in microbial fuel cells. High substrates of sugar when fermented then it can produce hydrogen gas through this biological process at more concentration of 60% [4]. By eradicating oxygen at the cathode and the addition of a small voltage through the biochemically aided microbial reactor (BEAMR) process, the MFCs can yield hydrogen gas when altered through this pattern. Bacteria produces an anode working potential of approximately –0.3 Volt. The electrons as well as protons that are fashioned at the anode can combine at the cathode to produce hydrogen gas with only an additional total cell potential of 0.11 Volt. Though in practice 0.25 V or more than this should be placed into the circuit to make hydrogen due to the reason of overpotential at the cathode. Through the process of BEAMR the production of biohydrogen is not constrained or limited to glucose only. Any substrate which is biodegradable and generates electricity in an MFC will work in the system of BEAMR. This pattern works with domestic wastewater, but the issue is that the hydrogen recoveries in current reactor designs are still too low to make hydrogen production with BEAMR likely to be as viable as electricity production with MFCs. High-strength wastewaters appeared to be the most instant potential for hydrogen recovery in the BEAMR process [5].

1.1.4. PRODUCTION OF ELECTRICITY

Microbial fuel cell is given importance Because of the ability of MFC to produce sustained current for longer duration it has given much importance. At present MFC is considered as the active method of current generation. The series of algae or aquatic plants acts as a feedstuff are used where the hydrogen production in manure plants and the greenhouse gases are used in electric current. The processed sugars and other compounds that caused the explosion of steam for corn wastage could be used as feedstuff of MFC while these microbial cells don't follow the Carnot cycle because the chemical energy from the fuel particles to oxide reliably converted into electricity instead of conversion into heat. In the hypothetical opinion, the proficiency of microbial fuel cell is over 70% though still producing electricity from MFCs in a very small quantity. The solution of this issue in a one way is to perform practically by storing it in the rechargeable power device. The appropriate method for MFCs offering energy is the automated communications process that consumes less energy. For example, for power production the wireless sensors for signal broadcast in distant areas. These MFCs can also be used as natural power distribution systems in areas which are not much developed all over the world.

1.1.5. BIOSENSOR

MFCs can be used hypothetically as a sensor for monitoring the in-situ process, directing and for inspection of contaminants [6]. The organic matter content in the wastewater is usually assessed in relations of biological oxygen demand (BOD). The examination of BOD according to ancient procedures take 5 or 7 days incubation period at 20◦C +1 or -1 in the dark. For the guesstimate of water and to make a guess of the wastewater quality the best procedures are BOD5 and BOD7. Most of the time the samples of wastewater are calculated through this conventional procedure. The expensive equipment's are not

required here. The limitations involve that it consumes time and needs expertise and much experience to get the reproducible results. So, the ancient procedures of BOD are not good for on-line monitoring and control of biological wastewater treatment processes due to the important need of rapid feedback. Consequently, it is important to change such old methods and introduce a method that could give fast measurements to demonstrate the lively changes in the process of treatment. To construct biosensors the concept of MFC has been applied for fast BOD estimation, in which a biological sensing element that is bacteria in anode compartment and a transducer that is electrodes and proton exchange membrane, they are combined. In 1977 Karubeetal developed an MFC biological oxygen demand biosensor consuming the hydrogen which is produced by the help of Clostridium butyricum restrained on the electrode. Many kinds of MFC-based BOD biosensors have been developed by following this procedure, for instance, mediator-less MFCs and microbial fuel cells with electron transfer mediated. Such sensors which are based on microbial fuel cell have the benefit of lasting stability and can be used continuously for operational monitoring of wastewater [7].

1.1.6. MFCS AS A POLLUTANT DEGRADER

Those compounds which are degraded by bacteria are converted into current. The capability of the microbial communities of microbial fuel cell to reduce a large number of pollutants environment could be more appreciable than electricity generation itself in some situations, exclusively when this technique could be used for the cleanup of environment in-situ. For the anaerobic deprivation of petroleum components and landfill leachate pollutants in ground water some species such as Geobacter has been shown as important. In soil, the utilization of electrode which acts as an electron acceptor is very attracting because the degradation causing microbes will co-localize with the pollutant at the anode that is of graphite. When in place those electrodes can deliver a continuous lasting electron sink for the deprivation of the destructive environmental pollutants. In such situation, the electrons that are produced by the microbes in the form of electricity is inappropriate when related to the enlarged bioremediation rate.

1.1.7. FERMENTATION INHIBITOR REMOVERS

Similarly, experimentations have revealed that microbial fuel cells may likely be able to eradicate inhibitors of fermentation which gather in process water after the pretreatment of cellulosic biomass. The exclusion of the inhibitors permits for enlarged fermentation product profit while providing small quantities of energy.

1.1.8. IMPLANTED MEDICAL DEVICES EMPOWERMENT

A rare application for this MFC technology is to rule the fixed medical devices using glucose and oxygen from blood. An implanted MFC could provide power indefinitely and refute the necessity for surgery to substitute batteries. Based on noble metal catalysts the abiotic fuel cells and activated carbon have been established to produce energy from blood glucose in vitro and based on enzymatic catalysts in the fuel cells have also been shown to work under functional conditions but still require much improvement to become feasible [8].

1.2 CURRENT PRODUCING MICROORGANISMS

Exo-electrogenic bacteria are those organisms that have the ability to transfer electrons to the extracellular electron acceptors that are insoluble and also have the potential to be used in devices like microbial fuel cells. Presently, these exo-electrogens have been recognized in the Firmicutes and Acido-bacteria and also in Alpha-, Beta-, Gamma- and Deltaproteobacteria. Even though most current MFCs perform optimally when they contain a mixed microbial community, some pure cultures that exhibit strong electrogenic activity in the environment of MFC have been characterized. The properties of electrogenic and some characteristics of extracellular electron transfer have been well-defined for pure cultures of organisms such as Rhodopseudomonas palustris DX-1, Ochrobactrum anthropi YZ-1, Geobacter sulfurreducens, Escherichia coli, Shewanella putrefaciens and Rhodoferax ferrireducens. The existing list of confirmed exoelectrogens includes representatives of four of the five classes of

Proteobacteria in which the Epsilon proteobacteria and the representatives of Firmicutes and Acidobacteria are not characterized. Though, it is likely that innovative electrogenic bacteria remain to be revealed [9].

1.2.1 MECHANISM

The statistic that some bacteria can transport electrons beyond their cell wall and for that reason they electrically intermingle with the environment is known for over a century. This property can be utilized to grow advanced electrically enhanced bioprocesses. In such bulk electric systems, organisms interact with electrodes through exchanging of electrons which are sometimes supplied or removed sometimes through an external electrical circuit. In MFCs the microbes donate electrons to electrodes and produce electrical current. In bioremediation of groundwater and sediments (aquatic) where microbes that are metal reducing catalyzes the conversion of organic contaminants to carbon dioxide. Within such systems the oxidation of anode by bacteria is joined to the construction of chemicals such as hydrogen or methane on the cathode

and they are denoted as microbial electrolysis cells. Electrons are transferred from a low potential electron donor to an acceptor having more positive redox potential by some reactions such as redox reactions in a microbial electron transport chain. Usually, these reactions are catalyzed by compounds that are membrane bounded and uses the energy difference between donor and acceptor to launch an ion gradient across the membrane which is used for the synthesis of ATP and does conversion of the difference in electrical potential into chemical energy [10]. Bioelectrochemical systems (BES) which are typical MFCs have arisen as hopeful technologies for bioremediation as well as energy generation. Those microorganisms that are attached to the electrodes as biofilms has a main role in electric current generation and in biosynthesis in Bioelectrochemical systems. Because of the capacity of extracellular electron transfer to electrodes weather directly or indirectly the electricity generating bacteria can be well defined as exoelectrogens. The transfer of electrons from exoelectrogens to electrode can combine with the energy conservation and can support their growth so it can be considered as respiration of electrode/anode. Motivated by the increased interests in bioelectrochemical, bacterial electrode respiration and electromicrobiology has received much attention in current years. The c-Type cytochromes that are well-thought-out as one of the most significant electron transfer strategy in electricity generation by exoelectrogens are the heme-containing proteins in most archaeas as well as bacterias. Biofilms contains many microbial cells which are compactly stacked and disseminated spatially in the extracellular polymeric substances. A network of complex electrons that involves numerous components of electron transfer can be assumed in an electric current generating biofilm. Some other factors such as the diffusion co-efficiency, the prearrangement of the electron transfer components in EPS and pH gradient can have an important impact on the electron transfer in biofilms. A proper electron shuttle in bioelectrochemical system can be dissolvable, stable, reusable, environment friendly, and can have a proper redox potential. Many bacterias most importantly G− bacteria has the ability of secreting electron shuttles in bioelectrochemical systems. Also, it has been recommended that the bacterial shuttle secretion can be stimulated by electricity generation in microbial fuel cells [11]. Producing power in microbial fuel cells depends on the redox chemistry. Microbial fuel cells contain anode and cathode compartments in which each of which grasps an electrode that is separated by a cation-permeable membrane. In the anode chamber, microbial substrates such as acetate (an electron donor) are oxidized in the absence of oxygen by respiratory bacteria, producing protons and electrons. The electrons are passed through an electron transport chain (ETC) and protons are translocated across the cell membrane to generate adenosine triphosphate (ATP). Electrons and protons exiting the ETC typically pass onto a terminal electron acceptor such as oxygen, nitrate, or Fe (III). However, in the absence of such acceptors in an MFC, some microorganisms pass the electrons onto the anode surface. Difference in redox potentials (i.e., the ability of a compound to donate or accept electrons, denoted E_o and measured in volts) between the electron donor and the electron acceptor is the determinant of the potential energy available to the microorganism for anabolic processes. In an MFC the electrochemical redox potential difference of the anode and cathode determines for bacteria to produce electricity in MFCs, the cells need to transfer electrons generated along their membranes to their surfaces. Very little information is known about bacterial interactions with electrodes. While anodes and cathodes can function in bacterial respiration, research has been focused on understanding microbial anodic electron transfer. Anode-respiring bacteria catalyze electron transfer in organic substrates onto the anode as a surrogate for natural extracellular electron acceptors (e.g., ferric oxides or humic substances) by a variety of mechanisms [12].

II. DESIGN OF MFCS

There are basic components of MFCs which are important in constructions. Electrodes, wirings, glass cell and salt bridge have an important role. Salt bridge is replaced with Proton exchange membrane in PEM fuel cell. Though it enhances the cost but handling and the power generation both get enhanced, thus increasing the portability and efficiency of the system. Apart from that fuel cells can be classified in two types on the basis of number of compartments or chambers.

2.1.1 DOUBLE CHAMBERED MICROBIAL FUEL CELLS

Both the cathode and anode are housed in different compartments or chambers connected via a proton exchange membrane (PEM) or sometimes salt bridge. PEM or salt bridge mainly functions as medium for transfer of proton to make the circuit complete. This not only completes the reaction process but also prevents anode to come in direct contact with oxygen or any other oxidizers. They are run in batches and can be used for producing higher power output and can be utilized to give power in much inaccessible conditions. It can be suitable designed to scale up to treat large volume of wastewater and other source of carbon. These particular types are called up flow mode of microbial fuel cell. They practically fall between the classification of single chambered and double chambered microbial fuel cells. They are mediator-less and

sometimes membrane-less and can be used for large scale production of electricity from the wastes.

2.1.2 SINGLE CHAMBERED MICROBIAL FUEL CELLS

They are simple anode compartment where there is no definitive cathode compartment and may not contain proton exchange membrane. Porous cathodes form one side of the wall of the cathode chamber utilizing oxygen from atmosphere and letting protons diffuse through them. They are quite simple to scale up than the double chambered fuel cells and thus have found extensive utilization and research interests lately. The anodes are normal carbon electrodes but the cathodes are either porous carbon electrodes or PEM bonded with flexible carbon cloth electrodes. Cathodes are often covered with graphite in which electrolytes are poured in steady fashion which behaves as catholytes and prevent the membrane and cathode from drying. Thus, water management or better fluid management is an important issue in such single chambered fuel cells.

2.1.3 STACKED MICROBIAL FUEL CELLS

These are another type of construction in which fuel cells are stacked to form battery of fuel cell. This type of construction doesn't affect each cell's individual Coulombic efficiency but in together it increases the output of overall battery to be comparable to normal power sources. These can be either stacked in series or stacked in parallel. Both have their own importance and are high in power efficiency and can be practically utilized as power source [13].

III. MATERIALS AND METHODS

This research took place at Biotechnology Research Laboratory, Centre of Biotechnology and Microbiology (COBAM), University of Peshawar, from April 2017 to June 2017. The samples were collected from pond as a source at University of Peshawar. In this experiment, double chamber and stacked cell microbial fuel cells were designed using fed batch and batch mode of feeding microbes for the purpose of maintenance and amplification of voltage generation for longer duration. Different mediators were checked to optimize the voltage generation and results were recorded to find out the most functional one.

All the equipment's, chemicals and materials that were used in this study were provided by the Centre of Biotechnology and Microbiology and the Department of Physics, University of Peshawar.

3.1. DOUBLE CHAMBERED MICROBIAL FUEL CELL CONSTRUCTION

Two double chamber microbial fuel cells were built in this experiment using different terms and conditions and were examined to find best out of them. For feeding microbes the modes selected was Fed Batch mode.

3.1.1. SAMPLE SOURCE

In this microbial fuel cell, the sample used was the sludge of pond; all the microbes present in the sludge were used as the anaerobes and were placed in the anaerobic half of the MFC. The sludge was collected from the bottom of Botany department pond at University of Peshawar. The reason of selecting from that area was that the anaerobic concentration is higher at the bottom because oxygen is in lesser amount in such areas. The collection of sample was done with the help of shovel in sterile bucket. The soil sample was dugged up to 10 inches and then the sample was collected by using clean gloves and was stored in a sterile zipper bag of polyethylene.

3.1.2. AN ANAEROBIC COMPARTMENT CONTRUCTION

A container made up of plastic having lids was taken and with the help of a driller, hole was made on one side of the container for salt bridge. All the ingredients such as sludge, table sugar and peptone in amounts 500 g, 30 g and 2 g respectively were added into the container. Electrode of Carbon of dimension 2 inches width x 3 inches length were attached with copper wire and was placed into the container in such a way that it was dipped in the sludge. The container was covered with the lid to make it airtight.

3.1.3. AEROBIC COMPARTMENT CONSTRUCTION

A hole drilled in a plastic container with the help of driller on one side was taken. Ingredients such as sodium chloride salt and distilled water in amounts 100 g and 500 ml respectively were added to container. The electrode of carbon of dimension 2 inches width x 3 inches length were attached with the copper wire are dipped into the electrolyte solution in the container. A hole in the lid of the container was also drilled to allow the air to enter into it. Despite that the container was covered with the lid to prevent the entrance of excess air into the container.

3.1.4. SALT BRIDGE SOLUTION CONSTRUCTION

Then salt bridge solution was made by taking 40 grams of table salt and 8.5 grams of Agar were weighed using digital weight balance. 300 ml of water was taken in the beaker and heated till boiling by using a burner. The salt and Agar were mixed into the boiling water. This mixture was carefully poured into the plastic pipe of about 1.5 Meter length with one end closed by using a tape. The pipe was shifted for some time in the refrigerator so that the mixture gets solidify. Once it gets solidified, the salt bridge is ready to use.

3.1.5. MICROBIAL FUEL CELL DESIGN

To design a fuel cell all parts were joined. The aerobic compartment was connected with the anaerobic compartment through salt bridge with the help of holes drilled in the compartments. The anaerobic compartment was sealed properly by applying plaster of Paris.

The anaerobic compartment acted as anode whereas the aerobic compartment as the cathode. The wires of the anode and cathode were connected to the positive and negative terminals of the multimeter. In anaerobic compartment sludge of pond was used. After designing the fuel cell no voltage was measured initially but with the passage of time the voltage started increasing gradually. The maximum voltage was measured at day 3 and was maintained for up to 50 hours. After 50 hours, the voltage decreased slowly but still the cell was generating voltage for up to 4 weeks and then the voltage became zero.

Our main focus was to amplify and sustain the current for longer time so the methodology was improved to maintain and amplify the voltage generated for longer time. The changes made were by altering the feed of microbes, by altering the concentration of feed of microbes, by changing the mode of feeding microbes such as batch or fed batch, by the Use of different mediators, by Changing dimensions and materials of the electrodes or by Increasing the quantity of sludge to amplify the voltage generated.

3.2. MICROBIAL FUEL CELL USING FED BATCH MODE OF FEEDING

In this case the basics were same as that of the previously described but only the concentrations of sludge were changed and instead of batch, fed batch mode for feeding the microbes was adapted. The amounts used in this case were two times more than used previously.

3.2.1. ANAEROBIC CHAMBER

1-liter mixed culture of pond's sludge, 12 grams of Glucose and 3 grams of Yeast extract was used as energy sources. Electrode of copper having dimensions 4 inches widthx3 inches length was used. Here the electrode concentration was increased.

3.2.2. AEROBIC CHAMBER:

1 Liter of 10X TBE Buffer was taken in the aerobic half of MFC. An electrode of copper with a copper wire was dipped into the buffer.

3.2.3. DESIGNING A MICROBIAL FUEL CELL

The aerobic and anaerobic compartments were connected together by means of a salt bridge which acts as a proton exchange membrane. Aerobic act as cathode and anaerobic as anode. The wires that were attached to the electrodes were connected to the multimeter. No voltage was measured right after the designing of a cell. After approximately 6 hours the voltage was generated by the cell and reading was measured then. In this case, the microbes were fed after every 24 hours with 12 grams of Glucose and 3 grams of Yeast's extract. The voltage generated was sustained for a longer time now and did not decline because the energy source was there in cell this time. After 72 hours, Methylene blue (a mediator) was added and it showed increase in voltage generated up to some extent. This cell generated maximum voltage for almost 4 weeks, after that the value of voltage started decreasing and after that negligible voltage was generated by the cell.

3.3. MICROBIAL FUEL CELL UTILIZING NON-FED BATCH MODE

When the food materials are added at once in the start of the process and then the chambers are properly sealed it's called the non-fed batch mode of feeding. Below is the process involved in making this MFC.

3.3.1. MAKING OF AN ANAEROBIC CHAMBER

Anaerobic chamber was made by taking a lid containing container made up of plastic and by the use of a driller a hole was made on one side of the container for salt bridge entrance. The composition of anaerobic chamber is sludge, table sugar and peptone in amounts 500g, 30g and 2g respectively. All these ingredients were added into the container. Electrode of copper attached with copper wire was placed into the container in such a way that it was dipped in the sludge. The container was then covered with the lid so that air do not enter into it.

3.3.2. MAKING OF AEROBIC CHAMBER

Aerobic chamber was made by drilling a hole by driller into a plastic container on one side of it for salt bridge entrance. Ingredients such as sodium chloride salt and distilled water in amounts 100g and 500 ml respectively were added to the container. Dipped the electrode of copper attached with the copper wire into the electrolyte solution in the container. A hole in the lid of the container was also drilled so that air can enter into it. The container was covered with the lid to prevent the container from excess entrance of air.

3.3.3. SALT BRIDGE COMPOSITION

40 grams of table salt and 8.5 grams of Agar were weighed using digital weight balance.

300ml of water was taken in the beaker and heated till boiling by using a burner. The salt and Agar were mixed into the boiling water. This mixture was carefully poured into the plastic pipe of about 1.5 Meter length with one end closed by using a tape. The pipe was shifted for some time in the refrigerator to make the mixture solidify. After that it was used.

3.3.4. DESIGNING OF A MICROBIAL FUEL CELL

All the parts were combined to design a fuel cell. The anaerobic and aerobic compartment were joined by means of salt bridge through the holes drilled in the compartments. The anaerobic compartment was sealed properly by using the plaster of Paris. The anaerobic compartment was labeled as the anode and aerobic compartment as the cathode. The wires of the anode and cathode were connected to the positive and negative terminals of the multi meter. The source of microbe was pond's sludge. The moment, at which the cell was designed, no voltage was measured but slowly and gradually as the time passed the voltage started increasing. The maximum voltage was measured at day 8 and was maintained for up to many hours in case of sludge. After that the voltage decreased slowly.

3.4. MAINTAINANCE

The operating conditions for MFC was room temperature. In the cathodic chamber of microbial fuel cell of both units, oxygen was allowed to enter (that will combine with H ions and electron from the anodic chamber to form water molecule). 7g yeast and 40g glucose (feed of bacteria/for maintaining the growth of bacteria in an aerobic chamber) was added to anodic chamber of both MFC units on day 1. Current was measured of the units individually and then in stacked form. Immediately after MFC construction, no current was recorded.

After sometime current was produced as a result of microbe's accumulation on anode (biofilm formation). After 24 hours, 7g yeast was added again in anaerobic chamber of both units and then change in current was detected.

3.5. IDENTIFICATION OF THE ISOLATES

For identification of isolates gram staining and different biochemical tests were performed.

3.5.1. GRAM STAINING

For gram staining small colony was picked from the plate and smear was made on the slide.23 drops of crystal violet stain were added on the smear for 1-2 mins. After that the slide washed gently with the water. Then the smear was covered with gram iodine and slide was left for 1-2 mins. Then the slide was decolorized using ethanol. Safranin dye was added for 2 mins and after that the slide was washed gently with water, air dried and was observed under the microscope using oil immersion.

3.5.2. MACCONKEY – AGAR

MacConkey agar was prepared by dissolving proper amounts of all ingredients in distilled water as recommended by the manufacturer. The medium was autoclaved and poured in petri plates in laminar flow hood. To check the sterility the petri plates were placed in incubator for overnight at 37°C, medium was initially red in color. Sterile plates were then streaked and incubated.

3.5.3. CATALASE TEST

In Catalase test with the help of inoculation loop we have picked the inoculum from the center of pure colony and were placed on a clean glass slide. A drop of 3% hydrogen peroxide was added to cover the colony completely. Immediate bubbling (gas liberation) was recorded as a positive result.

3.5.4. ENDOSPORE STAINING

In endospore staining, inoculum of organism to be tested was taken and smear was made on a clean microscopic slide for presence of endospores and the slide was air dried. He slides was passed over flame 2 to 3 times to fix the smear. The slide was heated gently by putting the slide on a beaker that has been placed over Bunsen burner. The malachite green was added 12 drops, after 5 mins the slide was removed and allowed to cool to room temperature for 2 mints. The slide was rained with tap water. Then for 2 mints the smear was stained with safranin; the secondary was removed with the help of rinsing. Then the slide was dried and observed under microscope.

3.5.5. TRIPLE SUGAR IRON TEST

With a sterilized straight inoculation needle was touched the top of a well-isolated colony and TSI Agar was inoculated by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant was done. The cap was left on loosely and the tube was incubated at 35°C in ambient air for 18 to 24 hours.

IV. RESULTS

The aim of this research was to remodel a working Microbial Fuel Cell and to sustain and increase the current generated. Out of three different Microbial Fuel Cells designed, two showed satisfactory results. The voltage produced by individual cell was measured at different intervals of time. As stated earlier, inoculum from a single source (pond sludge of Botany department, University of Peshawar) was used, two out of three were built using pond's sludge. During this research, different parameters were used in order to optimize and amplify the voltage for maximum possible time. Fed batch gave best results out of two modes of feeding microbes. MFC built using pond sludge as inoculum gave suitable results.

4.1. VOLTAGE GENERATION FROM POND SLUDGE USING DOUBLE CHAMBERED MFC (1st design)

Double chamber MFC using pond sludge was performed on 28th April, 2017. Results are shown in table 1.

Table 1. Voltage generation in a double chamber microbial fuel cell using pond sludge

Date	Time (in hours) after the cell was designed	Voltage generated by cell built up from pond's sludge (in mV)
28/4/2017	18	30
28/4/2017	3	33
28/4/2017	2	34
28/4/2017	2	36
02/5/2017	65	18
03/5/2017	24	75
03/5/2017	5	79
03/5/2017	1	80
04/5/2017	18	92
05/5/2017	27	119
08/5/2017	60	90
08/5/2017	7	87
09/5/2017	18	90
10/5/2017	20	98
10/5/2017	7	93
12/5/2017	18	88
15/5/2017	74	44
16/5/2017	24	23
17/5/2017	23	14
18/5/2017	24	3
19/5/2017	23	2.7
22/5/2017	25	2.3
23/5/2017	24	2
24/5/2017	24	2.1
25/5/2017	27	2
26/5/2017	22	2.2
29/5/2017	24	16

This experiment performed that electricity can be generated by using pond sludge and bacteria involved in current production were already present in pond sludge. It was shown that a voltage of 30mV was generated within only 18 hours. The voltage gradually increased with time because of biological activity of bacteria. The maximum voltage (119mV) was recorded on day 8 (after 192 hours of cell construction). The voltage was generated by cell over a long period and it was observed that whenever we feed it, an increase had occurred in it (Fig 1).

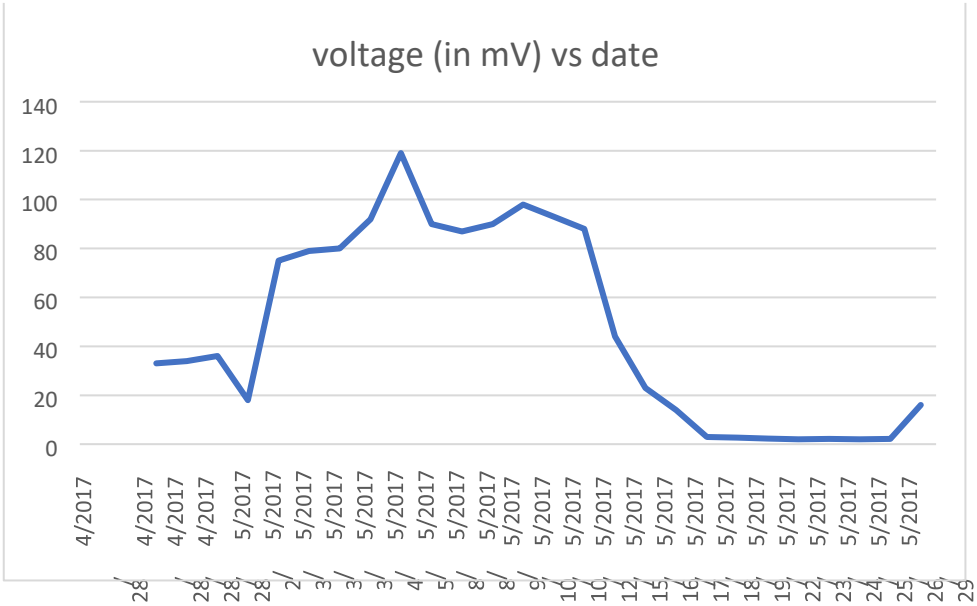

Fig 1. Voltage generation from pond sludge in double chambered MFC (1st design)

4.2. VOLTAGE GENERATION FROM POND SLUDGE USING DOUBLE CHAMBERED MFC (2nd design)

A 2nd Double chamber MFC design using pond water for electricity generation was constructed on 16th May,2017. The resulting voltage generated is given in table 2.

Table 2. Voltage generated in Double chamber MFC from pond sludge (2nd design)

Date	Time (in hours) after the cell was design	Voltage measured from the cell made from pond sludge
16/5/2017	25	31
17/5/2017	22	82
18/5/2017	23	104
19/5/2017	24	119
22/5/2017	72	66
23/5/2017	24	9.6
24/5/2017	26	34
25/5/2017	23	49
26/5/2017	23	56
29/5/2017	71	137
29/5/2017	4	88
15/6/2017	17 days	169

After constructing a new design, double chambered MFC, a voltage of 31mV was given at 25th hour. After this period of approximately 22h, cell voltage exponentially increased over the next 50hrs reaching an initial maximum voltage of 119V. With the passage of time, nutrients were metabolized by bacteria, decrease in voltage occurred. Then we feed it again and again so that the microorganisms do not die and the current is sustained. After a long time if there is enough food and enough anaerobic environment is provided then an increase in current is seen like after 17 days the current measured was 169. The power generation in double chamber MFC used in our experiment was limited by internal resistance (min et al. 2005) and nutrients un availability (as mode of feeding was some time batch and no nutrients were added during experiment (Fig 2).

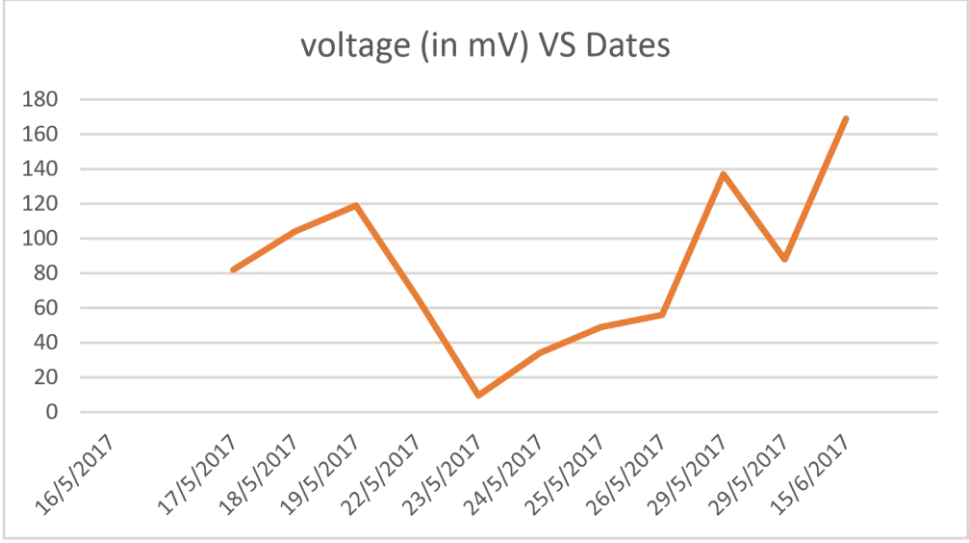

Fig 2. Voltage generation from pond sludge in double chambered MFC (2nd deign)

It is clear from Fig. that initial voltage given by the MFC using pond sludge (31) was higher than that of given by 1st design (30 mV). In addition to that, voltage generated by using pond sludge was maintained for longer period of time as compared to MFC using other samples.

4.3. COMPARISON OF VOLTAGE GENERATION IN DOUBLE CHAMBER MFC USING 1st DESIGN AND 2nd DESIGN

Double chambered MFC using 1st design MFC and 2nd design MFC was constructed to examine the voltage generation. The anaerobic chamber was fed with glucose and yeast (as carbon and energy source) in increments after every 24 or 48 hours. The resulting voltages from 1st and 2nd design recorded are shown. The maximum recorded voltage was from pond sludge. A comparison between voltage generated from pond sludge and soil is given in Fig 3.

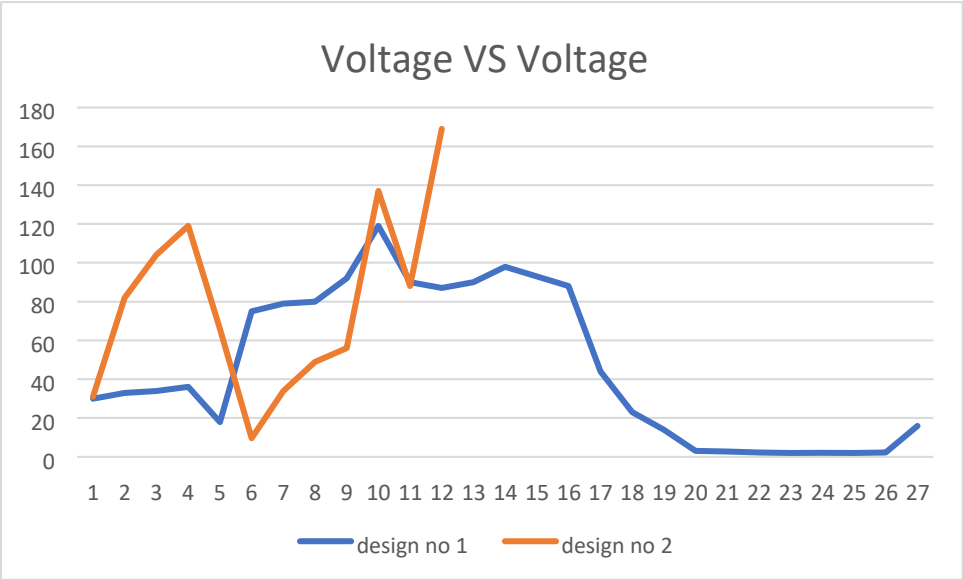

Fig 3. Comparison of voltage generated from 1st design and 2nd design

4.4. OTHER FACTORS AFFECTING VOLTAGE PRODUCTION

Several other factors were also examined with respect to voltage output like dimensions and materials of electrodes were changed, energy sources were improved and mediators were used in building double chamber cells. This time the results obtained were very good. Three different type of electrodes were used in this experiment for construction of three different MFC. Carbon electrodes showed good results as compared to aluminum electrodes used. The voltage produced using electrodes of different surface area is compared in below fig4.

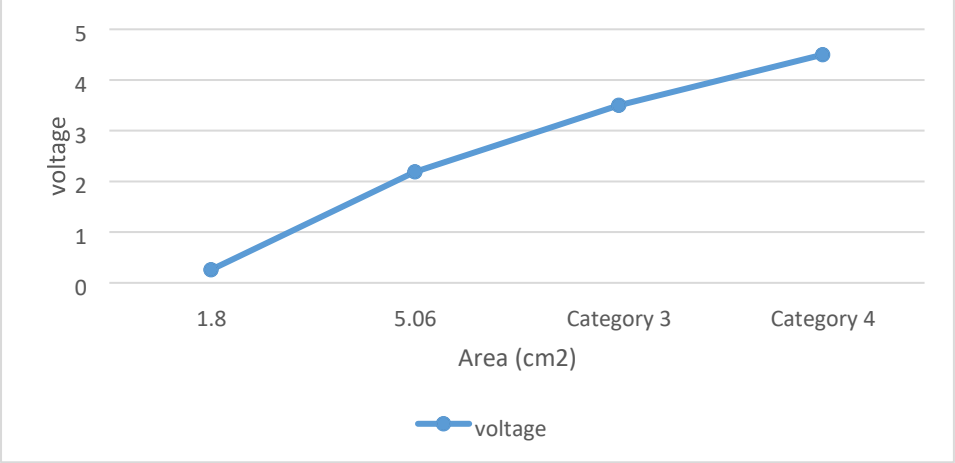

Fig 4. Comparison of voltage generated by graphite electrodes of different dimensions

The experimental findings that voltage generation is directly proportional to surface area has also been reported previously. Three mediators (methyl red, neutral red and methylene blue) were used in double chamber MFC in this experiment. Methyl red showe best results increasing the voltage from 34mV to 74mV(3 times more than the initial). Result by methylene blue was also satisfactory and the final voltage (after adding methylene blue) was approximately two times of the initial.

Results by ethyl red were disappointing and despite of increase in voltage, tremendous decrease was shown(Fig below).

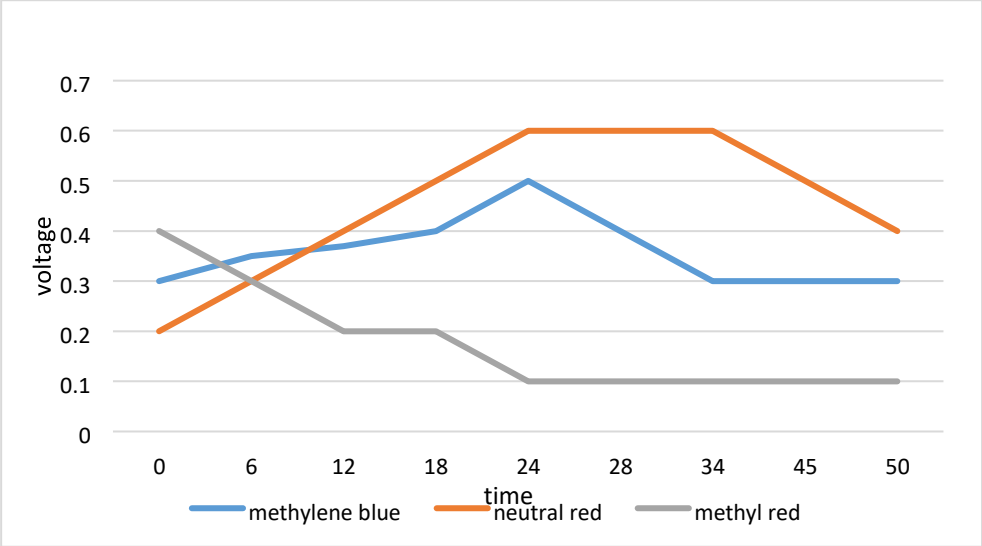

Fig 5. Effect of different mediators on voltage generation

4.5. IDENTIFICATION RESULTS

4.5.1. GRAM STAINING RESULTS

After subculturing, seven different colonies were obtained. For their identification gram staining was done and the seven isolates that are 1,2,3,4,5,6 & 7found following results and shapes.

Table 3

Plates no	Gram staining	Shape	Catalase+$_{ve}$	Spore forming	Oxidase test	TSI agar test
1	+ve	Rod (filamentous)	+ve	Greenish spores (reddish pink rods)	-ve	Red slant +red butt
2	+ve	Coccobacilli (non-filamentous)	-ve	Greenish spores (reddish pink rod)	-ve	Yellow slant +red butt
3	-ve	Coccobacilli (non-filamentous)	+ve	Greenish spores	-ve	Yellow slant +red butt
4	+ve	Coccobacilli- rods	-ve	Greenish spores	-ve	Yellow slant +red butt
5	+ve	Rod (filamentous)	+ve	Greenish spores	-ve	Yellow slant +red butt
6	-ve	Coccobacilli	-ve	Greenish spores	-ve	Yellow slant +yellow butt
7	+ve	Rods	+ve	Greenish spores (reddish pink rods)	-ve	Yellow slant +yellow butt

V. DISCUSSION

In this study, an increase in voltage from 31 mV to 169 mV was observed when the sludge conc. was increased from 500mg/l (in case of batch mode) to 1000mg/l (in case of fed batch).

Similar observations have been reported by researchers while using sludges of different conc.

i.e. 1g/l, 2g/l, 3g/l, 4g/l and 5g/l etc. to find out there effect on the voltage generated by microbial fuel cells [30]. So, sludge conc. is also an important factor in amplification of voltage generated by MFC and working of MFC can be improved by considering this.

To determine the effect of PEM surface area on power output salt bridges with different sizes such as 3.5 and 6.5 were used. Similar observations have been reported by researchers used different the sizes of the anode and cathode were varied in two-chambered MFCs having PEMs with three different surface areas (APEM = 3.5, 6.2, or 30.6 cm2). This showed an increase in voltage generation due to more transference of H ions.

To find out the effect of surface area of electrode on voltage, electrodes of different dimensions were used. An increase in voltage occurred when the surface area was increased. The voltage obtained for carbon electrode (5.06 cm2) was 6 times more than that obtained by using carbon electrodes (1.8cm). The experimental findings that voltage generation is directly proportional to surface area of cathode has also been reported previously e.g., the voltage obtained by using ferricyanide cathode and Pt coated carbon cathode (22.5 cm2) was reduced from 200-300mV when the surface area was reduced from 22.5 cm2 to 5.8 cm2. [31]

The effects of different mediators in different concentrations on the current generation in the cell was also observed by different researchers. In a study three different types of mediators i.e. methyl red, 2-hydroxy-1, 4-naphthoquinone and methylene blue in different concentrations (50, 100, 200, 300 and 400 µM) were used and it was found that different mediators are effective in different concentrations. [16] In our study, best results were shown by methylene blue. The reason of methyl red poor performance is that as mediators are specie specific so may be neutral red was not effective for the strains present in our inoculums.

Some scientists focused on building low cost MFC's, they used different construction methods and built different designs of MFC's. Cheap and simple materials were used in designing the MFC as compared to the previously designed MFC's. They used different methods for reducing resistance and power loss, building single chamber MFC and making economical membranes. They designed a simple and economical single chamber MFC and were able to generate the voltage of about 0.59 V[32]. In our study, MFC's were built up using cheap and easily available materials. Double chambered MFC's were constructed and a current maximum of 169mV was measured.

VI. CONCLUSIONS

1. A microbial fuel cell is a device that utilizes the power of microorganisms to convert chemical energy into electrical energy.
2. Stacked cell was more efficient one because large amount of voltage was generated by the stacked cell microbial fuel cell followed by double chambered and then single chambered MFC's.
3. Mediators are specie specific and only help in amplifying voltage of specific species; it was found that one type of mediator is not effective for all types of microbes.
4. Different substrates can be used as feed for microbes; it was observed that mostly organic matters such as peptone, yeast extract, glucose, etc. help in increasing the voltage if they are used as a substrate in MFC.
5. When the substrate was added in increments, an increase in voltage produced by the cell was observed, and also the life of the cell increased as compared to those in which the substrate was added as a whole in the cell.
6. Different metals can be used as electrodes such as carbon, copper, platinum, aluminum, etc.; it was concluded that when the electrodes having large surface area were used in building a cell, the voltage generation in cell increased i.e. more was the surface area of electrode, more will be the current generated by cell.
7. Different parameters were studied to maintain and amplify the current generated by the cell, it was also observed that by increasing the slurry concentration in the cell, the current production by cell can also be enhanced.

VII. RECOMMENDATIONS

Following recommendations should be suggested:

1. The anaerobic compartment should be properly sealed to avoid the entrance of air in the chamber.
2. Electrodes having large surface area should be used in making a cell.

3. The site rich with facultative anaerobe should be selected for sampling.

4. The substrate should be added in increments into the anaerobic compartment.

5. Minute amounts of mediators should be used.

6. Wires having minimum resistance and high conductance should be used.

FUTURE PROSPECTS

1. To design the chambers for anaerobic compartment that could properly provide the anaerobic conditions to the microbes within the cell.
2. To build up the techniques that utilized cheap and easily available materials and generate huge amount of current for us.
3. To design an MFC that is one level higher than the present one (by changing MFC design, by using new strains, by changing concentration of substrate, by reducing power dissipations and by improving the strains)

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