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CONSTRUCTION AND OPTIMIZATION OF MICROBIAL FUEL CELL

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ABSTRACT

The effect of several parameters that affect the activity of a microbial fuel cell (MFC) was studied in this work. The bacterial cells, act as the biocatalyst and work as an electron mediator and known as mediated electron transfer (MET) microbial fuel cell. The influence of the bacterial concentration, the effective electrode, the volume of the cell was analyzed. Our results indicate a proportional energy production to the bacterial concentration present in the anodic chamber. A highest power density of 0.789 m W cm^2 was obtained with a MFC that consist of electrodes of an effective area 40cm^2 with a 2.1 X 108 CFU / ml of bacterial concentration in the anodic chamber. Standardized power showed that the increasing area of electrode does not result in a linear increment of the output power and the increase in volume of the cell negatively affected the power produced by our optimized cells.

Keywords: Energy, Electrode, Microbial Fuel Cell, Anode, Cathode, Optimized Cells.

INTRODUCTION

Bioelectricity is described as the electrical potentials and currents occurring within or produced by living organisms. It results from the conversion of chemical energy into electrical energy. The difference between the bioelectric currents in living organisms and the electric current that is used to produce light, heat or power is that bioelectrical current is the flow of ions(atoms or molecules carrying an electrical charge), while standard electricity is the movement of electrons **[1].**

Bioelectricity(biomass derived electricity) come to exists to meet the rising, domestic demand for energy. Energy saving programs is being implemented as the global demand for energy is rapidly increasing day by day. Biomass is recognized as a 'green' source of energy and the waste streams are regarded as the most optimum substrates for bio energy production **[2]**. Microbial Fuel Cells (MFCs) are a type of biofuel cell which has recently attracted considerable interest. More than 70 years after William Grove in 1839 built the first Microbial Fuel Cell **[3].**

Microbial fuel cell (MFC) is a promising biotechnology capable of converting organic substrates in wastewaters (e.g. domestic wastewater, swine wastewater, leachate, and urine) to electricity. The electro-genic microorganisms colonized on the anode surface degrade organic substrates and generate electrons, which then transfer to the cathode through external circuit and complete reduction reactions **[4]**. The transfer of electrons obtained from an electron donor to the anode electrode is occurred either through direct contact, nanowires, or mobile electron shuttles in the anode compartment. During electron production protons are also produced in excess. The protons migrate through the cation exchange membrane into the cathode chamber **[5].**

Microbial Fuel Cell(MFC) is a device that converts chemical energy into electrical energy released from the reaction by using microorganisms. MFC holds a key in green technology for the production of bioenergy simultaneously treating wastes **[6]**.

MFC are the biochemical system in which electricity is generated via oxidation of biodegradable organic matter in the presence of active biocatalyst. The active biocatalyst or enzyme can generate electrons and protons from organic substrate **[7]**.

The generated electrons are transferred by anode to cathode through an external circuit. In the cathode compartment protons have to diffuse and react with oxygen to liberate water molecules [**8,9**].

The MFCs have two portions in their design *i.e.* theanode portion, where the microbial action produce electronsand protons, and the cathode portion where the electronsand protons from the anode produced by microorganism are transferred and form water by a chemical reaction with oxygen which act as electron acceptor **[10]**. The microorganisms that act as biocatalysts oxidize organic and in organic substrate to carbon dioxide and generate electrons at the anode. It requires transferring these electrons from inside the cells to the anode(surface) in anoxic conditions to produce electric current **[11]**.

In MFC, electron transfer chiefly occurs in two directions: at the anode, from microorganisms to electrode, and at the cathode, from electrode to microorganisms when bio-cathodes are used to catalyze oxygen reduction **[12,13]**.

MATERIALS AND METHODOLOGY

Samples collection:

The sewage sample was collected for the isolation of potential bacterial **[14]**.

Isolation of bacteria from samples:

The sewage sample were serially diluted in NaCl solutions and then diluted samples were spread on sterilized nutrient agar plates and then pure culture plates were prepared by selecting the cultures on the basis of different morphological parameters **[15]**.

Strain identification of isolates:

Screening of desired culture was carried out by observing the zone of hydrolysis after performing the congo red test on cultures streaked minimal agar media with CMC plates **[16]**.

Strain identification of isolates:

Various biochemical tests such as grams staining, endospores staining, glucose fermentation test, mannitol test etc. as were performed and strains were identified by using Bergy's manual **[17]**.

Construction and optimization of microbial fuel cell(MFC):

H-shaped double chambered microbial fuel cell was prepared. Each chamber was made up of autoclave plastic material. The anodic and cathodic chamber was connected by using an agarose salt bridge. Comparative estimation of different electrodes like copper, aluminum, carbon was performed **[18,19,20]**.

Operation of MFC:

Sterilization of MFC assembly was done. Anodic chamber was filled with substrate and cathodic chamber was open air cathode chamber containing phosphate buffer. Optimum conditions- temperature and pH was maintained.

Designing of MFC:

Compartments:-MFC was constructed with 2 compartments one is Anodic Compartment and other is Cathodic Compartment. The anodic chamber consist of the microbes with selected media and substrate where the microbial action produce electrons and protons. In the cathodic chamber electrolyte was added.

Proton Exchange Membrane:-Both the compartments are separated by Proton Exchange Membrane(PEM) which was prepared by using an agarose salt bridge.Through the PEM the protons transferred from the anodic chamber to the cathodic chamber.

Electrodes:- MFC Anode:- In the anodic chamber one electrode was used as a anode. The material used as the anode was Copper.
MFC Cathode:- One cathode was used in the cathodic chamber. The cathodic material used was Copper.

Electrolytes:-Different types electrolytes was used in the preparation of the MFC. Electrolytes were added to the cathodic chamber. The electrolytes that were used are listed below:- Sodium Chloride(NaCl)**,** Potassium Chloride(KCl)**,** Copper Sulphate($CuSO_4$)**,** Potassium Nitrate(KNO_3)**,** Sodium Acetate(CH_3COONa)

Substrates:-The source of substrate used in the MFC matters in the electricity generation.Substrates were added to the anodic chamber. The waste products were taken as the substrates.

RESULTS

Collection of sample:

1ml of waste water sample was collected in micro centrifuge tubes from a nearby drain

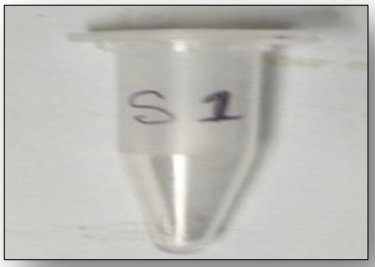

Figure 1: Collected sample

Bacterial isolation by serial dilution:

Total 15 bacterial cultures were isolated from all three drainage collected samples using serial dilution and spread plate method. These cultures were selected based on their different morphological characteristics. Five cultures were obtained from each sample 1, 2 & 3, and four cultures from sample 4. These cultures are named as C1, C2….. C15.

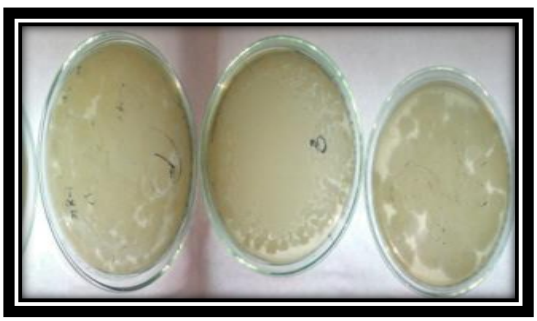

Figure 2: Bacterial cultures on agar plates after the serial dilution and spreading.

Bacterial purification:

Bacterial purification was done using the streak plate method by streaking the selected cultures in Petri plates.

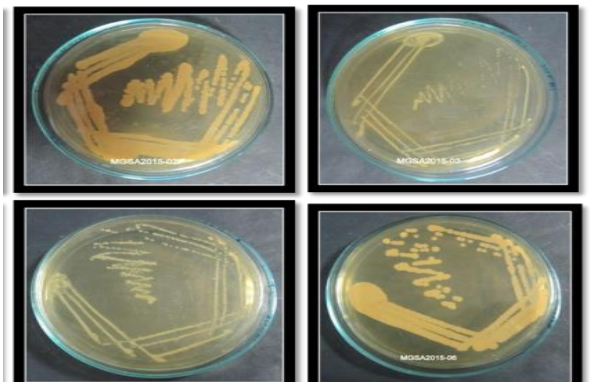

Figure 3: Few pure bacterial culture in agar plates after streaking.

Colony morphology

The cultures obtained from different samples were differentiated based on their morphology. The morphology of all the selected colonies is given in the table below.

Table 1: The morphology of all the selected colonies.

Culture name	Shape	Margin	Elevation	Pigmentation	Surface	Texture	Opacity
C1	Circular	Lobate	Flat	Off white	Smooth	Soft	Opaque
C2	Irregular	Lobate	Raised	Off white	Rough	Hard	Opaque
C3	Circular	Curled	Raised	Off white	Rough	Soft	Opaque
C4	Circular	Lobate	Flat	Yellowish	Convex	Soft	Opaque
C5	Circular	Curled	Raised	White	Smooth	Gummy	Opaque
C6	Irregular	Entire	Convex	Off-white	Convex	Hard	Opaque
C7	Circular	Lobate	Flat	Off white	Convex	Hard	Opaque
C8	Irregular	Lobate	Raised	Off white	Smooth	Soft	Opaque
C9	Circular	Curled	Raised	Off white	Smooth	Soft	Opaque
C10	Circular	Lobate	Flat	Yellowish	Rough	Hard	Opaque
C11	Filamentous	Curled	Convex	Off-white	Rough	Soft	Opaque
C12	Punctiform	Lobate	Pulmonate	Green	Smooth	Soft	Opaque
C13	Circular	Discrete	Flat	White	Rough	Hard	Opaque
C14	Irregular	Entire	Flat	Off white	Rough	Soft	Opaque
C15	Circular	Lobate	Convex	Yellowish	Convex	Soft	Opaque

Table 2: Showing results of Gram's Staining, Endospores staining.

Culture	Gram's staining	Shape	Endospores staining
C1	Gram Negative	Rod	Negative
C2	Gram Positive	Rod	Negative
C3	**Gram Positive**	**Rod**	**Positive**
C4	Gram Positive	Coccus	Negative
C5	Gram Negative	Rod	Negative
C6	Gram Negative	Rod	Negative
C7	Gram Negative	Coccus	Negative
C8	Gram Negative	Rod	Negative
C9	Gram Positive	Rod	Positive
C10	Gram Positive	Rod	Positive
C11	Gram Positive	Coccus	Negative
C12	Gram Negative	Rod	Negative
C13	Gram Negative	Rod	Negative
C14	Gram Negative	Rod	Negative
C15	Gram Positive	Rod	Positive

Table 3: Showing results of Catalase test, Mannitol Test, Starch hydrolysis test.

Culture	Mannitol test	Catalase test	Starch hydrolysis test
C1	Negative	Negative	Negative
C2	Positive	Positive	Positive
C3	**Positive**	**Positive**	**Positive**
C4	Positive	Negative	Negative
C5	Positive	Negative	Negative
C6	Negative	Negative	Positive
C7	Positive	Positive	Negative
C8	Positive	Positive	Negative
C9	Negative	Negative	Negative
C10	Positive	Negative	Negative
C11	Positive	Positive	Negative
C12	Positive	Negative	Negative
C13	Positive	Positive	Negative
C14	Positive	Negative	Negative
C15	Positive	Negative	Negative

Table 4: Showing results of Methyl red, Voges Prausker, Glucose Fermentation test, Citrate test.

Culture	MR Test	VP test	GF test	Citrate test
C1	Negative	Negative	Negative	Negative
C2	Positive	Negative	Positive	Negative
C3	**Positive**	**Negative**	**Positive**	**Negative**
C4	Positive	Negative	Negative	Negative
C5	Negative	Negative	Negative	Negative

Culture	MR Test	VP test	GF test	Citrate test
C6	Negative	Negative	Negative	Negative
C7	Negative	Negative	Negative	Negative
C8	Negative	Negative	Negative	Negative
C9	Positive	Negative	Positive	Positive
C10	Positive	Positive	Positive	Positive
C11	Positive	Positive	Negative	Positive
C12	Negative	Negative	Negative	Negative
C13	Negative	Positive	Negative	Negative
C14	Negative	Negative	Negative	Negative
C15	Positive	Negative	Positive	Positive

Table 5: Biochemical properties of culture C3

S no.	Tests	Results
1	Gram's staining	**Positive**
2	Shape	Rod, *Bacillus*
3	Catalase test	Negative
4	Spore forming test	Positive
5	Methyl red test	Positive
6	Voges Prauskeur test	Negative
7	Citrate test	Negative
8	Starch hydrolysis test	Positive
9	Glucose fermentation test	Positive

Preparation and optimization of microbial fuel cell (MFC)

The Dual Chambered Microbial Fuel Cell(MFC) was prepared as shown below. Different types of electrolytes and salt bridges were used which were showed in the table below.

Figure 4: Dual Chambered MFC with Voltmeter

MFC with different types of electrolytes and salt bridges or protein exchange membrane (PEM) are shown below in the table:-

Table 6:- Showing the generated power by using different Electrolytes and salt bridges.

ELECTROLYTE	PEM	CURRENT	VOLTAGE	POWER
NaCl	NaCl + Agar	**070**	**006**	**0.42mW**
KCl	NaCl + Agar	**020**	**16**	**0.32mW**
$CuSO_4$	NaCl+ Agar	**254**	**219**	**55mW**
KNO_3	NaCl + Agar	**12**	**166**	**1.9mW**
CH_3CooNa	NaCl + Agar	**181**	**45**	**8mW**
NaCl	KCl + Agar	**004**	**15**	**00.6mW**
KCl	KCl + Agar	**007**	**016**	**0.012mW**
$CuSO_4$	KCl + Agar	**143**	**175**	**25mW**
KNO_3	KCl + Agar	**047**	**142**	**6mW**
CH_3CooNa	KCl + Agar	**009**	**030**	**0.027mW**
NaCl	$MgSO_4$+Agar	**15**	**23**	**0.34mW**
KCl	$MgSO_4$+Agar	**090**	**180**	**16mW**
$CuSO_4$	$MgSO_4$+Agar	**95**	**202**	**19mW**
KNO_3	$MgSO_4$+Agar	**40**	**145**	**5.8mW**
CH_3CooNa	$MgSO_4$+Agar	**90**	**133**	**11mW**
NaCl	KNO_3 + Agar	**070**	**110**	**7mW**
KCl	KNO_3 + Agar	**045**	**33**	**1.4mW**
$CuSO_4$	KNO_3 + Agar	**112**	**168**	**18mW**
KNO_3	KNO_3 + Agar	**92**	**136**	**12mW**
CH_3CooNa	KNO_3 + Agar	**51**	**194**	**9.8mW**

Table7:- Showing different substrates with the power generated

SUBSTRATE	ELECTROLYTE	CURRENT	VOLTAGE	POWER
WoodPulp Waste	2.5% $CuSO_4$	**181**	**221**	**40mW**
HuskWaste	2.5% $CuSO_4$	**157**	**067**	**10mW**
Wastewater	2.5% $CuSO_4$	**121**	**033**	**3.9mW**
Vegetable Waste	2.5% $CuSO_4$	**110**	**058**	**6.3mW**
Municipal Waste	2.5% $CuSO_4$	**119**	**069**	**8.2mW**

The highest power generated in the lab using the dual chambered MFC was 55mV with a current of 254mA and a voltage of 219mV. The electrolyte used was Copper Sulfate($CuSO_4$) and the salt bridge or Protein Exchange Membrane used was a agarose salt bridge using Sodium Chloride(NaCl) and Agar Agar.

DISCUSSION

In this study at first the bacteria was isolated by serial dilution from the waste water sample further purification was done. The purified cultures were then examined for biochemical tests for the optimization of the bacteria in which out of 9 cultures, culture 5,6 & 9 gives Gram negative results, Culture 3,5 & 7 gives Endospore positive results, Culture 2,5,8 & 9 gives Catalase positive results and Culture 2,3 & 8 gives Mannitol Test positive. Amylase, Cellulase and Urease showed negative for all the cultures and Carbohydrate test positive for all the cultures.

After bacterialoptimizationmodified media was optimized in which 1.5% Dextrose was selected as carbon source, 1% Yeast was selected as the nitrogen source, Magnesium sulphate($MgSO_4$) was selected as the metal ion with apH of 7.1.

Dual Chambered Microbial Fuel Cell(MFC) was prepared by using twochambers(Anodic &Cathodic) which was connected by a proton exchange membrane(PEM). In the anodic chamber optimized modified media was used and in the cathodic chamber electrolyte was used. The optimization of the MFC was done by using different electrolytes and Proton exchange membrane with different salt concentration.

The power produced on the MFC was measured by using a Voltmeter. The maximum amount of power generated was 55mV in which a current of 254mA and a voltage of 219mV was observed in the voltmeter.

CONCLUSION

The MFC configuration proposed in this study was able to produce electricity from waste water, household waste, vegetable waste, husk waste & wood pulp waste used as a substrate. Bioelectricity was successfully generated in a dual chambered MFC.

Highest power achieved was 55mV using mix bacterial cultures and electrolyte used was Copper sulphate with a proton exchange membrane of Sodium Chloride and Agar-agar.

MFCs are considered as a Next-generation Energy Source. The main areas of application for MFCs have been waste treatment and electricity generation. Researchers have attempted to use microbes from the genera *Geobacter, Saccharomyces, Desulfurmonas,* and *Escherichia* for power generation.

Cambrian Innovation is working with the US army to test an MFC that could turn 2,250 liters of sewage into clean water and generate enough electricity to power itself.

MFCs are suitable for powering electrochemical sensors and small telemetry systems to transmit signals to remote receivers. Microbes have been used as biological oxygen demand sensors. However, MFC's have been observed to have better operational sustainability and reproducibility, with an operational lifetime of almost five years.

MFC development is still in its nascent stage, and the power density that current systems achieve needs further improvement. Researchers are still fine-tuning the process' efficiencies, especially in areas that involve "scaling up" power generation through higher volumes of substrates. MFC's application in wastewater treatment also depends on significant variables such as the concentration and biodegradability of organic matter, wastewater temperature, and the presence of toxic chemicals.

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