Production and Enhancement of Bioelectricity from Wastes using Microbial Fuel Cell

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Abstract— Electricity produced by microorganism is called bioelectricity. Microorganisms use different components of waste and convert chemical energy into electric energy. In this present study we have worked on production of bioelectricity by using different microbes.

Keywords: Bioelectricity, Chemical Energy, Microorganisms, Electrical Energy, Anode, Cathode, Wood Pulp

I. INTRODUCTION

Bioelectricity is the electricity which is produced with the help of organisms and the wastes. It is defined as the electrical potentials and currents arising within or produced by living organisms. It results from the transformation of chemical energy into electrical energy. The basic difference between the bioelectric currents in living organisms and the electric current is,it's used to produce light, heat or power is that bioelectrical current is the flow of ions(atoms or molecules carrying an electrical charge), while average electricity is the movement of electrons [1].

Bioelectricity (biomass derived electricity) come to occurs to meet the rising, domestic demand for energy. Energy saving programs is being executed as the global demand for energy is rapidly increasing day by day. Biomass is known as a 'green' source of energy and the waste streams are considered as the most optimum substrates for bio energy production. Microbial Fuel Cells (MFCs) are a category of biofuel cell which has recently attracted considerable interest. More than 70 years after William Grove in 1839 built the first Microbial Fuel Cell[1].

Microbial fuel cell (MFC) is a favorable biotechnology, which is 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 reduce organic substrates and generate electrons, which is then transfer to the cathode through external circuit and complete reduction reactions occurs. The transmission 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 section.

During the electron production, protons are also produced in additional. The protons migrate through the proton exchange membrane (PEM) into the cathode chamber[2].

MFC's are the captivating biological fuel cells that typically contain two compartments, i.e; the anode and the cathode and use biological catalyst (mostly bacteria) to produce electric energy from organic matter present naturally in the environment or in wastes[4].The MFCs have two portions in their design i.e. theanode portion, where the microbial action create electrons and protons, and the cathode portion where the electrons and protons from the anode produced by microorganism are transferred and form water by a chemical reaction with oxygen which act as electron acceptor. The microorganisms that act as biocatalystsoxidize organic and inorganic substrate to carbon dioxide and generate electrons at the anode. It requires shifting these electrons from inside the cells to the a node(surface) in anoxic conditions to produce electric current.

In MFC, electron transfer mostly 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.

Electrons created from the bacteria may transfer from bacterial cell to electrode with the help of mediators like thionine, methyl viologen, and methylene blue. It could also be transferred without the using mediators, which makes them mediator-less MFCs, by consuming the electrochemically active bacteria (EAB).EAB has the potential of transferring the electrons to the electrode via cell wall by making biofilm over electrodes. Different kinds of substrates have been used in MFC's for the production of bioelectricity which includes food, agriculture and domestic waste water and many others[5]

Microbial Fuel Cell can be constructed by combining two vertical compartments which act as compartments on either side by a Proton Exchange Membrane(PEM). Two electrodes (anode & cathode) are placed inside the respective cathodic and anodic chamber. Further the electrodes are connected to a multimeter for checking the potential voltage as well current.

II. MATERIALS AND METHODS

A. Sample Collection

Wastewater sample was collected in micro-centrifuge tubes from a nearby drainage of MRD LifeSciences, Vibuti Khand, Gomti Nagar, Lucknow.

B. Bacterial Isolation

Bacteria were isolated from waste water sample by serial dilution. 91 ml normal saline (0.85%) was prepared and it was transferred 10 ml in first test tube and 9 ml in remaining 10^{-1} 10^{-2}…..10^{-10}marked test tubes. It was sterilized and after cooling 1 ml sample was added to first test tube, then serially diluted by taking 1 ml sample from the same test tube up-to 10^{-10} marked test tube.

50µl sample was spread on nutrient agar plates from 10^{-8} 10^{-9}& 10^{-10} marked test tubes.

C. Colony Morphology Study

In order to distinguish between large numbers of different colonies grown on the Petri plates, colony morphology study has been taken out. It helps in identification of organism and gives important information regarding microbe.

Parameters for classification:

1) Colony Shape:

Colony may vary in their shape. Colonies can be circular, spindle, rhizoidal, filamentous, punctiform and irregular.

2) Colony Margin:
They can be entire, discrete, curled and lobate.
3) Colony Elevation:
They can be flat, raised, convex, pulvonate and urbanite.
4) Colony Texture:
It may be soft, hard or gummy.
5) Colony Surface:
It can be either smooth or rough.
6) Opacity:
Colonies can be opaque or transparent.
7) Colony Pigmentation:
Colonies can be yellow, cream, off-white and white.

D. Culture Purification

After spreading mix culture were obtained then it was converted in pure culture by streak plate method by using nutrient agar media.

E. Identification of Culture by Biochemical Test

Bacteria were identified by different biochemical test. For this biochemical test one day old culture was taken

F. Media Selection and Optimization

Media optimization- minimal media was optimized

S no.	Factors	Modified media	Standard media
1.	Minimal Salt Media	PM1	KH_2PO_4- 3g/l
			Na_2HPO_4 - 6g/l
			NaCl -5g/l
			NH_4Cl - 2g/l
			$MgSO_4$ - 0.2g/l
			Dextrose - 8g/l
2.	Nitrogen source		
	Peptone	MM1	5g/l
	Tryptone	MM2	5g/l
	Yeast extract	MM3	5g/l
3.	Carbon source		
	Lactose	MM4	10g/l
	Fructose	MM5	10g/l
	Maltose	MM6	10g/l
	Sucrose	MM7	10g/l
	Dextrose	MM8	10g/l
4.	Metalions		
	$MgSO_4$	MM9	0.2 g/l
	$ZnSO_4$	MM10	0.2 g/l
	MgS04	MM11	0.2g/l
	$CuSO_4$	MM14	0.2 g/l
	$CaCO_3$	MM15	0.2 g/l
6	Different pH on the growth of the bacteria		
	pH	MM27	3
	pH	MM28	5
	pH	MM29	7
	pH	MM30	9

G. Preparation and optimization of Microbial Fuel Cell(MFC)

1) Construction of 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 agar salt bridge.Comparative estimation of different electrodes like copper, aluminum, carbon was performed.
2) 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.
3) Designing of MFC:
a) Components of MFC:
1) 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.
2) Proton Exchange Membrane:
Both the compartments are separated by Proton Exchange Membrane (PEM) which was prepared by using an agar salt bridge. Through the PEM the protons transferred from the anodic chamber to the cathodic chamber. The percentage constituents of PEM were:

S no.	Components	Concentrations
1.	Agar-agar	10%
2.	Salts	1%

Table 1: Components used in the preparation of PEM / Salt Bridge.

3) Electrodes:
1) MFC Anode:- In the anodic chamber one electrode was used as a anode. The material used as the anode was Copper.
2) MFC Cathode:- One cathode was used in the cathodic chamber. The cathodic material used was Copper.
4) Electrolytes**:**
Different type'selectrolytes were used in the preparation of the MFC. Electrolytes were added to the cathodic chamber. The electrolytes that were used are listed below:-

S no.	Electrolytes
1	Sodium chloride(NaCl)
2	Potassium chloride(KCl)
3	Copper sulphate($CuSO_4$)
4	Potassium nitrate(KNO_3)
5	Sodium acetate(CH_3COONa)

Table 2: Different electrolytes which was used in MFC

5) Substrates**:**
The source of substrate used in the MFC matters in the electricity generation. Substrates were added to the anodic chamber. The substrates that were used are as follows:-

S no.	Substrate
1	Municipal Wastewater
2	Household Vegetable Waste
3	Municipal Waste
4	Corn Husk Waste
5	Wood Pulp

Table 3: Different substrates which was used in MFC

III. RESULTS

A. Collection of Sample

1ml of waste water sample was collected in micro centrifuge tubes from a nearby drain in Vibhuti Khand, Lucknow.

Fig. 1: Water sample in the micro-centrifuge tube

B. Bacterial Isolation from Serial Dilution Method

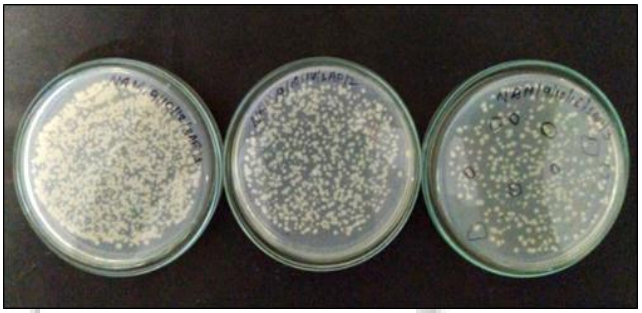


Fig. 2: Bacterial colonies in nutrient agar plates

C. Morphological Characteristics of the Potent Bacterial Isolates

Bacterial Isolates	Shape	Margin	Pigmentation	Elevation	Surface	Texture	Opacity
PSSA01	Circular	Lobate	Non-Pigmented	Flat	Smooth	Soft	Opaque
PSSA02	Irregular	Entire	Non-Pigmented	Flat	Smooth	Soft	Opaque
PSSA03	Filamentous	Entire	Non Pigmented	Flat	Smooth	Soft	Translucent
PSSA04	Spindle	Discrete	Non Pigmented	Raised	Rough	Hard	Translucent
PSSA05	Circular	Lobate	Yellow	Umbonate	Rough	Hard	Opaque
PSSA06	Circular	Entire	Non Pigmented	Convex	Smooth	Hard	Opaque
PSSA07	Irregular	Curled	Orange	Flat	Rough	Hard	Opaque
PSSA08	Filamentous	Discrete	Non pigmented	Umbonate	Smooth	Gummy	Translucent
PSSA09	Rhizodes	Lobate	Yellow	Flat	Rough	Hard	Opaque

Table 4: Morphological classifications of the selected cultures.

D. Purification of bacteria from mixed culture plates

Purification of bacteria was done by streaking the selected culture in the petri plate by using streak plate method.

Fig. 3: Bacterial colonies in nutrient agar plates

E. Identification of culture by biochemical test

1) Gram Staining

CULTURES	TYPE	SHAPE
Culture 1	Gram Positive	Coccus
Culture 2	Gram Positive	Rod
Culture 3	Gram Positive	Rod
Culture 4	Gram Positive	Rod
Culture 5	Gram Negative	Rod
Culture 6	Gram Negative	Rod
Culture 7	Gram Positive	Rod
Culture 8	Gram Positive	Rod
Culture 9	Gram Negative	Rod

Table 5: Gram's staining of the selected cultures

2) Endospore Staining:

CULTURES	ENDOSPORE
Culture 1	Negative
Culture 2	Negative
Culture 3	Positive
Culture 4	Negative
Culture 5	Positive
Culture 6	Negative
Culture 7	Positive
Culture 8	Negative
Culture 9	Negative

Table 6: Endospore's staining of the selected cultures

3) Catalase Test:

CULTURES	CATALASE
Culture 1	Negative
Culture 2	Positive
Culture 3	Negative
Culture 4	Negative
Culture 5	Positive
Culture 6	Negative
Culture 7	Negative
Culture 8	Positive
Culture 9	Positive

Table 7: Catalase test of the selected cultures

4) Mannitol Fermentation Test

CULTURES	MANNITOL
Culture 1	Positive
Culture 2	Negative
Culture 3	Negative
Culture 4	Positive
Culture 5	Positive
Culture 6	Positive

Culture 7	Positive
Culture 8	Negative
Culture 9	Positive

Table 8: Mannitol fermentation test of the selected cultures

5) Media selection and optimization

For media optimization minimal media was selected.

Media	OD 620 nm			
	24 hours	48 hours	72 hours	96 hours
PM 1	0.65	0.90	0.69	0.67

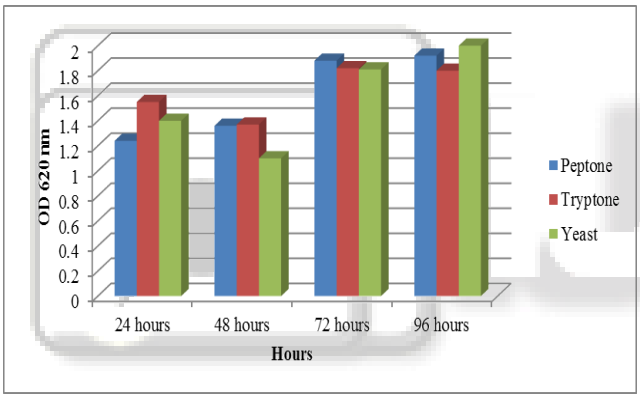
Table 9: Minimal Media of the selected cultures

a) Effect of different nitrogen sources

Four different types of nitrogen sources were selected for the optimum growth of the bacteria. Their optical density values are taken for 4 consecutive days and the values were observed. The results for the best nitrogen sources were showed with the help of a bar diagram.

Nitrogen Source	OD 620 nm			
	24 hours	48 hours	72 hours	96 hours
1. Peptone	1.24	1.36	1.88	1.92
2. Tryptone	1.55	1.37	1.82	1.80
3. Yeast	1.40	1.10	1.81	2.00

Table 10: effects of nitrogen sources is done on minimal Media.



Graph 1: Bar diagram showing values of different nitrogen sources.

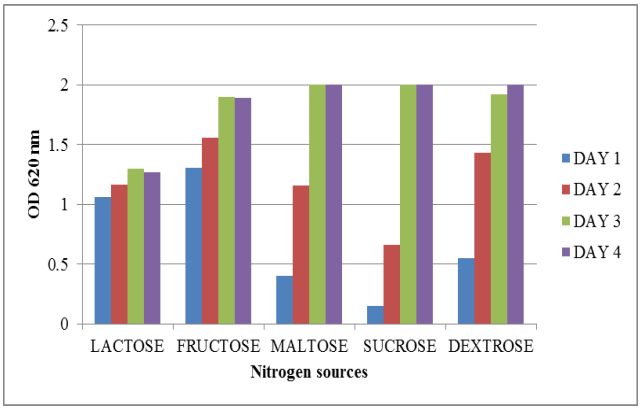
From the above bar diagram we can conclude that Yeast was the nitrogen source out of the 4 which increased gradually day by day. So we selected Yeast as our potent nitrogen source.

b) Effect of different carbon sources

For the optimum growth of the bacteria 5 different types of carbon sources was selected and their optical density values were observed for 4 consecutive days. The optical density values were putted in a bar diagram as shown below:-

Carbon Source	OD 620 nm			
	24 hours	48 hours	72 hours	96 hours
1. Lactose	1.06	1.17	1.20	1.27
2. Fructose	1.31	1.56	1.90	1.92
3. Maltose	0.40	1.16	2.00	2.00
4. Sucrose	0.15	0.66	2.00	2.00
5. Dextrose	0.55	1.43	1.85	2.00

Table 11: effects of carbon sources is done on minimal Media.



Graph 2: Bar diagram shows values of different carbon sources.

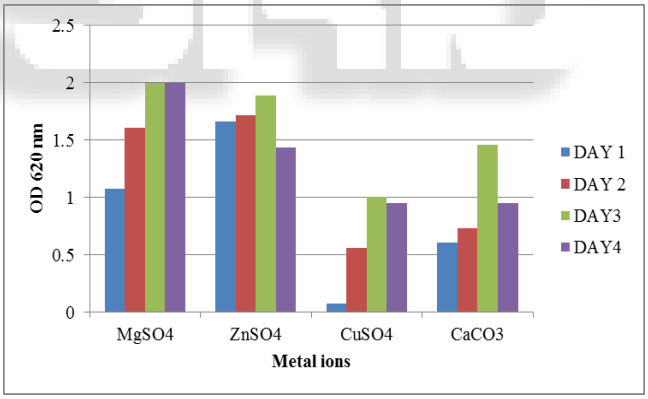
From the above bar diagram we concluded that dextrose gradually increased day by day as compared to the other carbon sources. So Dextrose was selected as the potent carbon source.

c) Effect of different metal ions

Four different types of metal ions are selected for the optimum bacterial growth. The optical density values were taken for 4 consecutive days for each of the metal ions. The result was showed with the help of a bar diagram.

Metal Ions	OD 620 nm			
	24 hours	48 hours	72 hours	96 hours
1. MgSO4	1.08	1.61	2.00	2.00
2. ZnSO4	1.66	1.72	1.89	1.42
3. CuSO4	0.08	0.53	1.00	0.93
4. CaCO3	0.61	0.73	1.46	0.92

Table 12: effects of metal ions is done on minimal Media.



Graph 3: Bar diagram showing values for Metal Ions

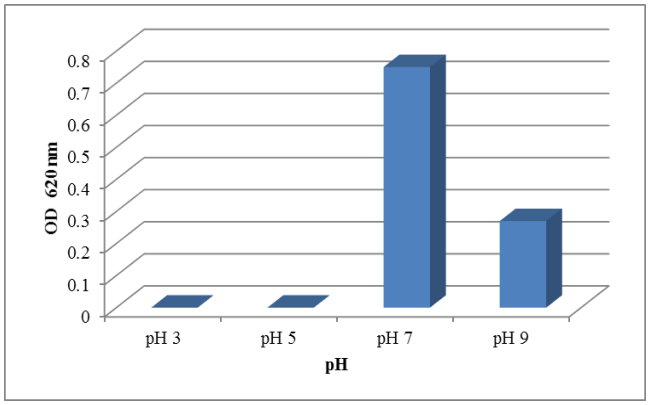
From the above bar diagram we conclude that $MgSO_4$ increased day by day as compared to other metal ions. So we selected $MgSO_4$ as our potent Metal Ion.

d) Effect of different pH on the growth of the bacteria

To know the effect of different pH on the growth of the bacteria, the cultures are inoculated on selected modified media which was prepared under 4 different pH. The result was observed by taking optical densities as shown below.

S. No	PH value	OD 620 nm
1.	3	0.00
2.	5	0.00
3.	7	0.75
4.	9	0.27

Table 13: effects of pH is done on minimal Media.



Graph 4: Bar diagram showing values for pH

e) Effect of different temperature on bacterial growth

The effect of different temperature on bacterial growth of the bacteria was done by incubating the cultures at 4 different temperatures(4ºC, RT,37ºC & 50ºC). The results are shown below-

Fig. 3: Petriplates showing results of effect of temperature

The best result was given by the petri plates which were incubated at a temperature of 37ºC.

IV. 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.

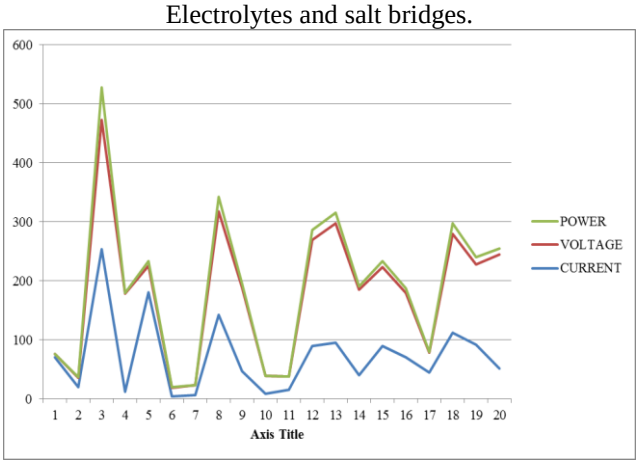
Fig. 4: Dual Chambered MFC with Voltmeter

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

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	9.8 mW

Table 14: Showing the generated power by using different Electrolytes and salt bridges.

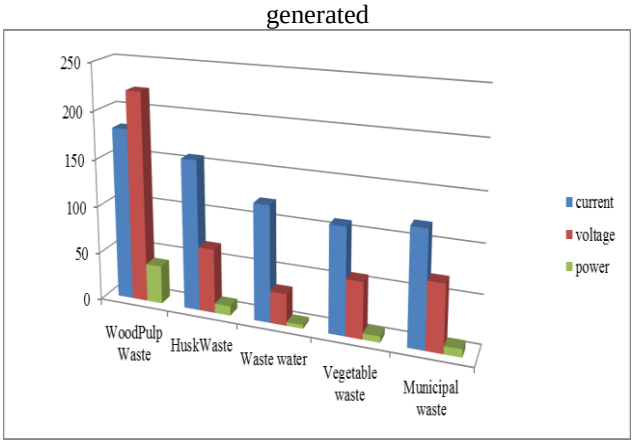


Graph 4: showing relation between current voltage power.

B. MFC prepared with different types of Substrates are shown in the table:

Substrate	Electrolyte	Current (mA)	Voltage (mV)	Power (mW)
WoodPulp Waste	2.5% $CuSO_4$	181	221	40
HuskWaste	2.5% $CuSO_4$	157	067	10
Wastewater	2.5% $CuSO_4$	121	033	3.9
Vegetable Waste	2.5% $CuSO_4$	110	058	6.3mW
Municipal Waste	2.5% $CuSO_4$	119	069	8.2

Table 15: Showing different substrates with the power generated



Graph 5: different substrate were used, wood pulp waste has produced maximum voltage.

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. The current and the voltage wasshown below:-

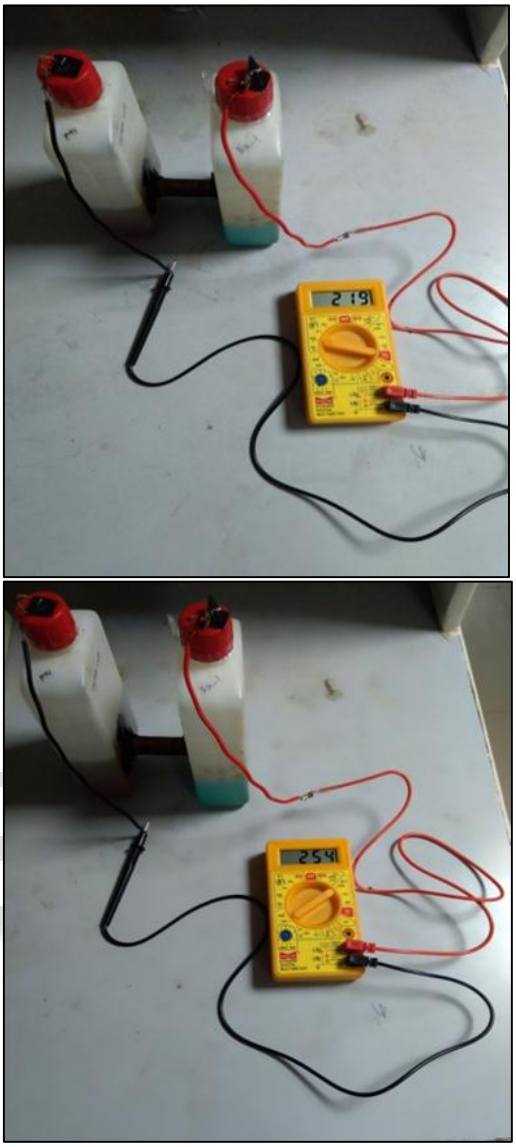


Fig. 5.19: MFC with voltmeter showing voltage and current

V. 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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