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Microbial Fuel Cell – Production of Bio Electricity

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Abstract: *Electricity is a form of energy resulting from charged particles (such as electrons or protons), either statically as an accumulation of charge or dynamically as a current. Microorganisms are ubiquitous and are used in almost all industries to produce specific products. They are termed as "the degraders" of the environment. They utilize a wide range of substrates in order to survive. This property is harnessed for the production of electricity. The biochemical interactions are converted into electricity. They act as catalysts for the production of electricity utilizing a wide range of substrate which helps generate power.*
Microorganisms were isolated from air and water sources. It was identified based on morphology and further confirmed by biochemical tests. Isolated organisms were examined for electricity production. Standardization procedures were carried out for increasing the efficiency of electricity production. Sewage water was used as media in which organism were grown which transformed the substrate into electricity and in the process the sewage was treated and the water was clarified. The MFC generated Alternating Current (AC). It cannot be used directly hence it was converted into Direct Current (DC) with the help of a capacitor. A battery like device was used to store the produced electricity. This was used to operate small gadgets like the LED bulb.
Keywords: *MFC, LED, Degraders, DC, AC, Cooum.*

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I. Introduction:

"Power shortage" a condition that occurs in electric power system when the total real or reactive power of the Power Plants in the system is insufficient to supply all consumer with electric power of required quality. The demand for the power is always higher than that of supply. Especially in the developing countries where the population rate is notably higher, the demand is more and to produce sufficient electricity becomes an uphill task. Burning of gasoline to generate electricity releases harmful gases that causes air pollution.It is of utmost necessity to generate electricity at high rates and that too eco friendly. Usingmicrobes for power generation would be the best alternative for this purpose viz the cost effectiveness, utility and production in small scale are some of the advantages of this study.

Microbial Fuel Cell (MFC) is a 'BIO ELECTRO CHEMICAL' system that derives an electric current using bacteria and mimicking bacterial interactions found in nature. It converts Chemical Energy by the action of micro organisms to electrical energy. It harnesses the power of respiring microbes to convert organic substances directly into electrical energy. Nature has been taking organic substances and converting them into energy for billions of years. Cellular respiration is a collection of metabolic reactions that cells use to convert nutrients into Adenosine Triphosphate

(ATP) which fuels cellular activity. The overall reaction is considered as redox in which electrons are being move around. These produced electrons can be used for the Power Generation.

II. Materials And Methods:

MEDIA AND CHEMICALS

- Nutrient agar media
- Nutrient broth media
- Marine broth 2126
- Catholyte (sodium chloride)
- Geobacter specific media

SAMPLE COLLECTION:

Arabian Sea sample:

Sample was obtained from NIOT (National Institute Of Ocean Technology) in a sterile container. It was collected from 50m depth. East 75.3817 ^{o} and North 9.863 ^{o,} pH of the sample was 6.6.

Cooum river sample:

This river is about 72 km in length, flowing 32 km in the urban part and the rest in rural part. It is highly contaminated with waste water and fecal contamination. Sample was collected from the nearby water body in a sterilized container. pH of the sample was 6.5.

ISOLATION:

Two basic method were opted for the process of isolation.

1. Isolation from Air:

Air is the prime source of micro organism. It contains different type of micro organisms. To isolate different micro organisms nutrient agar plate was prepared. It was opened in air for 5 to 15 minutes. The plates were incubated for 48 hours at room temperature. It was stored for MFC studies.

2. Isolation from water :

Water contains innumerable microbes.

- Contaminated water - Cooum

➢ Sewage water was used for isolation of microbes. Pour plate technique was followed normally for counting the number of organisms present in the liquid specimen.100µl of sample was taken in a sterile petri plate using sterile pipette. Sterilized nutrient agar was poured into the petri dish containing sample. It was mixed well. Once the agar solidified it was incubated for 24 to 48 hours. It was stored for MFC studies.

➢ 100µl of sample was inoculated into the Geobacterbacter specific media. It was incubated inside anaerobic chamber for 4 to 7 days.

- Arabian Sea water – Marine water

Aquatic organisms would be so versatile. It was utilized for power generation. 100 µl of Arabian sample was inoculated into the marine broth 2126. It was incubated at room temperature for 10 days. After growth loopful of broth containing organism was streaked on the nutrient agar plate. It was stored for MFC studies .

CULTURE CONDITIONS:

The isolates were stored in refrigerator at 40^{0} c. The culture was retrieved by streaking on nutrient agar plates and incubated at room temperature. For MFC operation a loopful of isolated colonies were inoculated in 100 ml of sterilized nutrient broth and incubated at room temperature in static condition.

Confirmation of Micro organisms:

After the growth organisms were identified based on their morphological character and confirmed based on their biochemical test.

MFC DESIGN AND COMPONENT:

Electrode:zinc electrode of dimension 12cm / 2.5 cm was used for anode . Carbon electrode of dimension 12 cm / 2.5 cm was used for cathode. Both the ends were immersed and fixed with the container containing media, culture and NaCl.

Cathodic chamber:Cathode chamber of MFC was made up of 10cm borosilicate bottles filled with 3% NaCl as Catholyte.

Anodic chamber:10cm fabricated borosilicate bottles were used for this purpose. The bottles were surface sterilized by washing with 70 % ethyl alcohol followed by UV exposure for 15 minutes. Then the autoclaved nutrient broth containing 24 hours old culture was introduced into it. Every time it is sterilized before adding the 24 hours culture.

Salt bridge**:**Two borosilicate bottles were connected by blowing the glass . Salt bridge was prepared by dissolving 3 % agarose in 1M NaCl. The mixture was boiled for 2 minutes and casted in the chamber in aseptic condition. It was left in the room temperature for solidification purpose.

Circuit Assembly:

Two chambers were internally connected by salt bridge and externally the circuit was connected with copper wires which were joined to the two electrodes at one end while the other end was connected to multimeter.

Measurement of potential difference:
The potential difference generated by the fuel cell was measured by using multimeter from Haoyue model number DT830D.

MFC Operation:
All the components of MFC were connected i.e via salt bridge internally and externally with wires to the multimeter.
The isolated colonies were aseptically transferred to 100ml of sterilized nutrient broth and incubated at room temperature for 24 hours. The bottles were sterilized prior to operation of MFC by 70% ethyl alcohol. Then it was exposed to UV for 15 minutes. Salt bridge was poured into the connection of the chamber. 100ml of nutrient broth containing over night culture of organism was introduced into the anodic region of the chamber. 3% Nacl was introduced into the cathodic chamber. The MFC was operated at room temperature (25 degree Celsius) . It was kept in the static conditions. Isolated organisms were checked one after the another to analyze the efficiency of power production. MFC was operated for 24 hours and voltage difference was noted in all the cases.

Standardization of MFC:
Different combination of organisms was used to analyze the efficient power production. pH was altered for highest producers and efficiency was examined.

SEM ANALYSIS: (SCANNING ELECTRON MICROSCOPY)
It is a type of electron microscope that produces images of the test sample by scanning the surface with a focused beam of electrons. The electrons would interact with the atoms present in the sample produce signals. These signals would be detected in the detector and further intensified.

Anodes were immersed in the nutrient broth containing organism. It was checked for adherence of the microbes. Zinc sample of size 12/2.5 was used for SEM analysis. This metal plate was placed in the sample loader and the machine was switched on. After the electrons were passed through the sample scanning of the surface was performed. High voltage of 30.00kv was used.

Width was 34.3 mm and 13.5mm. Whole metal electrode was scanned and the signals were detected and intensified.

BIOREMEDIATION OF COOUM WATER:
Cooum is a river heavily contaminated with domestic and municipal waste .Although 70% of the world is covered with water only 3% can be used for drinking purpose. The availability of water is drastically decreasing due to climate change, global warming and decreased rainfall. To manage this water crisis waste water treatment would be a boon. This treated waste water can be used for cleaning and gardening purpose. But the amount of electricity spent in the water treatment would be high. Best alternative to solve this problem is by adopting Microbial Fuel Cell technology. This can treat the waste water as well as generate electricity.

Method:
Collected Cooum water was sterilized and loopful culture of *Bacillus 1*was added and incubated at room temperature for 24 hours. After the incubation it was introduced into the anodic chamber of MFC and efficiency of its power generation was analysed. Various physio chemical properties of the water such as TDS, COD,DO and BOD were analysed before and after treatment of the water. Results were noted.

ELECTRICITY GENERATION:
Generated charges were stored in the capacitor as a temporary storage and permanently stored in the battery. There were totally 3 chambers having Anode A1,A2 and A3 and Cathode B1,B2 and B3. A1, A2 and A3 and B1,B2 and B3 were connected respectively. At the end both anode and cathode were connected to the capacitor. Negative end of the capacitor was connected to the negative end of the battery. In such a manner they were connected in a series fashion. Various appliances can be connected via the negative terminal of battery to the negative terminal of appliances while the positive terminal was connected to positive of capacitor. After connection working of the appliances were observed.

III. Results and Discussion:

Isolation of organisms:

After incubation different colored colonies were obtained.

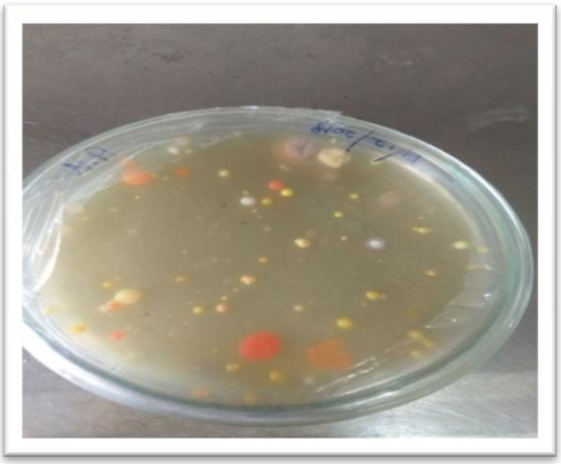

Fig 1: Open air plate

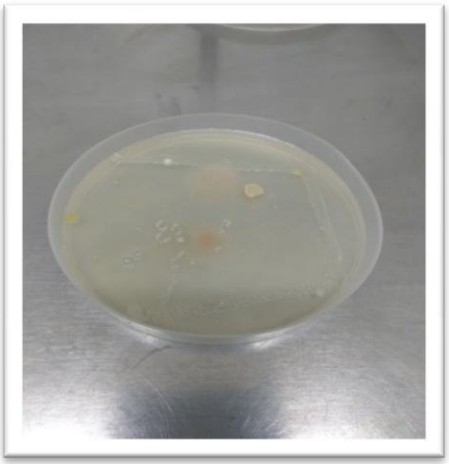

Fig 2: Marine agar plate

Figs 1 &2 show the culture plates which were developed by exposure to air, marine sample and sewage from Cooum.

CONFIRMATION OF ORGANISMS:

Colony morphology was noted. Staining was performed to identify the shape of the organism. Bio chemical tests were performed for further confirmation.

Colony morphology & staining characteristics

Organism	Colony morphology& staining characteristics
Bacillus 1	White colored ,rod shaped, flat ,entire ,flat and Gram positive rods
Bacillus 2	white colored, rod shaped, branched at ends and Gram positive rods
Bacillus 3	Flat or slightly convex with irregular edges and Gram positive rods
Bacillus 4	White colored, circular colony and Gram positive rod
Bacillus 5	White or slightly yellow ,rough, opaque , fuzzy and Gram negative rod
Bacillus 6	White or slightly yellow colored, granular, flat colonies and Gram positive rods
Serratia species	Red colored non diffusible pigment, entire margins and slightly elevation and Gram Negative rods.
Staphylococcus species	Golden yellow colonies, round , pinpoint and cluster of Gram positive cocci
*Sarcina*species	Orange colored, raised, slightly rough colonies and Gram positive cocci
*Geobacter*species	White colored, slimy, pin point colonies and Gram negative rods

Table 1: colony morphology and staining characteristics

BIOCHEMICAL TEST:

Table 2:

Organism	Catalase	oxidase	MR	VP	Citrate utilization	Triple sugar iron	Lactose utilization	Nitrate reduction	Gelatin	Indole
Bacillus 1	+	+	+	+	+	+	+	+	+	+
Bacillus 2	+	+	+	+	+	+	+	+	+	+
Bacillus 3	+	+	+	+	+	+	+	+	+	+
Bacillus 4	+	+	+	+	+	+	+	+	+	+
Bacillus 5	+	+	+	+	+	+	+	+	+	+
Bacillus 6	+	+	+	+	+	+	+	+	+	+
Serratia species	+	-	-	+	+	+	-	+	+	-
*Sarcina*species	-	-	-	+	-	+	-	+	+	-
Staphylococcus species	+	-	+	+	+	+	+	+	+	-
*Geobacter*species	+	-	+	-	-	+	+	+	+	+

+: Indicates positive

- : Indicates negative

Table 2 shows the results obtained from biochemical test done to confirm the micro organisms. All the species of Bacillusshowed positive result. Serratia species showed negative for oxidase, MR, lactose utilization and indole test. Sarcina species showed negative for Catalase, oxidase, MR, Citrate utilization, lactose utilization and gelatin. Staphylococcusspecies showed negative for oxidase and indole test.*Geobacter* species showed negative for oxidase, VP and citrate utilization test and it showed H_2s production.

Organism isolated:

S.NO	METHOD OF ISOLATION	ORGANISM ISOLATED
1	Air	*Serratia* species
2	Air	*Staphylococcus* species
3	Air	*Sarcina* species
4	Water – Cooum	*Bacillus 1*
5	Water – Cooum	*Bacillus 2*
6	Water – Cooum	*Bacillus 3*
7	Water – Cooum	*Bacillus 4*
8	Water – Cooum	*Geobacter species*
9	Arabian Sea – Marine water	*Bacillus* 5
10	Arabian Sea – Marine water	*Bacillus* 6

Table 3: Shows the organisms that were isolated from various sources namely air, contaminated sewage water Cooum and marine water.

GENERATION OF ELECTRICITY:

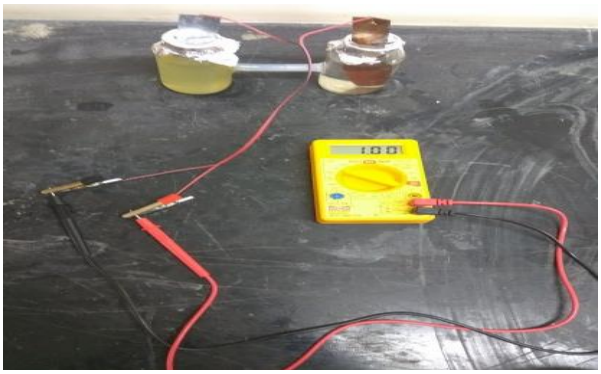

Fig 3: *Serratia* species

Fig 4: *Bacillus 2*

Figs 3 and 4 : show the electricity generation by isolated organisms

Table 4: Shows the generation of electricity by isolated organisms.

SL.NO	ORGANISM	ELECTRICITY GENERATED IN VOLTS
1.	*Bacillus 1*	0.75 to 0.84
2.	*Bacillus 2*	0.91 to 0.98
3.	*Bacillus 3*	0.78 to 0.82
4.	*Serratia* species	0.90 to 1.00
5.	*Sarcina*species	0.01 to 0.05
6.	*Staphylococcus* species	0.45 to 0.90
7.	*Bacillus 4*	0.83 to 0.89
8.	*Bacillus 5*	0.65 to 0.87
9	*Bacillus 6*	0.69 to 0.88
10	*Geobacter*species	0.30 to 0.85

PRODUCTION OF ELECTRICITY BY COMBINATION OF MICRO ORGANISM:

SL.NO	ORGANISM	ELECTRICITY GENERATED IN VOLTS
1.	*Bacillus 2* + *Serratia* species	0.84 to 0.88
2.	*Serratia* species + *Bacillus 3*	0.78 to 0.92
3.	*Serratia* species + *Bacillus 5*	0.80 to 0.89
4.	*Serratia* species + *Bacillus 4*	0.82 to 0.85
5.	*Serratia* species + *Staphylococcus* species	0.85 to 0.88
6.	*Serratia* species + *Bacillus 3* + *Bacillus 2*	0.75 to 0.78
7.	*Serratia* species + *Bacillus 6*	0.79 to 0.80
8.	*Serratia* species + *Bacillus 1*	0.80 to 0.84
9.	*Serratia* species + *Staphylococcus* species + *Bacillus 1*	0.82
10.	*Serratia* species + *Bacillus 1*+ *Bacillus 2* + *Bacillus 4*	0.77 to 0.78

Table 5: Shows the values of voltage generated by mixture of organisms.
The amount of voltage generated drastically decreased when two or organisms are cultured together .The production of electricity did not increase as expected as probably the grouped organisms were not antagonist to each other instead they dwell in adjustment with each other.

VARIATION IN pH:

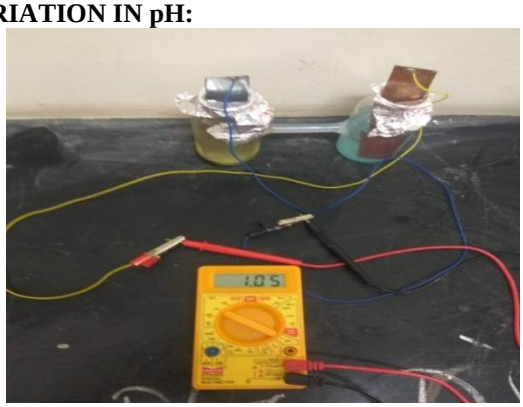

Fig 5: *Bacillus 1 species*

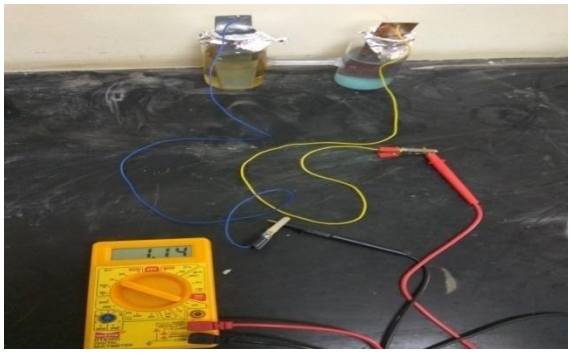

Fig 6: *Serratia* species

Figs 5 and 6 show the generation of electricity at altered pH.

Table 6

S.No	Organism	pH 3	pH 5	pH 12
1	*Serratia* species	0.88 to 1.19 v	0.80 to 1.02v	0.97 to 1.05 v
2	*Bacillus* 2	0.82 to 0.99 v	0.90 to 0.98 v	0.78 to 1.02 v
3	*Bacillus 1*	0.76 to 0.98 v	0.74 to 0.80	0.80 to 0.88 v

Table 6: Shows the production of electricity at various pH range .
Optimum pH is the condition at which growth of an organism would be appropriate. It can work at its best and show all its properties only at optimum pH. When the optimum condition is lost functioning of the organism becomes difficult. Organisms were subjected to extreme pH and examined for electricity production.

SEM ANAYLSIS (SCANNING ELECTRON MICROSCOPY)

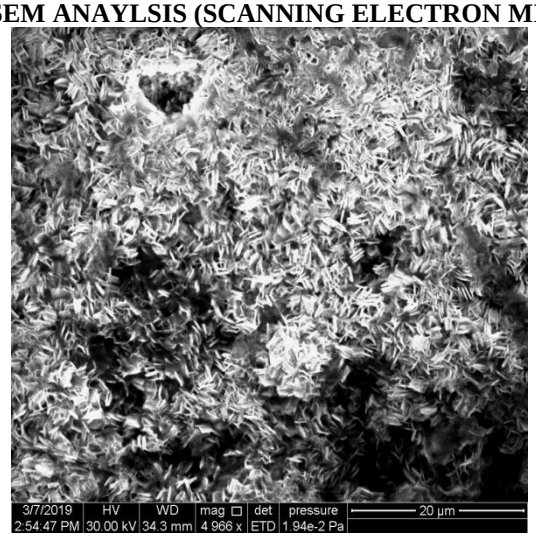

Fig 7: Adherence of rod shaped bacteria

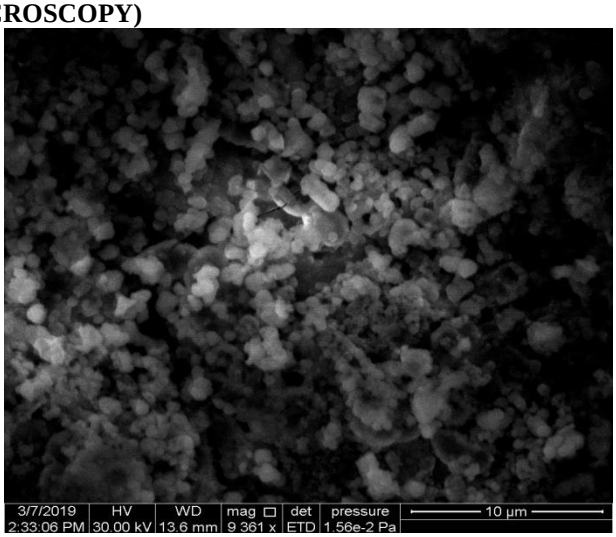

Fig8: Adherence of cocci shaped bacteria

Figs 7 and 8 : show SEM report for the cell adherence in the metal sheets. Results obtained indicated the presence of rod shaped cell and cocci which shows that the adhering cells (micro organism) are *Bacillus* species and *Staphylococcus* species.

BIOREMEDATION OF COOUM:

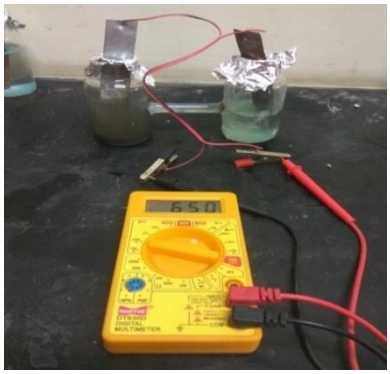

Fig 9: Electricity generation by *Bacillus 1*

Fig 9: Shows the production of electricity using the *Bacillus*1 species isolated from sewage water Cooum.

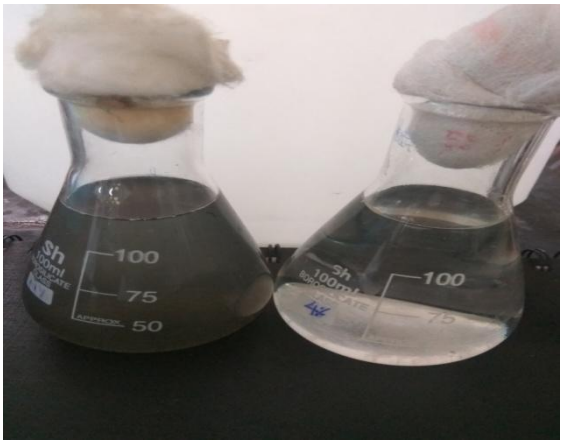

Fig 10 sewage water before and after treatment

Fig 10: Shows the difference in water clarity.
The water after production of electricity was crystal clear when compared to the water before production of electricity. This indicates that organism utilize substrate present in the sewage and transfer into electricity. It showcases the property of bioremediation.

Comparison:
Parameters of sewage were analyzed before and after water treatment. Results were noted.

Parameter	**Before**	**After**
TDS	0.8g	0.1g
DO	3200 mg/l	900 mg/l
BOD	2000 mg/l	960 mg/l
COD	5.5 mg/l	2.5 mg/l

Table 7: Shows the difference in the levels of DO, COD and TDS eventually decreased after treatment.

ELECTRICITY GENERATION:

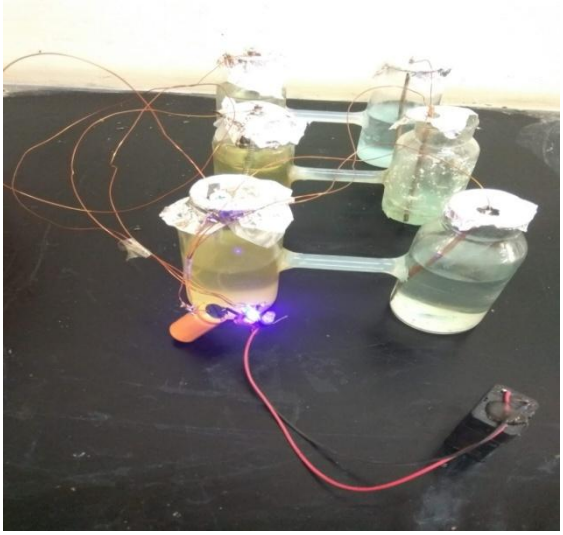

Fig 11: Glowing of blue LED bulb

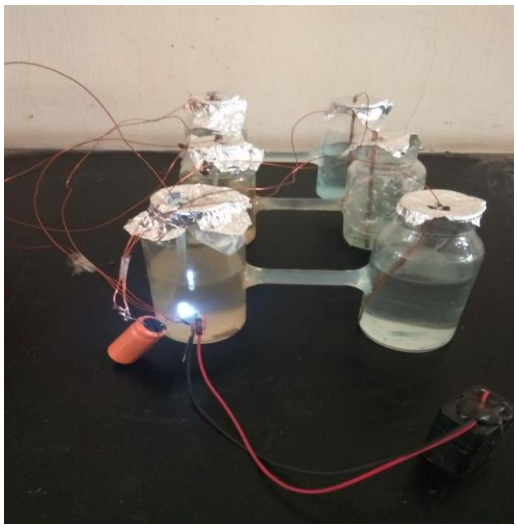

Fig 12: Glowing of white LED bulb

Fig 11 and 12 show the utilization of the electricity produced. Bulb which was lit by the electricity produced through series connection of organisms.

MFC produce Alternating Current. It cannot be directly utilized because voltage requirement of AC devices is more when compared when the generated voltage. So it was converted to Direct Current with the help of capacitor. For the constant supply of current generated voltage was stored in a storage tank like battery.

IV. Conclusion:

Arabian Sea Sample was collected from NIOT (National Institute of Marine Technology) and sewage water was collected from contaminated Cooum river. Two sources were opted for isolation. Isolation from air and isolation from water were carried out. Organisms were identified based on the morphology and further confirmed by biochemical test. Physio chemical parameters of the sample were analysed.SEM (Scanning Electron Microscope) was performed to analyze the adherence of the microbes to the electrode. Generation of electricity from isolated organisms was examined. Optimum conditions were investigated. About 73% of sewage is untreated in India. It can be used as a substrate for the generation of electricity. In this process the contaminated Cooum water was treated. Appliance like LED bulb was operated with the voltage generated by the isolated micro organisms. MFC generates Alternating Current. It cannot be utilized directly so it was converted to Direct current by using capacitor. A constant power supply is required so it was stored in a storage tank like battery.

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