MATERIALS AND METHODS
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Material for the Construction of the MFC Assembly:

1. Axenta boxes (Axiva)
2. Centrifuge tube (Poly tab)
3. Copper wire
4. Graphite electrode (Carbon Products)
5. Proton Exchange Membrane-CMI 7000 (Membrane International-NJ)
6. Multimeter (ORPAT ODM -200)
7. Agitator (Magnetic Stirrer)
8. Aquarium pump
9. Epoxy (M-seal)

Chemicals:

1. Phosphate buffer solution (see appendix)
2. Potassium Ferricyanide
3. Potassium dichromate (see appendix)
4. Potassium iodide (see appendix)
5. Sodium thiosulphate (see appendix)
6. Sulfuric acid (see appendix)
7. Starch (see appendix)
8. Ortho phosphoric acid (Use for maintain the pH)
9. Hydrogen peroxide (H₂O₂)

Media:

1. Nutrient Agar (see appendix)
2. Luria Bertani broth (see appendix)
**Microbial source:**

1. *Enterobacter aerogens* (MTCC-2824)
2. *Enterobacter cloacae* (MTCC-7097)
3. *E.coli* (MTCC-64)
4. Untreated municipal waste water (with sludge and oily material)
5. Primary treated municipal waste water (without sludge and oily material)
6. Pond water (stagnant water body)
7. Canal water (running water used for irrigation)
8. Yamuna river water (slow flowing water body)
9. Water from Sangam region (fast flowing water body)
10. Textile industry waste water (without any treatment)

**Substrate:**

1. Glucose (Polysaccharide)
2. Sucrose (Disaccharide)
3. Sodium acetate (Mild acid)

**Electrode:**

Graphite electrode is used in this work which is obtained from Carbon Product, Mumbai (Table 3.1). Dimension of electrode was 3 X 3 X 0.5 cm. Its surface was increased to 27.5 cm\(^2\) from 24 cm\(^2\) by making 5 through holes.

![Graphite electrode with 5 through holes](image-url)

**Fig. 3.1: Graphite electrode with 5 through holes**
Pure graphite electrodes without coating were used in the present study to minimize the cost of the experiment and the properties of the electrode are shown in Table 3.1.

**Table 3.1: Property of plain graphite electrode**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (g/cm$^3$)</td>
<td>1.70 to 1.74</td>
</tr>
<tr>
<td>Apparent porosity %</td>
<td>15 to 20</td>
</tr>
<tr>
<td>Hardness on Rockwell “R”</td>
<td>65 to 70</td>
</tr>
<tr>
<td>Compressive strength N/mm$^2$</td>
<td>38 to 42</td>
</tr>
<tr>
<td>Ash %</td>
<td>0.02</td>
</tr>
<tr>
<td>Transverse strength N/mm$^2$</td>
<td>23 to 25</td>
</tr>
<tr>
<td>Heat resistance ºC</td>
<td>600º in oxidising</td>
</tr>
<tr>
<td></td>
<td>2500º in Non-oxidising</td>
</tr>
</tbody>
</table>

**Membrane:**

During the present study Cation Exchange Membrane (CEM) was used (Fig. 3.2) that is cheaper than nafion membrane with minimum electrical resistance (Table 3.2). A cation exchange membrane CMI 7000 was obtained from Membrane International, USA.

![Fig 3.2: Membrane CMI (7000)]
Table 3.2: Property of CMI-7000 membrane

<table>
<thead>
<tr>
<th>Technical Specification</th>
<th>CMI-7000S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single Sheet</td>
</tr>
<tr>
<td>Functionality</td>
<td>Strong Acid Cation Exchange Membrane</td>
</tr>
<tr>
<td>Polymer Structure</td>
<td>Gel polystyrene cross linked with divinylbenzene</td>
</tr>
<tr>
<td>Functional Group</td>
<td>Sulphonic Acid</td>
</tr>
<tr>
<td>Ionic Form as Shipped</td>
<td>Sodium</td>
</tr>
<tr>
<td>Color</td>
<td>Brown</td>
</tr>
<tr>
<td>Standard Size : US : Metric</td>
<td>48in x 120in</td>
</tr>
<tr>
<td>Standard Thickness(mils) (mm)</td>
<td>18±1</td>
</tr>
<tr>
<td></td>
<td>0.45±0.025</td>
</tr>
<tr>
<td>Electrical Resistance (Ohm.cm²) 0.5 mol/L NaCl</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Permselectivity (%) 0.1 mol KCl/kg / 0.5 mol KCl/kg</td>
<td>94</td>
</tr>
<tr>
<td>Total Exchange Capacity (meq/g)</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>Water Permeability (ml/hr/ft²) @5psi</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Mullen Burst Test strength (psi)</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Thermal Stability (°C)</td>
<td>90</td>
</tr>
<tr>
<td>Chemical Stability Range (pH)</td>
<td>1-10</td>
</tr>
</tbody>
</table>
METHODOLOGY

3.1 Maintenance of the bacterial culture:

The ampoule containing bacterial culture was opened very carefully making mark on the ampoule near the middle of the cotton wool with a sharp file. The surface was disinfected around the mark with alcohol and thick cotton wool was wrapped around the ampoule and broken at the marked area. Top of the ampoule was removed gently and the cotton plug was drawn to one end. If the ampoule was opened quickly then fine particles of dried organisms will be released into the environment. 0.3 to 0.4 ml of nutrient agar medium was added to make a suspension of the bacterial culture. Few ml of the suspension was inoculated in nutrient agar medium and rest of the suspension was transferred in a test tube for further use. The suspension was kept in the shaker in anaerobic condition and growth of the culture occurred after few days. All the remains in the original ampoule were sterilized before discarding.
Fig 3.3: Instruction for opening the Freeze-dried ampoule

1. **Collection of water samples**: All water samples have been collected around 2 feet deep to avoid dust and oily layers in plastic containers.

2. **Maintenance of the pH**: In the whole operation, the environment was acidic. So pH was maintained at 4.5 or 5.5 using 88% orthophosphoric acid to reduce the methane production.

3. **Electrode pre-treatment**: Electrodes were pre-treated in deionised water for 24 hrs to enhance the porosity.

4. **Membrane pre treatment**: Membrane was immersed in 30% H₂O₂ after 6 hrs intervals also immersed in deionised water to enhance the porosity.

6. **Construction of dual chamber MFC Assembly**: Dual chambered assembly was constructed as given below:

   Assembly was constructed using two axenta box, made by up of polyplex material with the volume of 300 ml each and Boxes were joined together with the help of centrifuge tube. Both chamber were connected using one centrifuge tube made up of polyplex material with volume
50 ml and 9 cm total distance. These tube had and the diameter was 3 cm. Two plain graphite electrodes without coating were used. Surface area of each electrode was 24 cm² (3 cm × 3 cm × 0.5 cm). But the surface area was increased 27.5 cm² by making five through holes with 0.5 cm diameter each. Both electrodes were placed in each chamber; one act as anode and other one was work as cathode. Both were attached using metallic wire (copper wire) and an external resistor (100 _) made an external circuit. Which was formed the path for the transportation of electron. Cation exchange membrane (CMI 7000) act as separator, which was separate the chamber, anode chamber and cathode chamber. The membrane only allow to proton transportation from anodic solution to cathodic solution. Both anaerobic and aerobic conditions were maintained. Anaerobic environments was maintained in the anode chamber, was sealed with the parafilm and agitate at minimum speed with magnetic stirrer where as cathodic chamber was maintained in aerobic environment with the help of aerator continuous flow of air in the chamber. Agitation and aeration both process were simultaneously and continuously used for maintaining the environment.

3.2 MFC Operation:

During the present study, MFC has been operated for 15 days using three bacterial cultures at various densities (0.125, 0.25, 0.5 and 1.0 OD for voltage optimization) using 0.4 % initial concentration of three different substrates i.e. Glucose, Sucrose and Sodium acetate. Voltage optimization was then done using various substrate concentrations of 0.2 and 0.6% at pH 5.5 to obtain maximum voltage or power density.

Six different water samples – 4 inland and 2 waste water samples – were used to analyse the water remediation potential of the designed MFC. Dual chambered Microbial fuel cell has been operates with each water samples separately using sucrose at 5.5 pH. Optimization of electrical conductance and COD removal rate has been done with glucose at 5.5 pH (for lower electrical conductance producing inland samples) and with sucrose with 4.5 pH (for waste water and higher electrical conductance producing inland samples) using 0.4% concentration of substrate. Sodium acetate has been also used as substrate with highest electrical conductance producing water sample. Voltage recordings were used to derive other electrical parameters.

After getting optimum O.D. of selective bacterial culture and optimum concentration of selective substrate at optimum pH, textile industry waste water have been inoculated with pellets
from 50 ml, 100 ml, 200 ml and 300 ml (washed twice in PBS) of overnight bacterial culture to prove the increased COD rate and the improved coulombic efficiency of the MFC.

COD was calculated before starting the MFC operation using titration method given below. The anode chamber filled with bacterial culture/water sample suspension with 5.5/4.5 pH using 88% orthophosphoric acid in anaerobic condition and agitation has been done magnetic stirrer. Cathode chamber was filled with phosphate buffer saline (PBS) with 7.2 pH in aerobic condition that was maintained by aerator (aquarium pump). Agitation and aeration both process were simultaneously and continuously used for maintained the environment. The process has been done continuously till 15 days with above mentioned condition and media in anode chamber was enriched with same amount of substrate at each 3rd day interval. After the termination final COD was calculated (as calculated for initial COD) to count coulombic efficiency

### 3.3 COD removal rate and Coulombic Efficiency:

The increase of pollution by discharging large amount of various chemically oxidizable organic substance of different nature entering the aquatic system. The chemical oxygen demand (COD) is a better estimate of the organic matter, which needs no sophistication and is time saving. The amount of organic matter in the water is estimated by their oxidability by chemical oxidant such as potassium dichromate. The organic matter is first oxidized with a known volume of potassium dichromate ($K_2Cr_2O_7$) and then the excess of oxygen is allowed to react with potassium iodide to liberate iodine in amount equal to excess oxygen, which is estimated titrimetrically with sodium thiosulphate solution using starch as an indicator. Procedure is given as follows:

To 50 mL bacterial culture sample in a conical flask 5mL 0.02N potassium dichromate was added and kept in a water bath at 100°C for 1h. After cooling for 10 min 5 mL of 10% potassium iodide was added followed by 10 mL of 1.1% H$_2$SO$_4$. The solution was titrated with 0.1M sodium thiosulphate until a pale yellow colour appeared and 1mL of the 1% starch solution added till a dark blue colour appeared. The solution was again titrated with 0.01M sodium thiosulphate solution till the blue colour disappeared. Formula for COD determination is: COD = \[\frac{8 \times 100 \times (B-A)}{V}\] where, $B$= volume of titrant used for sample, $A$=volume of titrant used for distilled water and, $V$= volume of sample to be titrated.
The recovery of electrons is referred to as Coulombic efficiency, defined as the fraction (or percent) of electrons recovered as current versus that in the starting organic matter. Performance of MFC also evaluated by estimating the substrate removal efficiency during operation using following equation:

\[ \text{COD} = (\text{C}_{\text{so}} - \text{C}_{\text{s}}) \times 100 \]  \hspace{1cm} \text{.................... \ldots (16)}

Where

\begin{align*}
\text{C}_{\text{so}} &= \text{initial COD concentration} \\
\text{C}_{\text{s}} &= \text{final COD concentration}
\end{align*}

3.4 Power generation in Microbial fuel cell:

Microbial fuel cells (MFCs) exploit microbes to generate electricity directly from organic compounds. In MFCs, the power capability depends on the kinetics of the electron transfer between the bacterial cells and the fuel cell anode. The electron transfer can take place directly via proton exchange membrane alternatively via conductive Bacterial pili. The power output of Microbial fuel cell is calculated from the voltage (E) across the load and the current as:

\[ P = IE \]

And the current produced can be calculated by measuring the potential across the load (external resistor R) i.e.

\[ P = E^2 \]
\[ P = I^2 R \]  \hspace{1cm} \text{................................. (17)}

(Since I=E/R)

3.5 Power density in microbial fuel cell:

Power density is the amount of power (time rate of energy transfer) per unit volume. Power density produced with sucrose or acetate is much larger than those obtained with domestic wastewater. These differences in power densities arise from substrate concentration and form (soluble or particulate), intrinsic microbial kinetics, and the complexity of the microbial community in the biofilm that is needed to completely degrade the substrate. Power density of a MFC can be increased by chemically modifying the anode, improving the cathode Performance using ferricyanide.

\[ \text{PD} = P / V \]  \hspace{1cm} \text{................................. (18)}
PD = Power density

P = amount of power

V = volume of electrode

### 3.6 Current in Microbial fuel cell:

Since voltage, current, and resistance interrelate:

\[ V = IR \]

In this algebraic expression, voltage (E) is equal to current (I) multiplied by resistance (R). Using algebra techniques, we can manipulate this equation solving for I:

\[ \text{Current (I)} = \frac{V}{R} \]………………… (19)

### 3.7 Current Density in Microbial fuel cell:

Current density is a measure of the density of flow of a conserved charge. Usually the charge is the electric charge, in which case the associated current density is the electric current per unit surface area of electrode, but the term current density can also be applied to other conserved quantities. It is defined as:

\[ \text{CD} = \frac{I}{A} \]……………………………… (20)

CD = Current Density

I = current

A = surface area of electrode

This methodology was suitable and fulfills the objectives of the present work. The materials are easily available and occur at low cost in comparison to other methodology and used chemicals were easily available. In this method, every step was performed in the laboratory under sterile conditions (where needed).