
Miniature Ecological System for the Treatment of Hussain Sagar Waste Water with Associated Power Generation

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Abstract

Extensive development of science and technology in this scientific era has made the human life much easier and comfortable. However a simultaneous increase in the pollution due to the dumping of industrial effluents, Plastic utilities, Computer hardware etc has become one of the threatening problems in the present day. It's the time for the use of this science and technology in the direction of bioremediation or environment management. In association with this concept the present work involves the development of an eco-friendly miniature treatment system for the treatment of waste water. The major source used here are the microbial cells which act as bio scavengers or purifiers. The main principle of the work is the ability of microbes to act as behave as biological catalyst by donating their electrons and protons produced as a result of anaerobic respiration of organic substances. These electrons are accepted by acceptors resulting in the generation of power. The work also involves the simultaneous generation of power from this treatment system. Thus it is not only eco-friendly but also an important source of the generation of electricity. These kind of set ups are also called as Microbial Fuel Cells.

Key Words:

Microbial Fuel Cells, Bio reactors, Anaerobic, Proton and Electron Donor

Introduction

The use of microbes as biofuels in the treatment of waste water and generation of power is one of the most economic, environment safe and on demand technique required. The specially designed closed system in which bacteria are used as proton and electron donors so as to cause a potential difference resulting in power generation is called microbial fuel cells. There are several kind of microbial fuel cells running on different microbes and designed definitely.

Several substrates like Acetate, Lactate and glucose have been used as substrates in the production of microbial fuel cells (1). One of the Single chamber Microbial Fuel cell (SCMFC) was designed by a research team at The Pennsylvania State University, Pennsylvania. It contained eight graphite electrodes and a single air cathode. The continuous flow conditions were maintained in the system for which

the primary clarifier effluent is obtained from a nearby waste water treatment plant. Such bioreactor would reduce the COD of the water and generate electric current.

In view of the importance of microbial fuel cells there are certain organizations coming up which would supply the pure cultures and mixtures of these microbes which are capable of digesting the carbon sources of the waste thereby producing power as well as purification of water (2). Some of the microbes used for the purpose are: *Bacillus* species and *pseudomonas*. They are commercialized as concentrated blend of freeze dried microbes on some nutritious source like wheat bran.

Potter for the first time reported that the bacteria are capable of generating electricity which is almost hundred years ago (3). A Microbial Fuel Cell (MFC) is capable of generating electricity directly from a large variety of organic or inorganic compounds, using a microbe as a catalyst [4].

A typical microbial fuel cell contains an anode and a cathode which are separated by an ion exchange. The fuel source used here is a solution containing microbes and the organic matter acting as a carbon source for the microbe (5). These fuel cells function by converting the chemical energy obtained by the consumption of carbon source by microbes, into electrical energy obtained by the release of protons and electrons by the bacteria.

Increasing the efficiency of an MFC involves addition of the extra nutrients like monosaccharide's, nitrogen source and amino acids, these sources increasing the electron shuttle in the medium(6). One of the study conducted by the students of University of Medras showed that the increase in the yield of the power can be obtained by using Glucose (0.98 V), beef extract (0.85 V) and Leucine (0.92 V).

Renewable energy is an increasing need in our society. MFC technology represents a new form of renewable energy by generating electricity from what would otherwise be considered waste. This technology can use bacterium which already present in wastewater as catalysts for generating

electricity and simultaneously treats wastewater (Lui et al., 2004; Min and Logan 2004).

Materials and Methods:

Designing of the Tank:

The major component in the preparation of microbial fuel cell is the designing of the container or the chamber for the reacting material.

Three tanks were designed in the present protocol with the following dimensions.

Length	38cm
Width	25cm
Height	28 cm with free board of 3cm
Total Volume	26.6 L
Working Volume	23.75 L
Free Board Volume	2.8L

Contents of Tank 1:

- Eichornia Crassipes Collected from the Lake of Uppal
- Lake Bed sand is collected from Saroor Nagar lake
- Hussain Sagar water collected from IICT tank which is used for garden
- Snails are collected from an aquarium shop at Nagole.
- Blue green algae is obtained from the Sump of IICT
- 1 electrode is dipped under the sand bed, to act like an anode
- Another electrode is placed on the water surface to act as a cathode

Contents of Tank 2:

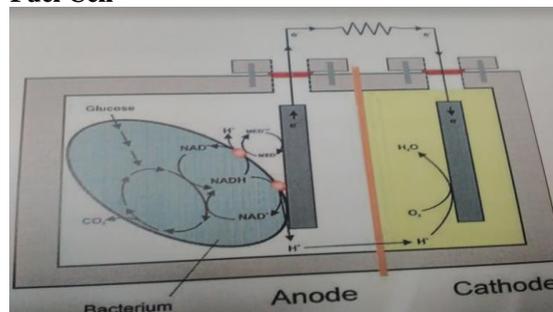
- Submerged plant namely Hydrilla was introduced
- Table rose is planted above the water level on a soil bed which is introduced on a section.
- Duck weed was obtained
- Snails are also collected from a nearby aquarium outlet at Nagole.
- One of the electrode is dipped under the sand bed acting as an anode
- Second electrode is placed on the surface of the water which acts as a cathode
- Lake bed was collected from the lake at Saroor Nagar

Contents of Tank 3:

- Two varieties of fishes were selected
 - 1) Gambusia and 2) Koyacarp which were introduced into the tank

- Two snails were also introduced which were collected earlier.
- Submerged plant Hydrilla was also introduced into the tank
- Along with these components Duck weed was also included into the tank.

Fig 1: Showing the Basic Structure of Microbial Fuel Cell



Methodology:

Domestic waste water was used for the water sample in the current research. As shown above the three perplex tanks were used for the study. The three were arranged in a specific sequence with heights raised one above the other. This arrangements helps in the flow of the wastewater through the three tanks at a specified rate of flow.

Fig 2: Showing the Setup for the Miniature Ecological System



Tank 1:

A feed composition of 20L Hussain Sagar water and 100ml distillery waste was included in the 1st tank. The first tank contains Eichornia crassipes which is a floating plant with a good root system. It helps in the oxidation of organic matter with the help of bacterial growth on the root system. The algae which were included in the first tank also aids in the oxidation reaction, which is a major cause for the utilization of organic material. This oxidation step

is also enhanced by the snails that are kept in the same tank.

Tank 2:

The water from the tank 1 enters the second tank at a specific flow rate (370ml/10min). The tank contains Marie gold, known for its specific root system supporting the oxidation step and the bacterial growth. Hydrilla a submerged aquatic plant helps in the same oxidation reaction thereby generating dissolved oxygen. This oxidation reaction utilizes the organic matter present in the feed composition.

Tank 3:

At a specific flow rate the water in the 2nd tank enters the third tank. The remaining organic matter present in the 3rd tank is further utilized by the fishes (Gambusia and Koyacarp) and the snails. The Hydrilla present also enables the generation of Dissolved oxygen. At specific time intervals analysis was conducted for the following parameters: The COD, DO, pH, ORP, Nitrates, Colour, Temperature, TDS to estimate the performance of the system (APHA 1998)

The Outlet from the tank 3 is used to collect the remnants and were subjected for the BioAssay.

Analysis

VFA (Volatile Fatty Acid Assay)

These VFA refers to butyric acid, Pyruvic Acid Acetate, Pyruvate and Butyrate.

5ml of the sample (collected from tank 3) was used to which 95 ml of distilled water was added. The pH of the media was determined followed by the titration.

Titration with 0.01N HCl: Adjust the reading to Ph -3. (Acids get ionized)

Titrate against 0.01N NaOH: Adjust the reading to pH -6.5 (Dissociation of NaOH occurs)

Calculation:

$$\text{VFA} = (\text{B} \cdot 101) - (\text{A} + 100) \cdot 20 \cdot 60 / 99.23$$

A = HCl Reading, B = NaOH Reading

Analysis of Chemical Oxygen Demand (COD)

The oxygen required for the oxidation of organic matter in the presence of strong chemical oxidant is called Chemical Oxygen Demand (COD). Determination of COD had an advantage over BOD where in the results can be obtained in about 5hrs as compared to 5 days required for BOD Test. The determination was done using COD apparatus using and following closed refluxing method as per APHA (2000) method. The organic matter gets

oxidised completely by potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) in the presence of Sulfuric acid (H_2SO_4) TO PRODUCE CO_2 and H_2O . The excess $\text{K}_2\text{Cr}_2\text{O}_7$ remaining after the reaction is titrated with ferrous ammonium sulphate. The dichromate consumed gives the O_2 required for the oxidation of the organic matter.



To remove chloride ions mercuric sulphate is added. The Hg^{2+} ions combine with chloride ions to form a poorly ionized HgCl_2 complex which is resistant to oxidation by dichromate. AgSO_4 is also used as a catalyst in the reaction.

Reagents

1. Standard Potassium Dichromate (0.1N): 2.45 gms of $\text{K}_2\text{Cr}_2\text{O}_7$ was dried at 103°C for 24hrs. Add 125ml of water and 83.5 ml of sulphuric acid. Make it upto 500ml with distilled water.

2. COD Acid reagent: 10mgs of AgSO_4 was dissolved in 1000 ml conc sulphuric acid and kept overnight for dissolution.

3. Standard Ferrous Ammonium Sulphate (0.025N): 9.8 gm of ferrous ammonium sulphate is mixed in 200ml distilled water, 20ml of concentrated sulphuric acid was added cooled and made up to 1litre.

4. Ferroin Indicator: 1.485gms of 1, 10 phenanthrolin monohydrate and 0.695gms of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was dissolved in distilled water and diluted to 100ml.

5. Mercuric Sulphate: Mercuric sulphate crystals were directly used.

6. COD Mix: 70ml of CXOD acid is taken in a beaker and 30ml of potassium dichromate is added.

Procedure:

2ml of the sample was taken in 10ml COD vials and to this 5ml of COD mix is added. The colour turns yellow. If the colour in the vials turned green dilute the samples (10X, 20X, 30X, ..) and repeat the procedure. The vials were kept in the COD block digester and refluxed for a period of 2 hrs at 148°C . After refluxing the samples were cooled and each of it was transferred to 100ml beaker. 7ml distilled water along with 2 drops of ferroin indicator was added to the sample solution and titrated against 0.1N FAS until brick red colour appeared. COD was calculated using the formula

$$\text{COD (ml/L)} = \text{B} - \text{S} \cdot \text{N} \cdot 8000 / \text{ml of sample taken}$$

B= Amount of FAS consumed for blank
S= Amount of FAS consumed for Sample

N= Normality of FAS.

pH:

The pH was determined by measuring the EMF of the cell containing an indicator electrode. Immersed in the test solution and reference electrode. The EMF of this cell is measured using the pH meter. The electrode system was calibrated against standard buffer solutions of known pH.

Buffers were prepared fresh. The standardization of the meter was performed after which the electrodes were immersed in the test sample and the pH was read.

Oxidation Reduction Potential:

Oxidation-reduction potential is the measure of the tendency of a chemical substance to oxidize or reduce another chemical substance and the units for which are millivolts (a).

Upon loosing of electrons a substance becomes oxidised. The same substance gets reduced upon acceptance of electrons. When a platinum electrode is immersed in a redox system a potential is developed on it depending on the ratio of oxidised and reduced sates. This potential is termed as oxidation reduction potential. It is a vital parameter in controlling the biological treatment of wastes. Anaerobic treatment requires a low ORP. It also depends on the pH of the solution.

The ORP values are also measured using the same pH meter as above.

Total Dissolved Solids (TDS):

Total amount of mobile charged ions including minerals, salts or metals dissolved in a given volume of water expressed in mg per Litre units also referred to as parts per million (ppm). This parameter is directly related to the purity of the water. This effects everything that consumes, lives in or uses water, weather organic or inorganic, weather for better or for worse.

Nitrates:

The nitrate concentration in the given waste water is estimated by UV method in spectrophotometer. Measurement of the UV absorption at 220nm enables rapid determination of nitrates.

Protocol for the Estimation of Nitrates:

The volume of standard KNO_3 required for concentration of 2.5, 5, 7.5, 10, 12.5 and 25 ppm range nitrate calibration standard was made. The solution was diluted upto 50ml using volumetric flask. The nitrate calibrations are prepared and labelled. Water sample was taken and 1ml of KNO_3 was added and diluted to 50ml. This was collected into fresh standard flask. To all the above standard flasks 1ml of 1N HCl was added. The absorbance of the above solution was read at 220nm. Graph was plotted using concentration on X axis and

Absorbance on Y axis, the unknown concentration of nitrate in water sample was calculated using calibration graph.

$$\text{Nitrate (mg/ml)} = (\text{OD} + 0.0431)/0.0588$$

Turbidity Determination:

Weigh 50gms of Hydrazine sulphate $\text{NH}_2 (\text{H}_2\text{SO}_4)$ In 500ml flask.

Add distilled water and stand for 4 hrs.

Weigh 30gms of Hexa methylene amine. In 500ml flak.

Mix equal volumes of the above 2 solutions to form formazene turbidity concentrate. Allow it to stand in a closed container at 250°C to 300°C for 48hrs. to produce insoluble white turbidity corresponding to 40,000 Ntu (Nephelometric Turbidity)

Dilute 25ml of formazine turbidity concentrate to 1 litre with turbidity free distilled water to obtain 100 Ntu formazine standard for setting 100 of the instruments.

Results and Discussion:

Samples were collected twice in a week from all the three tanks and is analysed for COD, Nitrates, TDS, Volatile Fatty Acid Test (VFA), Turbidity, pH and ORP. The inlet consists of 16 Lts of domestic waste and 4L of H_2 pilot plant outlet. Results have shown a major removal of COD Concentrations form Tank 1 to Tank 3. Inlet samples showed varying COD values with the change in time. The COD values in the inlet are pretty high even compared to the three tanks. HBR outlet is added to increase the COD levels in the inlet. The COD removal rate is high in tank 1.

Fig 3: The Graph showing the COD of Three tanks of EES system including inlet observed during 65 days of operation with domestic waste water and fermented food waste water

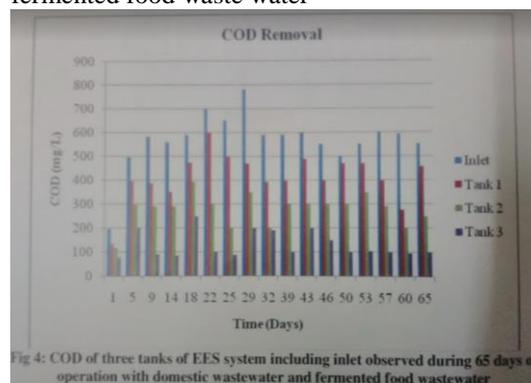


Fig 4: COD of three tanks of EES system including inlet observed during 65 days of operation with domestic wastewater and fermented food wastewater

This tank 1 also contains *Eichornia crassipes* a water plant which is known to grow in the nutrient rich water. *Eichornia* has a well-developed large root system which provides home for the large kinds of microbes. These microbes absorb the nutrients and supply them to *eichornia* which uses this energy for its growth. The removal rate had

been decreased when compared to tank 2 and 3. The Lemna, snails and fishes helps in the nutrient removal in these tanks. Due to the absorbance of heavy root system removal rate is less in these tanks.

pH Evaluation:

This is none of the important parameter to assess the quality of the waste. The whole duration of the work the pH varied between 6.5 to 8.7. The fig 5 depicts that the pH in tank 1 has decreased when compared to the inlet. This means that the water have changed to acidic. This indicates the presence of acido genic bacteria in the tank and also due to the release of acids during the process of digestion of substrate in the tank. At some points the inlet shows less pH values which is due to the changes occurring in the inputs of domestic wastes. After the reduction of pH in the tank 1 an increase in the pH is observed in tank 2 and tank 3. Thus the tanks 2 and 3 are more basic.

Fig 4: Graph showing pH of samples collected

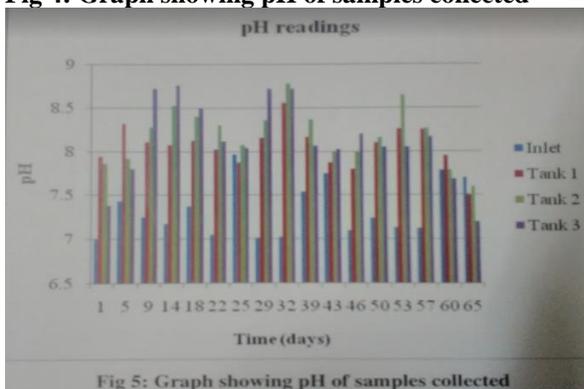
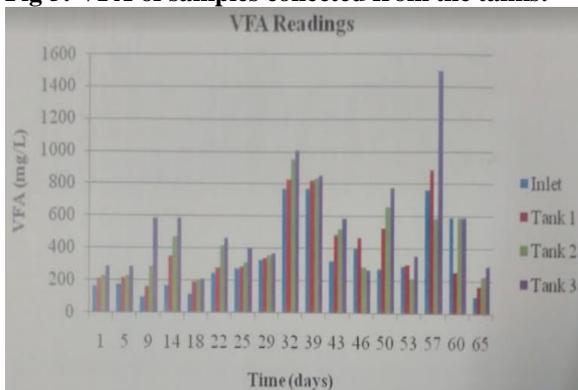


Fig 5: Graph showing pH of samples collected

Volatile Fatty Acids Evaluation (VFA)

The higher concentration of VFA was observed in tank 3 when compared to Inlet, tank 1 and tank 2. The accumulation of high concentration of VFA lowers the voltage production.

Fig 5: VFA of samples collected from the tanks:



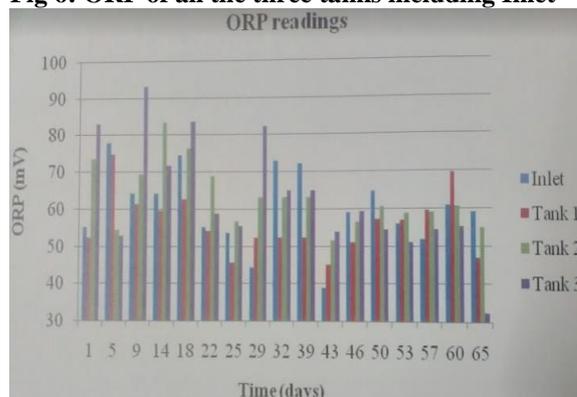
Relatively increased potential differences were observed at lower VFA concentrations. However VFA concentrations might not be influencing potential difference created in the given system as

the voltage generation depends on the number of factors such as mass transfer, Temperature and internal resistance. Maximum VFA was observed only in tank 3.

Oxidation Reduction Potential ORP Evaluation

Negative potentials are developed in water due to the digestion of substrates present in both domestic and HBR outlet by bacteria and microbes. These potentials developed would further vary which would provide the oxidation potential of the tank. From the result analysis it can be concluded that negative ORP is often generated in these tanks.

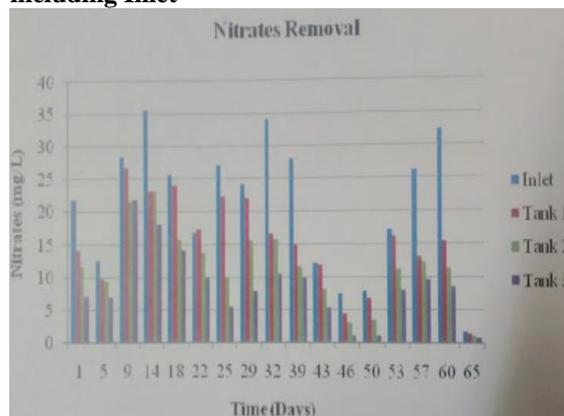
Fig 6: ORP of all the three tanks including Inlet



Nitrates Evaluation

There is an overall reduction in the concentration of nitrates form Tank 1 to 2. The nitrate concentration COD 550mg/Litre inlet is high when compared to COD 100mg/Litre. Added distillery waste to the domestic waste as the domestic waste contains run off from different places. Most of the treatment occurred in tank 1.

Fig 7: Nitrate Concentration in the 3 tanks including Inlet



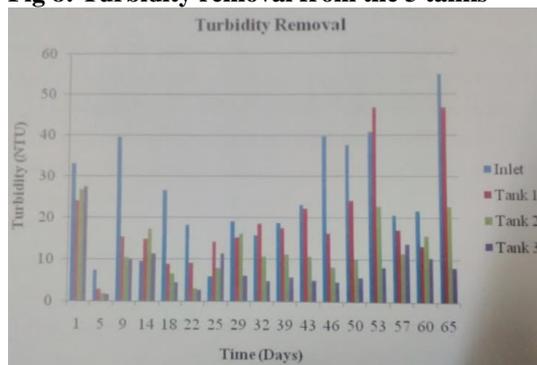
The nitrate concentration in tank 1 is always less than the nitrate concentration in the inlet. Treatment has also occurred in tank 2 and 3. The nitrate levels in tank 2 have decreased in from tank 1. Similarly the concentrations of nitrates have fallen in tank 3. Out of three tanks maximum nitrates removal

occurred in tank 1. This may be due to the presence of eichornia.

Evaluation of Turbidity:

Inlet shows maximum turbidity when compared to tank 1, 2 and 3. There is a gradual decrease in the turbidity from inlet to tank 3. About 60% of turbidity was cleared in tank 1 and 30% in tank 2. The overall system was proved to be efficient in the removal of turbidity.

Fig 8: Turbidity removal from the 3 tanks

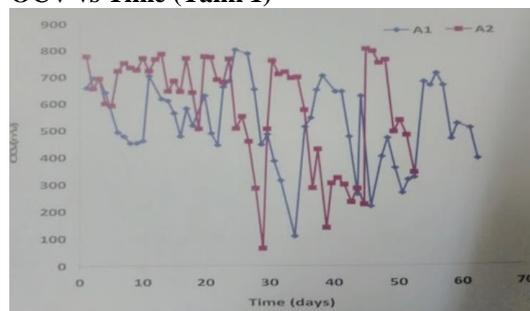


Power Generation and COD removal:

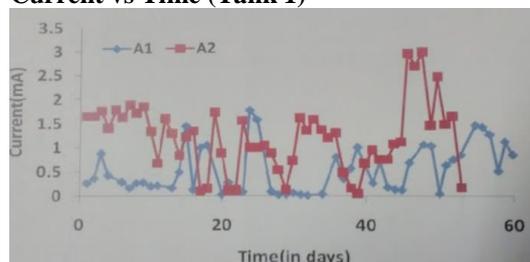
The ecologically designed system was operated under optimal temperature and continuous mode of operation. Domestic waste water from Hussain sagar was fed as the substrate. The components of this miniature ecological system would treat the waste water by diverse functions of the biological activities. Due to the processes like aerobic, anaerobic, anoxic metabolisms the Bacteria generates protons and electrons. The electrodes placed in the system would generate potential gradient and the closure of the circuit causes the flow of electrons which is called the **Electricity**. Initially in the first tank 589mV of voltage was generated. Later the voltage was found to be decreasing as the cell was acclimatizing to the waste water treatment. The maximum voltage registered was 981mV on 37th day of operation where the system was feeding on the domestic waste water supplemented with HBR outlet. During this period the operation COD was recorded to be 86%. At maximum point of power generation the percentage of COD (83%) removal is high. In the second tank the initial voltage generated was 492mV and little improvement was observed in the voltage generation. Maximum voltage was generated on 55th day which was 573mV. In the third tank the maximum potential was observed at 21st day of operation 796mV. Compared to the first and second tank third tank was efficient in generating highest electricity. This system was operated with two types of waste water Domestic waste water and same water supplemented with HBR outlet. When the system was operated under second condition higher voltage was generated with higher substrate

removal. The voltage generation was also found to depend on the strength of organic concentration.

Fig 9a, 9b: Graphs showing Open circuit voltage and current with relation to time in Tank 1 OCV vs Time (Tank 1)

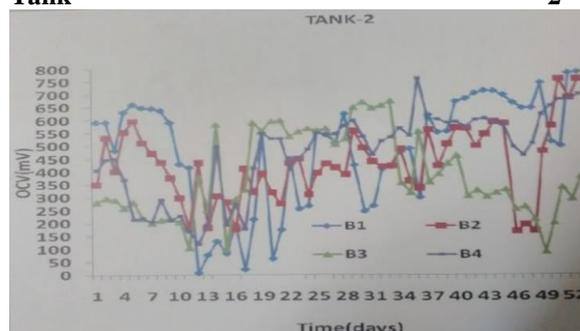


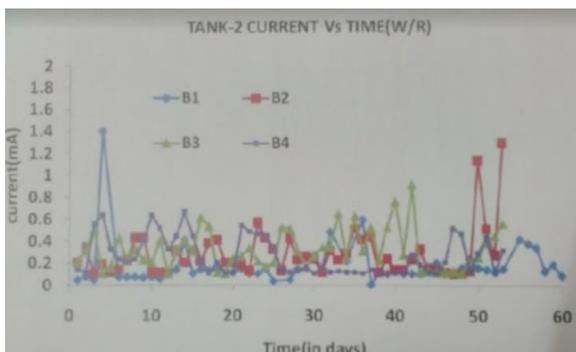
Current vs Time (Tank 1)



When OCV for individual tanks was observed in parallel connection tank 1 and tank 3 showed maximum OCV. In tank 1 maximum OCV was observed on 25th day i.e, 792mV. In tank 2 maximum OCV was observed on 55th day i.e. 753 mV. In tank 3 maximum OCV was observed on 21st day i.e. 794mV.

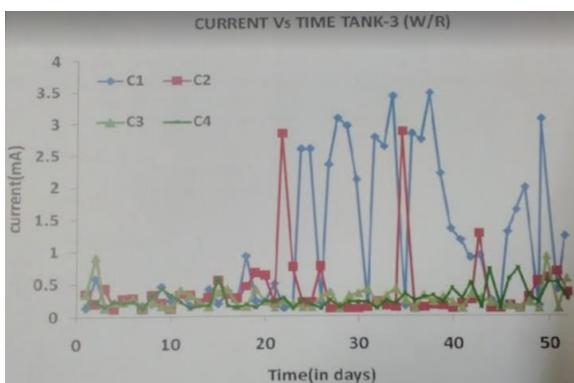
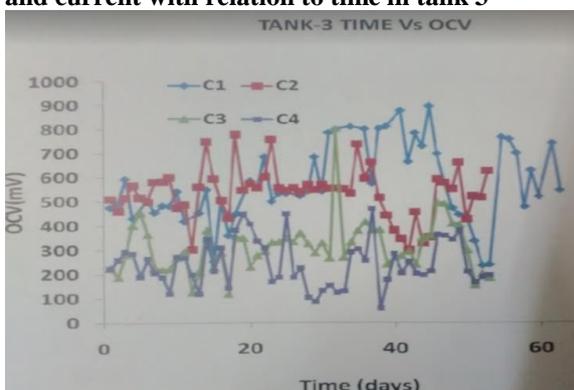
Fig 10a, 10b: Graphs Showing Open circuit voltage and current with relation to Time in Tank 2





Tank 1 containing 2 electrode assemblies A1 and A2 among which A2 showed higher power generation compared to A1. Power generation can be explained in all the three tanks. In tank 1 maximum current was observed on 55th day, 2.89mA (A2). In tank 2 maximum current was observed on 66th day. 1.89mA (B1). In tank 3 maximum current was observed on 45th Day 3.47mA (C1). Among all the three electrodes A2, B1 and C1 are showing maximum current. When individual tanks are compared, tank 3 shows Maximum current. Maximum current in tank 1 was observed on 63rd day (2.93mA). Tank 2 showed maximum current on 24th day (2.44 mA) and tank 3 showing maximum current on 5th day. (3.29mA)

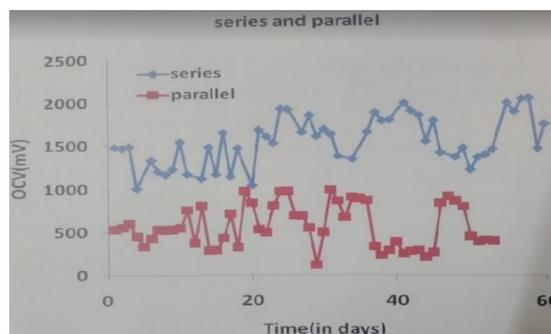
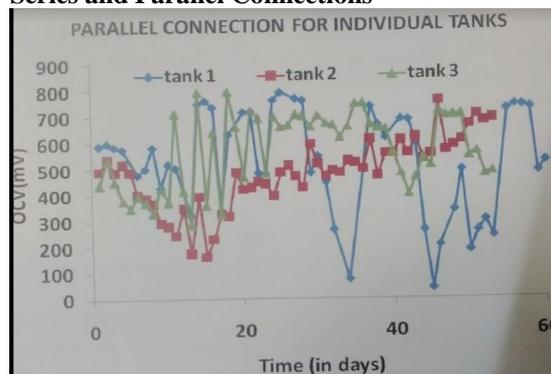
Fig 11a, 11b Graph showing open circuit voltage and current with relation to time in tank 3



When open circuit voltage was compared among series and parallel connections former was showing maximum OCV. Maximum OCV was observed on 58th day (2011mV) and maximum OCV in parallel connection was observed on 37th Day (981 mV).

When current was compared on series and parallel connections the series connection showed maximum current. Maximum current was observed on 62nd day i.e. 3.22 mA in series connection. Maximum current was observed on 45th day i.e. 2.56mA in parallel connection. When current was compared among individual tanks in parallel connection tank 3 was showing maximum current. In tank 1 maximum current was observed on 63rd day i.e. 2.93mA in tank 2 maximum current was observed on 24th day i.e. 2.44mA. In tank 3 maximum current was observed on 57th day i.e. 3.29mA.

Fig 12a 12b Graph showing OCV vs Time in Series and Parallel Connections



Polarization:

Polarization tells about the performance of the reactor in terms of efficient performance of the system and the load capability of the system. Polarization curve was plotted against potential and power density at different resistance (100 Ω to 30K Ω) to visualize current densities with respect to time in concurrence with the experimental variations studied. Under low resistance of the fuel cell circuit allows more electron flow compared to higher resistances and neutralizes the protons present at the cathode. This results in potential drop especially at lower resistances in spite of higher PD. Current generation showed decreasing trend with increase in the resistance which is in concurrence with the literature reported earlier (Venkata Mohan et al., 2008 a) At 30k Ω resistance, less current generation (<1mA) was recorded in both the MFC. Rapid stabilization of voltage was also observed at higher

resistances (30kKΩ). However relatively high potential drop was observed at low resistance. Effective electron discharge was observed at which might be the reason for higher potential drop and slow stabilization of the voltage at lower resistances. Substrate metabolism was observed to be more at lower resistances where microbes donate electrons to the anode which is discharged in closed circuit. At lower resistances the electrons move more easily through the circuit than at higher resistance. Oxidizing the electron carriers released by the microbes. Higher oxidation by microbes is expected with high ration of oxidized electron carriers at low resistance. The point at which maximum PD was observed on the polarization curve against voltage and CD is generally considered as cell design point of that particular fuel cell system. Effective performance with respect to power output can be obtained on the right side of the cell design point. When total EES system was considered as a single fuel cell by connecting the tanks in the series, maximum power density of 180Mw/m³ was observed. Whereas each tank was considered maximum power density and current densities were registered with tank 2 (128mW/m², 590 mA/m²) Compared to tank 1 (76 Mw.m²; 400mA/m²) and Tank 3 (34Mw/m²; 280mA/m²).

Conclusion:

Miniature ecological system was found to be an economical mode of treatment for low concentrated and fermented waste waters. The novel concept of designed system mitigates the natural ecosystem by using natural biological components in the different units of treatment processes. The study was performed with different living components like floating plants (Eichornia) Submerged plants (Hydrilla), Duck Weed, Snails and Fishes. This research documented the effective treatment of waste water Mainly by Eichornia in first stage, submerged plants in second stage and fish, duck weed and snails in the third stage of the process. The electrons accommodated in three tanks were able to generate bio electricity. Experiments documented the simultaneous potential of designed system to generate bio electricity apart from the treatment. Among the three tanks tank 1 and 3 showed higher power generation when compared to tank 2. On connecting the three tanks in series the current was found to improve. When electrodes were connected in parallel the voltage was further improved significantly.

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