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EFFECT OF ELECTRODES, AERATION, SALT BRIDGES AND SOURCE OF MICROBES IN A MEDIATOR-FREE DOUBLE CHAMBERED MICROBIAL FUEL CELL

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ABSTRACT

The potential of generating bioelectricity in a mediator-free double chambered microbial fuel cell is a dependant variable of the independent parameters stipulated in the cell construction mechanism. The effect of these stipulated independent variables as source of microbial colony, material of construction of electrodes and salt bridges, presence and absence of aeration was evaluated in the cell to have the best efficacy. The efficiency of the fuel cell was studied on the basis of the electricity generated via treatment of waste water and manure used as biowaste. The experiments showed that electrode of more conductive material provided more efficiency. Aluminium foil and carbon cathode were used to be compared which gives 25.1 μA current with 19% COD removal and 185.8 μA current with 53% COD removal respectively. Experiments reflected that microorganisms in a solid or semi solid source were less mobile than in the liquid source as in manure with respect to waste water and so the latter showed more efficiency than manure by giving maximum 185.8 μA current in waste water and maximum 28.9 μA current in manure for same setup. The efficiency fluctuated when we used agar salt bridge instead of NaCl salt bridge from 185.8 μA to 112.2 μA with 30% COD removal. When the aeration process was stopped in cathode chamber the setup of having the maximum amount of current reduced to an amount of 181.6 μA with 49% COD removal. The aeration in cathodic chamber was important to maintain the preferable pH for the microorganisms while the anodic chamber was to be anaerobic to sustain the biofilm to maintain the production of electrons in an efficient way.

KEYWORDS: Microbial Fuel Cell; Bioelectricity; Microbial colony, Aeration, Electrode, Salt bridges

1 INTRODUCTION

The world is enchained by the crisis of fuel energy which triggers the global warming and environmental instability. Alternative fuel source is needed to be emerged that will be amicable to environment. Renewable energy source as alternative fuel is to be harnessed as a solution to have the equilibrium in climate and drop down the level of existing sources. Production of electrical energy using microorganisms through microbial fuel cells (MFC) is one such renewable and sustainable technology that is considered to be one of the most promising, efficient and carbon neutral energy sources. Microbial fuel cells harness the power of bacterial respiration and convert energy released in metabolic reactions into electrical energy. The fact that bacteria can directly supply electrons or oxidize the substrates to produce electricity makes MFCs an ideal system for wastewater treatment and electricity production simultaneously. The specialty behind MFCs can be focused down to two words: cellular respiration. Cellular respiration is a collection of metabolic reactions that cells use to convert nutrients into adenosine triphosphate (ATP) which fuels cellular activity. Electrons are accepted by nicotinamide adenine dinucleotide (NADH) [1]. The protons generated are diffused through the salt bridge or any membrane to the cathode. Whenever there are moving electrons, the potential exists for harnessing an electromotive force to perform useful work. Bacterial respiration is basically a redox reaction in which electrons are being moved around. This bacteria had the ability to respire directly into the electrode under certain conditions by using the anode as an electron acceptor as part of its normal metabolic process or they can use mediator. The most promising MFCs for

commercialization in today's energy industry are mediatorless [2]. MFCs which use a special type of microorganism termed exoelectrogens. Exoelectrogens are more than vivacious to breakdown and metabolize the carbon rich sewage of a waste water stream to produce electrons that can stream into a cheap conductive carbon anode. The electricity generated from the MFC also offsets the energy cost of operating the plant. Bacteria that can transfer electrons directly and extracellularly, are called exoelectrogens. The direct communication of exoelectrogens like *Geobacter* species that are capable of oxidizing organic compounds and their efficiency in transferring electrons to electrodes creating biofilms [3],[4] via highly conductive filaments were considered remarkable in MFC research Mixed bacterial cultures can produce power densities equal to pure cultures and gradual increases in power densities accelerated the research interest on MFCs. As an additional fact, the bacteria metabolize a lot of the sludge normally present in waste water. MFCs don't only have to be used for power generation; MFCs can also be used as a convenient biosensor for waste water streams. Wastewater is evaluated based on the amount of dissolved oxygen required by aerobic bacteria to break down the organic contaminants present in a body of water. These waste streams are clearly richer in energy [5]. Microbial fuel cells appear promising for wastewater treatment and metal recovery by bioelectrocatalysis because metal ions can be reduced and deposited by bacteria, algae, yeasts, and fungi. Interestingly, treatment of heavy metal-containing wastewater can be attempted in both anode and cathode chambers of microbial fuel cells [6].

2 EXPERIMENTAL WORK

2.1 Experimental Design

In this experiment a mediatorless two chamber MFC is used. The chamber with anode in the experimental MFC was anaerobic. So, this is necessary for the anodic chamber to be air tight and anaerobic to get feasible results. The anode is a graphite electrode or aluminium foil electrode having about a dimension of 5 cm x 2.5 cm x 0.5 cm. Waste water from food industry was used as anolyte. In few experiments glucose (15g/L)[7] used as nutrients in anode chamber for the microorganisms. At the cathodic half-cell, the cathode is merged into the cathodic solution which contains oxidizing agent supplied through the salt bridge as hydrogen ion to produce water in the aerobic condition. The cathode is a graphite electrode having about a dimension of 5 cm x 2.5 cm x 0.5 cm. Both of the chambers were drilled about an inch at the same height for the salt bridge. Opening of the both side was covered with cotton or sealed with araldite. The inside of the pipe was filled with agar paste (china grass) or saturated NaCl solution. This solution worked as the salt bridge that acts as the second connection between two chambers. The electrodes were positioned at a distance of 3 cm on either side of the salt bridge. All experiments were conducted at least in duplicate, in a constant temperature room (30 ± 2 °C), and the average value was reported for all data. The pipe of the salt bridge was 10 cm. Two identical plastic bottle with same volume and height were used as anodic and cathodic chamber. We have used 500mL bottles in experiments of waste water. The chambers were covered with lids. The lid of the cathodic chamber had two holes. One was for aeration and another was for electrode connection. The lid of the anodic chamber had only one hole for electrode connection.



Figure 1: Set-up
one of the

sample.

of Microbial Fuel Cell for
experiments of wastewater

2.2 Function of cell

2.2.1 Electricity generation

The electron generated by the bacterial metabolism passes through the system to produce the electrical energy. The cell was bolstered by a multimeter to calculate and collect the data of current and voltage generated by means of bacterial cell metabolism.

2.2.2 COD removal

In environmental chemistry, the chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water (e.g. lakes and rivers) or wastewater, making COD a useful measure of water quality. It is expressed in milligrams per litre (mg/L), which indicates the mass of oxygen consumed per litre of solution. In this study, the COD was measured by maintaining the sequential steps:

1. 2 mL potable water was collected in a blank COD tube & then the tube was to be filled with 3 mL H₂SO₄ and 4 mL digestion solution.
2. Then the other COD tube of 2 mL raw sample was filled with 3 mL H₂SO₄ and 4 mL digestion solution.
3. The third COD tube of 2 mL treated sample was then filled with 3 mL H₂SO₄ and 4 mL digestion solution and collected for observation.
4. The three tubes were then heated up to 150 degree celcius for 2 hours in HACH reactor .
5. The tubes were then kept to reach at normal temperature.
6. Lastly, HACH machine (DR/4000U Spectrophotometer) at the automatic determination (2720) was used to read the value of COD of each sample with respect to the blank sample (calibrated as 0 mg/L).

2.3.1 Sample Calculation for Experiment 5 for manure sample

Blank Sample = 0 mg/L

Raw waste sample = 123 mg/L

Treated (in MFC anode) waste sample = 104.5 mg /L

Multiplying factor = 2.0929

%COD removal = $(123 \times 2.0929 - 104.5 \times 2.0929) \times 100\% / (123 \times 2.0929) = 15\%$

2.3.2 Sample Calculation for Experiment 7 for waste water sample

Blank Sample = 0 mg/L

Raw waste sample = 75 mg/L

Treated (in MFC anode) waste sample = 53 mg /L

Multiplying factor = 2.0929

%COD removal = $(75 \times 2.0929 - 53 \times 2.0929) \times 100\% / (75 \times 2.0929) = 29.33\% \cong 30\%$

2.4 Parameters Varied in Experiment for Electricity generation & COD removal

The aim of this experiment is the simultaneous electricity generation & removal of COD. Higher the current passes through the multimeter, higher is the COD removal. To improve the COD removal, experiments were run on several parameters. The parameters considered in this experiment were:

▪ **Material of Electrodes:**

The material of electrodes (e.g.: Aluminium foil, Graphite) was considered to compare the electricity generation and percentage of COD removal in order to observe the effect on the mediator-free double chambered MFC.

▪ **Aeration:**

The effect of the presence and absence of aeration in the cell was observed to collect the data of electricity generation and percentage of COD removal in order to take the respective decision. The aeration was maintained by using an aquarium water pump.

▪ **Salt Bridge:**

The salt bridge was one of the vital parameters of this study where the type of chemical reagents e.g.: NaCl salt bridge, agar salt bridge in salt bridges, along with the ion exchange efficiency were considered.

▪ **Source of Microbes:**

The source of microbes was another parameter to drive the study with the respective experiments. In the experiments, manure from a plant nursery and wastewater sample from a food industry were collected as two different sources of microbes.

3 RESULTS AND DISCUSSIONS

Table 1: Experimental Cases of treating waste water sample in MFC

Experiment number	Anode (Waste Water)	Cathode (Potable Water)	Aeration	Salt bridge	Nutrients	COD Removal %
01	Graphite electrode		Yes		Not added	16
02			No	NaCl solution		12
03	Aluminium foil		Yes			45
04			No			42
05	Graphite electrode	Graphite electrode	Yes	Agar paste		13
06			No			10
07	Aluminium foil		Yes			30
08			No			27
09	Graphite electrode		Yes	NaCl solution	added	19
10			No			15
11	Aluminium foil		Yes			53

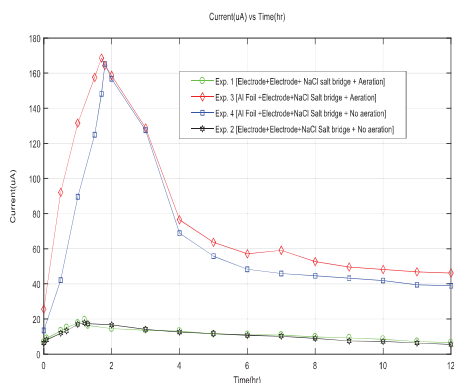


Figure 2: Current (μA) vs Time(hr) for graphite electrode + graphite electrode/Al foil + NaCl salt bridge + with and without aeration combination in waste water treatment in absence of nutrients.

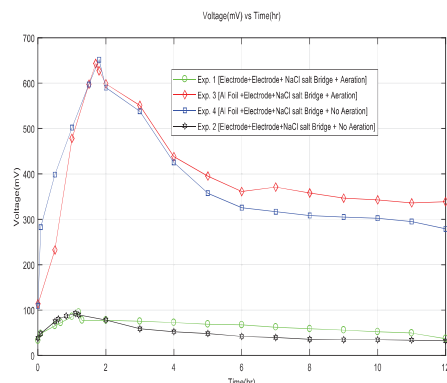


Figure 3: Voltage (mV) vs Time (hr) for graphite electrode + graphite electrode/Al foil + NaCl salt bridge + with and without aeration combination in waste water treatment in absence of nutrients.

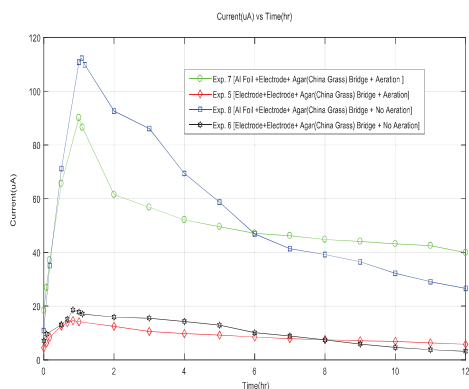


Figure 4: Current (μA) vs Time (hr) for graphite electrode + graphite electrode/Al foil + Agar salt bridge + with and without aeration combination in waste water treatment in absence of nutrients.

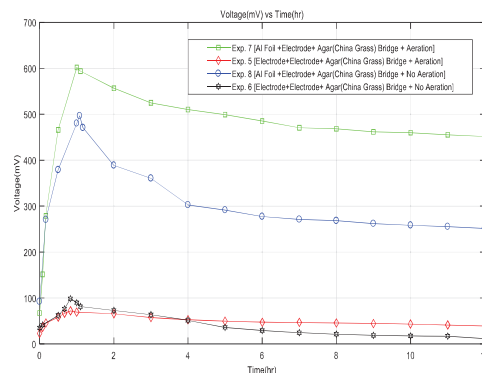


Figure 5: Voltage (mV) vs Time (hr) for graphite electrode + graphite electrode/Al foil + Agar salt bridge + with and without aeration combination in waste water treatment in absence of nutrients.

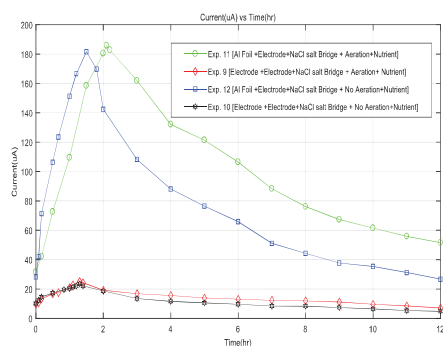


Figure 6: Current (μA) vs Time (hr) for graphite electrode+graphite electrode/Al foil + NaCl salt bridge + with and without aeration combination in waste water treatment in presence of nutrients.

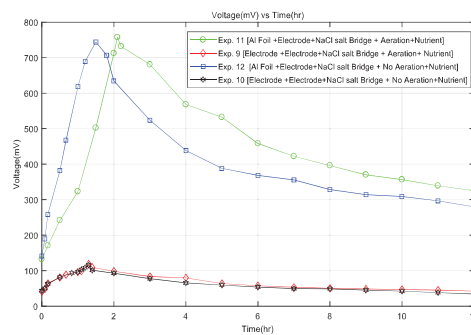


Figure 7: Voltage (mV) vs Time (hr) for graphite Electrode + graphite Electrode/Al foil + NaCl salt bridge + with and without aeration combination in waste water treatment in presence of nutrients.

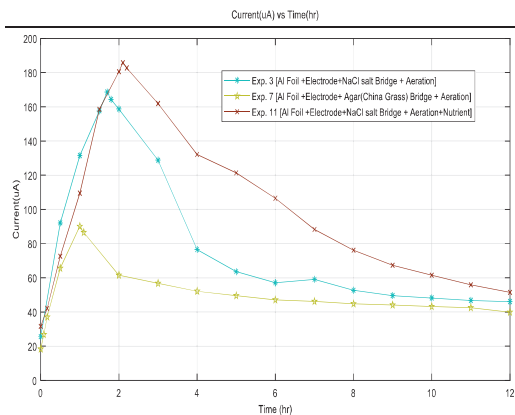


Figure 8: Current(μA) vs time (hr)graph for comparison of the best three combination in waste water treatment

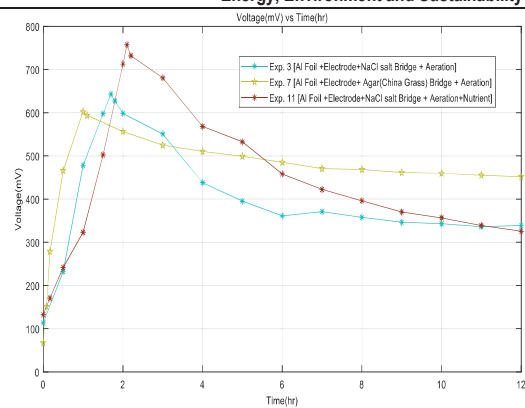


Figure 9: Voltage (mV) vs Current(μA) graph for comparison of the best three combination in waste water treatment

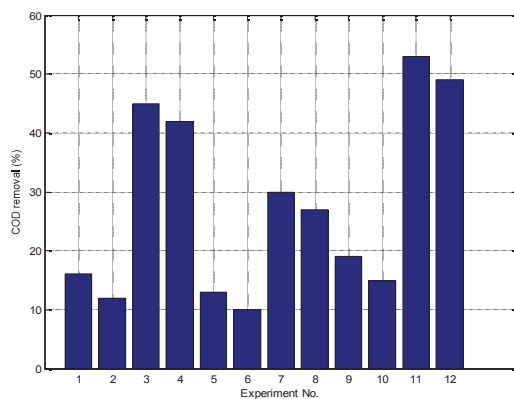


Figure 10: COD removal (%) comparison for the 12 combinations of waste water treatment.

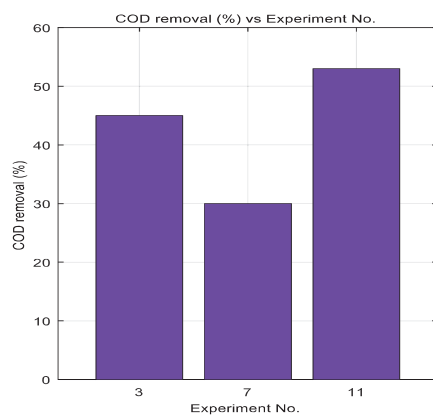


Figure 11: COD removal (%) comparison for the best 3 combination of waste water treatment

Table 2: Experimental Cases of treating manure in MFC

Experiment number	Anode (Manure)	Dilution of Manure %	Cathode (Potable Water)	Aeration	Salt bridge	Nutrients	COD Removal %
01	Graphite electrode	Dry	Graphite electrode	No	Agar Paste	No	2.5
02		Dry		Yes	Agar Paste	No	4
03		25		Yes	Agar Paste	No	6
04		55		Yes	Agar Paste	No	9
05		60		Yes	NaCl Solution	No	15
06		60		Yes	NaCl Solution	Yes	NaCl Solution

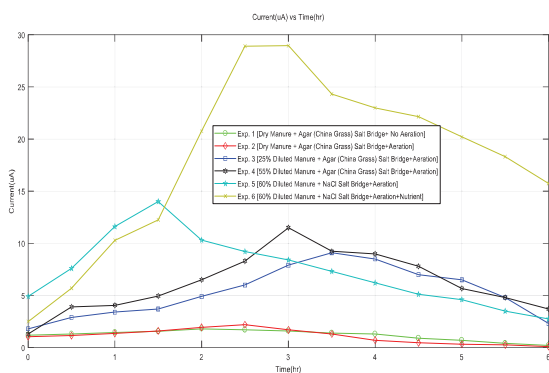


Figure 12: Current(μ A) vs Time (hr) graph for 6 combinations of manure sample in MFC

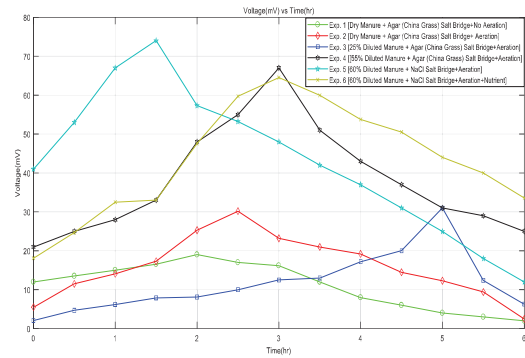


Figure 13: Voltage (mV) vs Time (hr) graph for for 6 combinations of manure sample in MFC

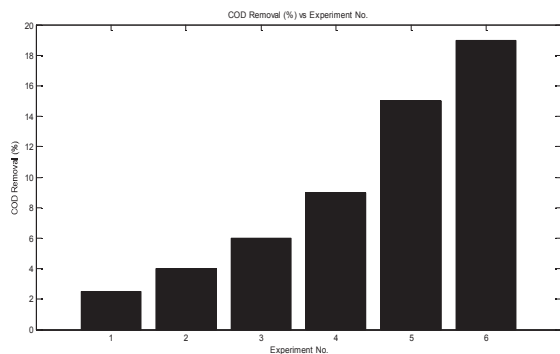


Figure 14: : COD removal (%) comparison graph for the 6 combinations of manure sample in MFC

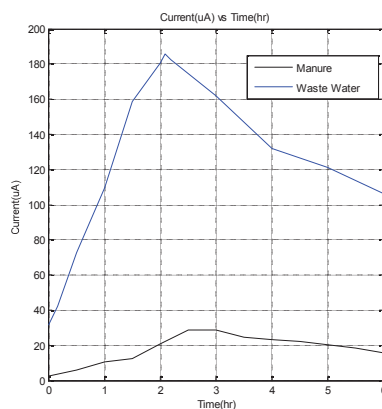


Figure 15: Comparison of bioelectricity generation between waste water and manure in MFC (same set-up, different anolyte)

From Figure 2 and 3 for experiment 1 to 4 where the NaCl salt bridge is used with graphite electrode and aluminium foil electrode in presence and absence of aeration in cathode. We can see in experiment 1 and 2 we have graphite electrode as anode and cathode and because of absence of aeration in cathode the experiment 2 has given less current than 1 with less COD removal. Aeration in cathode harnessing the reduction reaction by increasing the dissolved oxygen in catholyte. For experiment 3 and 4 Al foil is used as anode instead of graphite electrode and the current has been then increased than experiment 1 and 2 because of the presence of Aluminium electrode and ofcourse as earlier for Al electrode the aeration has given more current than absence of aeration. The current was recorded best at experiment 3 with 45 % COD removal it was 168.6 μA with 643.2 mV in the cell combination of Al foil anode, graphite cathode and NaCl salt bridge with aeration.

From Figure 4 and 5 the Agar (china grass) paste is used instead of NaCl salt solution in salt bridge. We can see in experiments 5, 6, 7 and 8 have the same effect as in the experiment 1 to 4 except they have less generation of current only because of the agar salt bridge instead of NaCl salt bridge having all other conditions unchanged. Figure 6 and 7 show in experiment 9 and 10 we have graphite electrode as anode and cathode and because of absence of aeration in cathode the experiment 9 has given less current than 10 with less COD removal. Aeration in cathode harnessing the reduction reaction by increasing the dissolved oxygen in catholyte as mentioned earlier.

For experiment 11 and 12 Al foil is used as anode instead of graphite electrode and the current has been then increased than experiment 9 and 10 because of the presence of Aluminium electrode and ofcourse as earlier for Al electrode the aeration has given more current than absence of aeration. The current was recorded best at experiment 11 with 53 % COD removal it was 185.8 μA with 757.4 mV in the cell combination of Al foil anode, graphite cathode and NaCl salt bridge with aeration having nutrient in all 4 experiments from 9 to 12 and it has been seen that this combination of experiment 9 to 12 has given more current than the same combination except nutrient in experiment 1, 2, 3 and 4. Then nutrients work on the microorganism's growth and make them more sustained in hours in their stationary phase than the experimental run without nutrients and with the growth they give more current for more time than other runs absence of nutrients. Figure 8 and 9 show the comparison among the best three combination in waste water treatment via the values of current and voltage generated with respect to time.

Now, from Figure 10 we have an overall idea of COD removal percentage for the 12 experimental run each of which had been run for 12 hours & experiment 11 has given the best value of 53% COD removal. According to the pattern of the graphs shown, the increasing voltage produced is due to the higher rate required for the microorganism to form the biofilm on the surface of the electrode. After the formation of the biofilm on the surface of the electrode, the rate of electric voltage decreases because the competition occurs between the microorganisms to obtain their food from the organic matter and nutrients in the media. This happens simultaneously while electrogens are on the biofilms and competition in the media. This phenomenon affects the rate of electric voltage production by the microorganisms. Thus, the rate of electric voltage produced becomes lower compared with the beginning of the MFC operation in which the formation of biofilm occurs. Figure 11 shows the COD removal (%) comparison for the best 3 combination of the treatment of wastewater.

We can have data for the experimental cases of treating manure in MFC in the table 2 above and from Figure 12 and 13 for experiment 1 to 6 where the dry manure has given the lowest current and voltage as recorded 1.80 μA & 19.0 mV while aeration can ascend the value a bit but with aeration if the dry manure can be diluted simultaneously with water. The increase in dilution makes the current increased providing the higher percentage removal of COD respectively. Experiment 6 has been run with 60% diluted manure providing NaCl salt bridge, aeration, and nutrient. It has given 19% COD removal with 28.9 μA current generation where NaCl salt bridge has been used with graphite electrode both in anode and cathode. Moreover, it has given 9% COD removal with 11.5 μA current generation where Agar salt bridge has been used instead of NaCl salt bridge in the same set-up of the cell. In the results, we can see that the NaCl salt bridge has given better removal of COD with current generation than the Agar salt bridge and in both salt bridge more the manure diluted more current is generated. When nutrient has been used then experiment

6 with the same dilution percentage of experiment 5 (where nutrient is absent) has given higher percentage removal of COD along with higher current generation has been increased than the absence of nutrient.

Figure 14 is capable of giving a comparison on COD removal percentage of the 6 experiments run for 6 hours in the MFC to treat manure and we can get that experiment 6 gives the best result. So the more the manure diluted in presence of aeration and nutrient along with NaCl salt bridge the more current generated with more COD removal occurred. At last, the Figure 15 has given a comparison between waste water and manure as anolyte and we can see that the waste water as liquid media has given more current and COD removal than solid or diluted manure in the same set-up because of the conductivity of the media and mobility of the microorganisms.

3.1 Impact of aluminium foil as anode

The main focus was on the aluminium foil; as anode on the total experiments. Aluminium can react with water but aluminium foil has protective coating on it and hence it doesn't react with water. So, this foil has been used as anode for its high conductivity as well as availability in comparison with the conductivity of carbon or graphite electrode.

3.2 Impact of salt bridge

Liquid phase saturated sodium chloride solution aids better than the semi-solid agar paste. As the comparison shown earlier that agar salt bridge is less conductive for proton than NaCl salt bridge because of its ion exchange efficiency with the better conductivity than the semi-solid agar paste.

3.3 Impact of aeration

Anodic solution must be air tight. If there exists external oxygen, the organic substances will produce CO₂ instead of electron. Moreover aeration in anodic chamber hinders the formation of biofilm [8]. Aeration is important for the cathodic chamber [9]. Proper aeration supplies oxygen that combines with the proton and electron to produce water to maintain the pH of the solution. The preferable pH range for bacteria is 6.8 to 8. If aeration isn't proper or absent, the solution will become acidic gradually which isn't preferable condition for bacteria and voltage drops quickly. As comparisons in the experiments runs mentioned above it is clear aeration gives more current than the absence of aeration.

3.4 Impact of nutrients

The more the sustainability of microorganisms in their stationary phase, higher is the removal of COD. To visualize the impact of nutrients, we can compare experiment 3 and 11 in case of wastewater treatment. Both of them are identical in setup except the presence and absence of nutrient. In addition of nutrient the electricity generation along with the percentage of COD removal have been increased.

3.5 Recommendation on distilled water as cathodic solution

Distilled water can be used as cathodic solution. But for the industrial purpose, preparation of huge amount of distilled water is tough. So, normal potable water is recommended as cathodic solution which is cheap and feasible.

4 CONCLUSION

MFC has been emerged as a promising yet challenging technology to extract energy from different sources and turn them into electricity with sustainability. The major challenge in the application of MFCs is its low current and voltage output i.e. low power density with negligible sustainability. In this study the

parameters as material of construction of electrodes, different electrolytes, varying the surface area of electrodes, varying the rate of aeration in cathodic chamber, type of nutrients, applying different types of salt bridge can be studied and researched more to get feasible values to have a viable amount of energy as current and to treat the waste water from industries and other sources simultaneously in a cost effective way in comparison with other technologies. Major advantages of energy produced from wastewater are the absence of environmental emissions, simultaneous recovery of energy and wastewater treatment. MFC (Microbial Fuel Cell) is an ascending technology which can have scopes to contribute in a great extent as a worth considering greener solution for energy of this world which can simultaneously deal with sustainable energy production and wastewater treatment in a very efficient way. Despite the rapid progress, there are some areas in which further research needs to be done to overcome the constraints associated with MFC.

5 ACKNOWLEDGEMENT

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