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Microbial Fuel Cells as Potential Power Generator

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Abstract : *The present research was done to establish waste-based microbial fuel cells as an efficient source of power generation. Two chambered cells were used. Methylene blue was used as the mediator. Potassium Ferricyanide was used as enhancer. L-cystiene was used as a scavenger of dissolved oxygen in the anodic chamber. The use of phosphate buffer in both the chambers and agar-KCl salt bridge in the Ananus comosus waste based cell of capacity 1000ml generated a potential upto 0.4V while using simple tap water instead of a buffer and a rope salt bridge with the same sample of same cell capacity gave a potential of just 0.25 V. It was found that the potential produced by a larger volume of cells (i.e.1000ml) was approximately 0.1-0.4 V as compared to the smaller volume of cells (i.e.200ml) with a potential of approx. 0.01-0.2 V, without any addition of microbial culture.This illustrated a clear relationship between the capacity of the cell and the potential generated by it. Addition of anaerobic isolates to the different fruit waste based cells showed a favourable effect on the efficiency of the cells. However, the effect varied with varying time duration. Among different samples used, which included waste generated out of Ananas comosus, Citrus limetta, Saccharum officinarum ; rice field soil and muck ; Ananas comosus proved to be the best sample to be used as substrate. The results demonstrate that a usable amount of bioelectricity can be generated by proper optimization of fruit waste based microbial fuel cells, thus providing a new dimension to sustainable electricity generation.*

Keywords: *Bioelectricity, salt bridge, phosphate buffer, microbial fuel cell, dissolved oxygen, L-cysteine.*

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Introduction :

Microbial Fuel Cells are a device that use microorganisms as catalyst to oxidize organic and inorganic matter and convert chemical energy into electrical energy. MFCs works on a similar principle of electrochemical cell. Bacteria can be used in MFCs to generate electricity while accomplishing the biodegradation of organic matters or wastes as studied by Kim *et al.* (2003); Logan *et al.* (2006a). Microorganisms being omnipresent. Therefore using Microorganisms as catalyst makes MFCs cost efficient as compared to other electrochemical cells. MFCs technology can potentially be applied to enhance subsurface bioremediation of contaminants by providing an inexhaustible source of terminal electron acceptors to a groundwater environment that is likely depleted in thermodynamically favorable electron acceptors. This was demonstrated by Morris *et al.* (2007).The earliest MFC concept was demonstrated by Potter (1911). The basic principle of the MFCs is that the electrons and protons produced during the oxidation(in absence or low partial pressure of oxygen) by the concerned enzymes possessed by the microorganisms, are used to generate voltage which ultimately when connected to an external resistor or load generates power.

Anodic reaction:

$$CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$$

Cathodic reaction:

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$$

The power can be calculated by: P = V*I. The internal resistance and potential loss can be calculated by: V=E –Ir ; Ir is the potential loss due to internal resistance, E= E.M.F of the cell, V= Net Potential generated, I =Current, r = Internal Resistance. The cells can be either two chambered or one chambered. In the two chambered electrochemical cell, the anodic and cathodic chamber is connected through a salt bridge. In one chambered cell, Proton Exchange membrane does the work of the salt bridge as stated by Logan *et al.* (2006b). MFCs can be divided into types based on construction and presence of mediators. On the basis of mediators, the first type is mediator free. MFC in which bacteria that can transfer electrons extracellularly, are called exoelectrogens. Kim *et al.*(1999). MFCs containing exoelectrogens do not require mediator and are therefore known as "mediator less MFCs". The other type is that in which mediators are used. In case of non-exoelectrogenic microbes, exogenic chemicals (mediators) such as Methylene Blue, Neutral Red, Natural Red etc. are essential for transferring the electrons from out of the cell walls of the microbes. Optimization of the MFCs is essential for studying their efficiency. The chemicals like Potassium ferricyanide in cathodic chamber increase the conduction. The use of carbon electrodes instead of metal electrodes reduces the ohmic loses. The conditioning of carbon electrodes before use, by 0.1 M sulphuric acid is important for increasing the conduction. The use of buffer in cathodes is also an important way of increasing the efficiency. The anodic chamber must be kept anaerobic and for this L-cysteine is used which is an oxygen scavenger, Min *et al.* (2005). Sparging nitrogen gas is also a way to ensure complete removal of dissolved oxygen in anodic chamber. The mediators used should be non-toxic to the microbial fauna of the anodic substrate. Potential of the cell can be increased by connecting more than one cell in series connection, as by connecting cells in series the total voltage is increased whereas capacity of each remains the same.

Materials and Methods :

MFC Set-up Components : Anodic chamber, cathodic chamber, salt bridge, graphite electrodes, potassium phosphate buffer, mediators: methylene blue, potassium ferricyanide, waste samples: (*Ananas comosus, Saccharum officinarum, Citrus limetta*, muck, rice field soil.

Method : Two chambered MFCs were constructed in this project

Anodic Chamber : Sample (substrate), cysteine, dextrose, methylene blue were weighed according to the decided volume of the cell set-up. Sterilised distilled water measured according to the volume of the cell set up was added to the above mentioned components. All these were then transferred to the anodic chamber. Anode was sealed properly to block the passage of air (Logan *et al.*(2006b).**Cathodic Chamber:** Methylene blue, potassium ferricyanide were weighed according to the volume of the cell set up. These were then added to the measured volume of tap water and added to the cathodic chamber. In case of buffer, weighed amount of

Methylene blue and potassium ferricyanide were added to the measured volume (according to the volume of cell set up) of 0.1 M phosphate buffer (Potter, 1910). Then, the solution was transferred to the cathodic chamber. Proper passage of air in cathode was ensured. **Electrodes** : Electrodes used in the MFC set-up were dipped in 1 N sulphuric acid for 24 hours. **Salt Bridge : Case A**: Rope dipped in super-saturated solution of NaCl, **Case B**: Agar salt bridge was used (Lovely, 2006; Oh *et al.* 2004).

Screening of the Samples

Initially waste samples i.e. *Ananas comosus* (pineapple peel), *Saccharum officinarum* (sugarcane waste), *Citrus limetta* (mausambi waste) muck and rice field soil were collected and used in different MFC set-ups. Set-ups were done accordingly: **CASE A:** Set-up was made using containers of 1000 ml capacity. Buffer as well as external microbes were not added.Salt bridgewas prepared using arope boiled with super saturated NaCl solution.Amount of the sampleused was 500g. *Ananas comosus ,* muck and rice field soil were used in this case. **CASE B:** Set-up was made using containers of 1000 ml capacity. Buffer was added with no external addition of microbes. Agar salt bridge was prepared using agar and KCl.Amount of the sampleused was 500g. *Ananas comosus*, *Saccharum officinarum*, *Citrus limetta* and mixed samples (combination of the three fruit wastes) were used in this case.

On comparison, fruit wastes gave better results over muck and rice field soil and so further work was done by selecting the fruit waste samples.

Comparison of Different Capacity Cell Set-ups

For comparing the efficiency of cells according to their capacity, set ups were done according to the following condition. **CASE C:** Set-up was made using containers of 200 ml capacity. Buffer was added with no external addition of microbes. Agar salt bridge was prepared using agar and KCl. Amount of the sampleused was 100g. *Ananas comosus, Saccharum officinarum*, *Citrus limetta* were used in this case.

Positve Control : Positive control was performed in order to check whether the amount of the sample affects the generation of potential or not. For the positive control 3 set-ups were assembled which were as follows:-Glucose along with yeast in addition to methylene blue was mixed in the anode.Lemon juice along with the methylene blue was taken in the cathode.Acetic acid was taken in the anode along with the methylene blue.In all the above three cases, tap water was taken in the cathode along with potassium ferricyanide.

Negative Control : Glucose without yeast: Glucose with addition of methylene blue taken in the anode. Tap water was taken in the cathode and potassium ferricyanide and methylene blue were added to it.

Hydrogen ion Concentration (pH) : Hydrogen ion concentration of different fruit samples were calculated with the help of pH meter. Two pH readings were taken for each sample i.e. one with fresh sample and other with the fermented sample taken from the anodic chamber. This was done to check the acid production.

Isolation of Anaerobic Microbes : Nutrient agar media along with cysteine was used . Inoculation was done using 10^{-3} dilution. Incubation was done at 37 degree celsius using the candle jar method to ensure anaerobic condition.

Addition of Microbes to the Cells : Mass cultivation was done of the anaerobic isolates after performing the biochemical tests. The mass cultivated culture was then added to the MFCs of volume 200 ml. Amount of inoculum added to each cell set up was 50 ml.

The data obtained from all the above cases was recorded and studied properly. The next step was to generate current and power.

Power Generation : A series connection between the anodes and the cathodes were made in order to obtain a potential difference high enough to generate enough current and hence power to light the LED. Anode of the first set-up was connected with the cathode of the second set-up and so on. Readings of the potential and current generated were thus recorded with the help of a multimeter. To generate power using the property of fermentation, different wastes were taken.

Result and Discussion :

To generate power using the property of fermentation, different wastes were taken. The following results were obtained based on the observations.

Screening of Samples :

Case A: Set-up was made using containers of 1000 ml capacity. Buffer as well as external microbes were not added.Salt bridge was prepared using a rope boiled with super saturated NaCl solution. Amount of the sample used was 500g.*Ananas comosus,* muck, rice field soil were used as samples in this case. The results are displayed in Fig 1, 2 and 3 respectively. According to the study done by Jang *et al.* (2004), electricity could be generated from sewage sludge by using it in MFCs. However, in this study, it was observed that muck showed a very inconsistent behavior in potential generation.

Case B: Set-up was made using containers of 1000 ml capacity. Buffer was added with no external addition of microbes. Agar salt bridge was prepared using agar and KCl. Amount of the sample used was 500g.*Ananas comosus, Saccharum officinarum, Citrus limetta*and mixed samples (a mixture of the above fruit waste samples) were usedin this case. The results are given in fig 4, 5,6 and 7 respectively.According tothe study done by (Khan *et al.* 2015), citrus fruits produced best results of power generation through MFC technique. In this study, *Ananas comosus* provided the best result of a potential upto 4V.

Case C: Set-up was made using containers of 200 ml capacity. Buffer was added with no external addition of microbes. Agar salt bridge was prepared using agar and KCl.Amount of the sample used was 100g.*Ananas comosus, Saccharum officinarum and Citrus limetta.*were usedin this case.The results are given in fig. 8, 9 and 10 respectively.A clear relation between the cell capacity and potential generation was observed.

Hydrogen Ion Concentration (pH) : Two pH readings were taken for each sample i.e. one with fresh sample and the other with the fermented sample that was taken from the anodic chamber. The pH of *Saccharum officinarum, Ananas comosus and Citrus limetta*are given in the table 1. The drop in pH indicated the production of acid.

Isolation of Microbes and Biochemical Analysis : Slide preparation was done and viewed (table 2). The biochemical tests were done to confirm the isolation of anaerobic colonies from the different samples. On the basis of biochemical tests,most probably the colonies that were isolated were anaerobic and were responsible for efficiency of the MFCs. These isolates were used to study their effect separately on the same sample. (Table 3 and 4)

Case D : Set-up was made using containers of 200 ml capacity. Buffer was added with further external addition of microbes. Agar salt bridge was prepared using agar and KCl.Amount of the inoculum used was 50 ml. Optical Density of Standard (Nutrient Agar Broth) was 0.244. **Case D1:** Sample used was *Ananas comosus*(100g). **Case D2:** Sample used was *Saccharum officinarum*(100g).The results obtained are given in table 5 and 6. (Sheikh *et al.* 2015) studied the effect of addition of anaerobic isolates to MFCs. A clear relation was observed between the microbial addition and potential generation. In this study, positive as well as negative effects of microbial addition were seen.

Conclusion :

Microbial fuel cell is a promising sustainable technology as it generates potential by utilizing wastes as substrates. Even if the generation of high levels of electricity from microbial fuel cells is a long way off, in future by providing more suitable environment and conditions the potential may be raised and an understanding of the coupling of organic matter oxidation to electron transfer to electrodes is likely to yield important insights into the diversity of microbial respiratory capabilities and might lead to applications in nano-electronics.

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Table 1. pH of different samples before and after fermentation

Sample	pH of fresh sample	pH after fermentation
Saccharum officinarum	6.8	5.2
Ananas comosus	5.2	3.5
Citrus limetta	5.9	4.6

Table 2. Identification of the isolates

Colony Characteristics	Isolate 1 (*Ananas comosus*) 10^{-5} dilution	Isolate 2 (*Saccharum officinarum*) 10^{-5} dilution	Isolate 3 (Fresh cow dung) 10^{-5} dilution	Isolate 4 (*Citrus limetta*) 10^{-5} dilution
Shape	Circular	Circular	Circular	Circular
Colour	Yellow	Cream	Cream	Cream
Elevation	Raised	Raised	Raised	Raised
Margin	Regular	Regular	Regular	Regular
Texture	Slimy	Slimy	Slimy	Slimy
Gram	Gram	Gram	Gram	Gram
Reaction	Positive	Positive	Positive	Positive
Slide View	*Bacillus*	*Bacillus, Coccus*	*Bacillus*	*Bacillus*

Table 3. Biochemical test results

Biochemical Tests	Result Shown by Different Isolates			
	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Amylase production	+	+	–	+
Fermentation	+	+	+	+
Catalase	–	–	–	–

Table 4. IMViC tests

Biochemical Tests	Result Shown by Different Isolates			
	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Citrate utilization	+	+	+	+
Indole production	+	+	+	+
MR	+	+	+	+
VP	–	–	–	–

Table 5. Potential of cell before and after adding microbial strains at different time period

Microbes	Time	Potential before adding microbes (V)	Potential After adding microbes (V)
Isolate 1 O.D. = 1.75	0 hour	0.1	0.12
	6 hour	–	0.1
	1 day	–	0.24–0.25
Isolate 2 O.D. = 1.78	0 hour	0.1	0.1
	6 hour	–	0.09
	1 day	–	0.7–0.8
Isolate 3 O.D. = 1.950	0 hour		0.04
	6 hour	–	0.1
	1 day	–	0.24
Isolate 4 O.D. = 1.440	0 hour	0.1	0.2
	6 hour	–	0.01
	1 day	–	0.09–0.1

Table 6. Potential of cell before and after adding *Pseudomonas* (O.D. = 1.383) strain at different time period

Time	Potential (before adding microbe)	Potential (after adding Microbes)
0 hour	0.1 V	0.2 V
1 Day	–	0.03 V

LIST OF FIGURES

Fig. 1. Graph showing variation in potential difference generation with time period with *Ananas comosus* as substrate

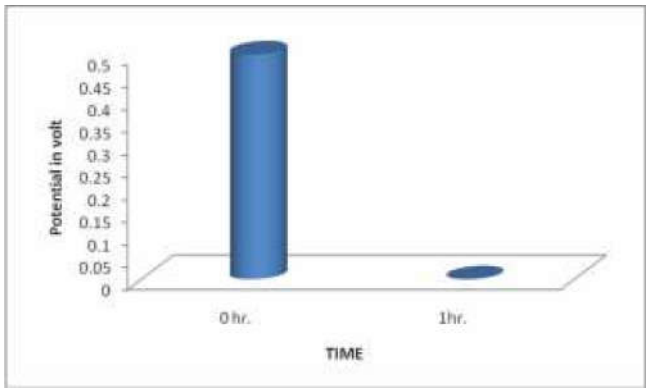

Fig. 2. Graph showing variation in potential difference generation with time period with muck as substrate

Fig. 3. Graph showing variation in potential difference generation with time period with rice field soil as substrate

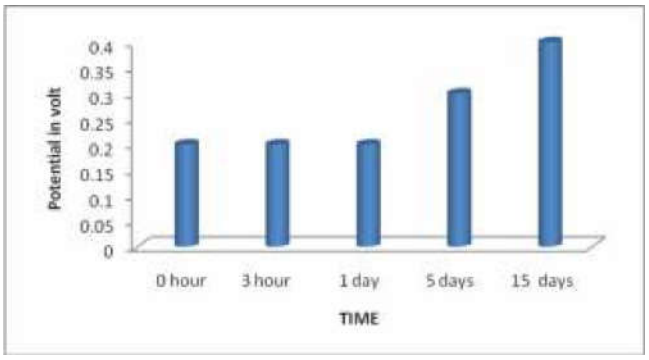

Fig. 4. Graph showing variation in potential difference generation with time period with *Ananas comosus* as substrate

Fig. 5. Graph showing variation in potential difference generation with time period with *Saccharum officinarum* as substrate

Fig. 6. Graph showing variation in potential difference generation with time period with *Citrus limetta* as substrate

Fig. 7. Graph showing variation in potential difference generation with time period with mixed sample as substrate

Fig. 8. Graph showing variation in potential difference generation with time period with *Ananas comosus* as substrate

Fig. 9. Graph showing variation in potential difference generation with time period with *Saccharum officinarum* as substrate

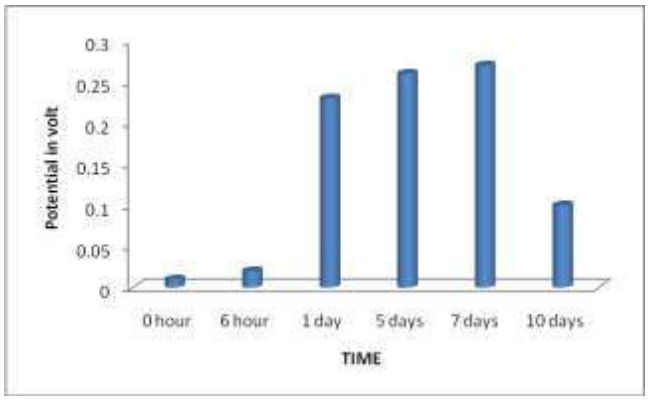

Fig. 10. Graph showing variation in potential difference generation with time period with *Citrus limetta* as substrate

Fig. 11. Figure showing Microbial Fuel Cell set up made using *Ananus comosus* as a substrate

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