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Fabrication and operation of a novel mediator and membrane-less microbial fuel cell

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Abstract

This present study deals with the Microbial Fuel Cells (MFC) using waste water and waste organic matter in its organic content to generate electricity using microorganisms. Microorganisms were able to utilize the carbon source in the substrate for generation of bioelectricity. This study is concentrated on the comparison of electricity generation by two different organic substrates like whey, rotten tomato juice, and electricity generation with Saccharomyces cerevisiae and Escherichia coli and also the comparative study on two different combinations of electrodes Carbon-Copper and Graphite-Copper. Electrodes play an important role in microbial fuel cells. In Carbon-Copper electrode whey water produced maximum voltage of 934mV at 2nd day of incubation. The result as electricity output was recorded as open circuit voltage (OCV) by Digital multimeter.

Keywords: Microbial Fuel Cell, electrode, *Saccharomyces cerevisiae, E. coli,* OCV.

Introduction

The real potential of application of microbial fuel cell techniques in treatment of wastewater has been shown by various researches in the fields of Environmental and Biological Engineering. The environmental, economical and social need of sustainable wastewater management plants and renewable energy has motivated the research in this domain. Microbial Fuel Cells or MFCs can be defined as the bio-electrochemical transducers that use the microbial reducing power to generate electrical energy. Hygienic and renewable energy has drawn attention from around the whole world due to the shortage of energy and need to protect the environment. Electricity consumption range in Tamilnadu was about 1,276.6 (kWh). The energy sectors of the developing countries assume a critical importance of huge investments to meet the ever increasing need of energy. The fossil fuels like coal and petroleum, which took three million years to form, are on the verge of depletion. We need to adopt energy efficient measures for sustainable development since we have consumed 60% of all the resources in last two hundred years. The non-renewable and fossil resources (coal, oil etc.) make up the 80% of primary energy source to the date. But these reservoirs are depleting with continuous consumption and hence will not exist in future. To overcome these problems, research focused on biological resources to generate electricity without affecting the environment and at low cost is required.

The new technologies that produce electricity from renewable energy sources with no a net carbon dioxide emission are desirable[1-2]. There are two types of basic models of MFCs that has been constructed. The Mediator microbial fuel cells are the first kind of MFCs. Electrochemical inactivity is the characteristic of most of the microbial cells. To facilitate the electron transfer from microbial cells to the electrode different types of mediators can be used such as methyl viologen, humic acid, thionine, methyl blue, neutral red etc.[3-4]. The mediators available have the limitation of being expensive and toxic. The ideal mediator should function as an electron shuttle by displaying reversible redox reaction and should be stable and appreciably soluble in an aqueous solution. Its penetration into the cell membrane should have low formal potential and should be free enough to capture electrons. The difference between the cathode and anode potentials, the formal potential, lowers with the increase in the cell voltage. The mediator-free microbial fuel cells are the second kind of MFCs that do not have need of mediator but instead use electrochemically active bacteria in order to transfer electrons to the electrodes where they are carried directly from the bacterial respiratory enzymes to the electrode. Generation of electricity using mediator-less Microbial Fuel Cell has been attempt in many laboratories.

A variety of organic compounds such as carbohydrates, proteins and fatty acids have been used to generate electricity in MFCs. One of the supreme advantages of MFCs over conventional fuel cells like methanol and hydrogen fuel cells is that a varied range of organic compounds can be used as fuels. Sugar when consumed by the microorganisms under aerobic conditions produces carbon dioxide and water, but when oxygen is not present the end product is carbon dioxide, electrons and protons which can be described as below.

$$C_{12}H_{22}O_{11}+13H_2O - 12CO_2-48H^+\_48e^-$$

Then the electrons pass across a loaded wire that can be considered as resistor to the cathode where they combine with oxygen and protons to form water. The current and voltage is generated to produce electricity when these electrons flow from anode to the cathode.

Presently, the reported works so far mostly rely on use of monocultures at laboratory level which helps in the research on these MFCs using wastewater as substrate and this is also in the primary stages of laboratory assessment around the world[5-7]. The use of energy resources and exploitation is increasing every day. Therefore the need to explore alternatives of fossil fuel is important because the utilization of waste organic sources for the production of renewable energy is the present field of active research and hence this view encourages extensive studies on bioelectricity generation through microbial fuel cell using a range of substrates.

The ability of microorganisms to produce fuel from organic materials is a well-known fact. The objective of the current study was to devise microbial fuel cells employing low-cost materials such as waste water and waste products rich in organic matter and ions that can be used as potential source to generate electricity using *Escherichia coli* and *Saccharomyces cerevisiae* as inoculums without using toxic mediators, from this study waste can be used to produce electricity while reducing the damage to the environment.

Materials and methods

MFC construction: The fuel cell was constructed by using transparent glass bottles which has electrode compartment of approximately 3 liters. Each chamber was equipped with a port for sample, wire point inputs at top of the chamber and ports for electrode wire. A salt bridge was supplied to connect the two compartments.

Freshly prepared, autoclaved 1.5 liter waste organic matter along with 5ml of microbial culture inoculums was added to the anodic compartment of the chamber and anaerobic condition was maintained. The cathodic compartment was loaded with 1.5 liter of water and continuously aerated. All MFCs were operated in batch mode using water as a substrate.

Preparation of salt bridge: The salt bridge was prepared by making 5-7% agar in KCl solution filled inside the PVC pipe to be used as separator in the MFC system. At first KCl solution was prepared by dissolving 7.45gm KCl in 100ml of Double Distilled Water (DDW) and then used this solvent to make 5-7% agar.

The solvent was heated and simultaneously the agar powder was added gradually until a clear homogenous suspension was obtained. Molten agar was poured into the PVC pipe having length of 35cm, width of 25cm and Internal Diameter of 20mm. The tube was sealed from both ends. Once the agar gets solidified on cooling, the seals were removed. This was used as the salt bridge in the MFC system.

Preparation of the substrate: whey water: Paneer whey is the lactose-rich watery by product of paneer manufacturing. Paneer whey was collected from local Dairy Industry. The initial pH of the whey was 5.5. The sample solution was sterilized by autoclaving at 121ºC for 15mins, then cooled down to room temperature.

The aggregated solids were removed by centrifugation at 11,000 x g in sterile tubes for 15mins. The pH of the collected whey supernatant was adjusted to and by using 1 M NaOH solution, as it is the major constituent of media for the growth of the microorganisms. The treated whey water was used as Carbon source in the MFC and lactose was the major carbohydrate in whey.

Rotten tomato juice: Rotten tomatoes were collected from local vegetables market. Tomato juice was prepared and the initial pH of the tomato juice was checked. Then the juice was sterilized by autoclaving at 121ºC for 30mins, and then cooled down to room temperature. The treated rotten tomato juice was used as a substrate.

Preparation of *Escherichia coli* culture: *Escherichia coli* culture was revived in 100ml nutrient broth and incubated at 37ºC for 24 hours. Then 5ml of this culture was inoculated in the substrate.

Preparation of *Saccharomyces cerevisiae* culture: *Saccharomyces cerevisiae* purchased from the local super market in Sivakasi and 5gm of *Saccharomyces cerevisiae* was employed in an anaerobic jar which acts as biocatalyst.

Studies on electrode materials: There are different types of electrode materials used for increasing the efficiency of the MFC. The type of materials used in the electrode construction has vital effect on MFCs efficiency. The different electrodes used in our study are Copper(Cu), Carbon(C) and Graphite(Gr). Copper electrodes were 14cm long and 0.1 cm in diameter. The dimension of Carbon electrodes was 12cm x 1.5cm and the Graphite electrodes were 20 cm long and 0.3cm in diameter.

Results and discussion

Experiment with *Escherichia coli:* The addition of *Escherichia coli* culture into anode chamber initiates the process. Substrates were fed to all the microbial fuel cells. The result as electricity output was recorded as open circuit voltage (OCV) by Digital multimeter. The biochemical activities of the microbes steadily increased the electricity production. During this phase, carbon sources are used up and different end products are produced. Whey water produced highest voltage of 934 mV at 2nd day of incubation, In Carbon- Copper electrode Rotten tomato juice produced maximum voltage of 582 mV at 4th day incubation (Figure-1).

Table-1: Total number of operated microbial fuel cell.

Bio catalyst	Anode compartment	Cathode compartment	Electrode	
			Anode	Cathode
Saccharomyces cerevisiae	Whey water	TAP water	Graphite	Copper
			Carbon	Copper
	Rotten Tomato juice		Graphite	Copper
			Carbon	Copper
E.coli	Whey water		Graphite	Copper
			Carbon	Copper
	Rotten Tomato juice		Graphite	Copper
			Carbon	Copper

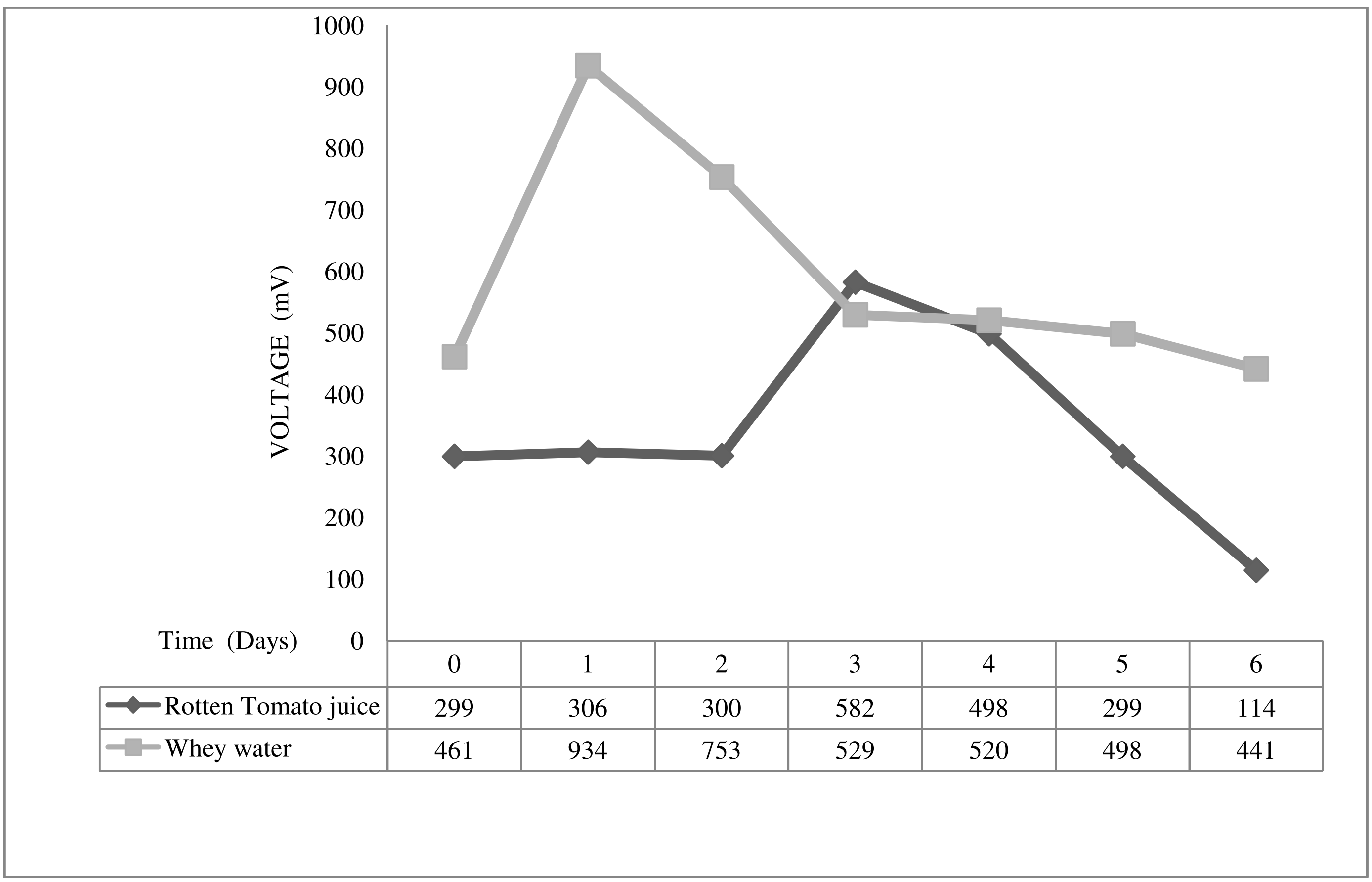
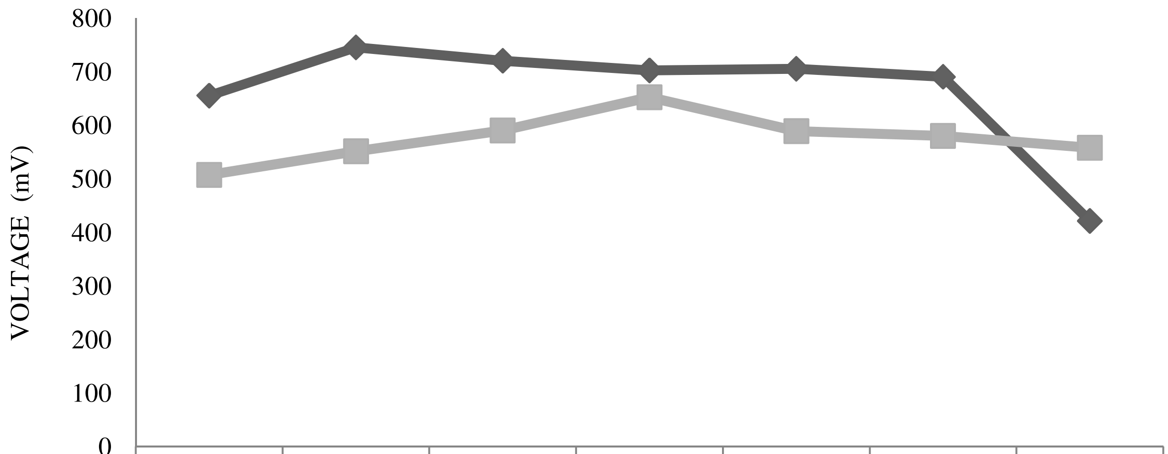


Figure-1: Comparison of power generation from whey water and rotten tomato juice by Carbon – Copper electrode.

Figure-2 illustrates that the variation of electricity production by graphite- copper electrode. During the initial stages, the voltage (V) increased sharply and then the increase became gradual till it reached the peak. In the initial phase the microbes got plenty of nutrients and their activity increased rapidly and thus the voltage increased sharply. The voltage then decreased gradually due to the depletion of the nutrients. During this phase, carbon sources are used up and different end-products are produced.

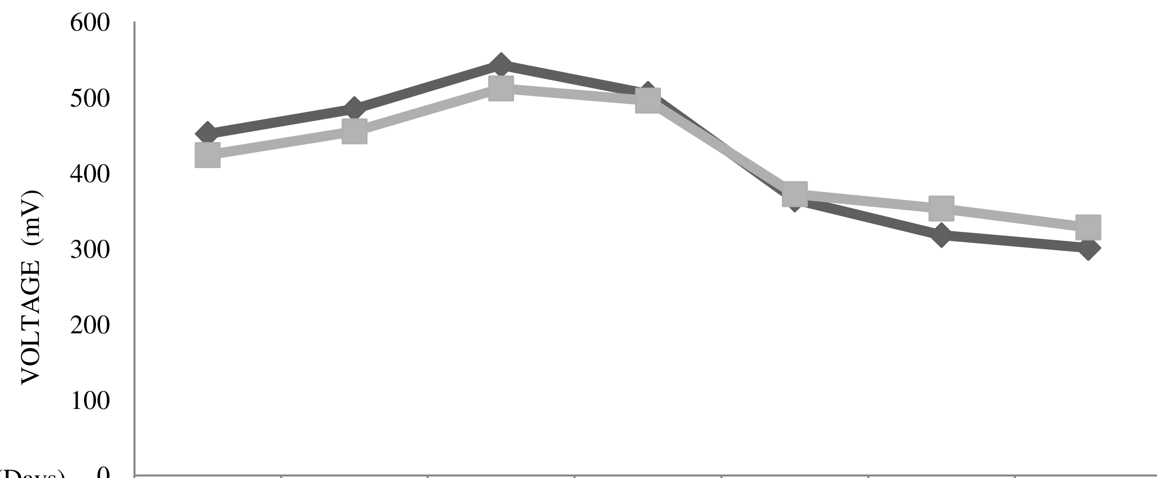
The open-circuit voltage peaked to the maximum level after a day of operation and then the Open-circuit voltage remains absolutely stable even after 24 hours. As a final point, swift utilization of the organic substrate and buildup of end-products leads to the stationary phase where the density of cells and the voltage remains constant. Consequently the decline phase occurs in which the biochemical activity of cells gradually decreases. At this stage, the depletion of lactose of whey water in the anode chamber results in the instability of voltage. In Graphite - Copper electrode Rotten tomato juice produced maximum voltage of 745 mV at 1^{st} day incubation. Whey water produced highest voltage of 652 mV at 3^{rd} day of incubation.

Experiment with *Saccharomyces cerevisiae:* The *S. cerevisiae* culture was added into anode chamber to start up the process. Substrates were fed to all the microbial fuel cells. The result as electricity output was recorded as open circuit voltage (OCV) by Digital multimeter. The biochemical activities of the microbes steadily increased the electricity production. During this phase, carbon sources are used up and different end-products are produced. The Open-circuit voltage remains absolutely stable even after 24 hours. In Carbon-Copper electrode whey water produced maximum voltage of 543 mV at 2^{nd} day of incubation and Rotten tomato juice produced maximum voltage of 512 mV at 2^{nd} day incubation (Figure-3).



Time (Days)	0	1	2	3	4	5	6
Rotten Tomato juice	655	745	720	702	705	690	421
Whey water	507	551	590	652	589	580	558

Figure-2: Comparison of power generation from whey water and rotten tomato juice by Graphite - Copper electrode.



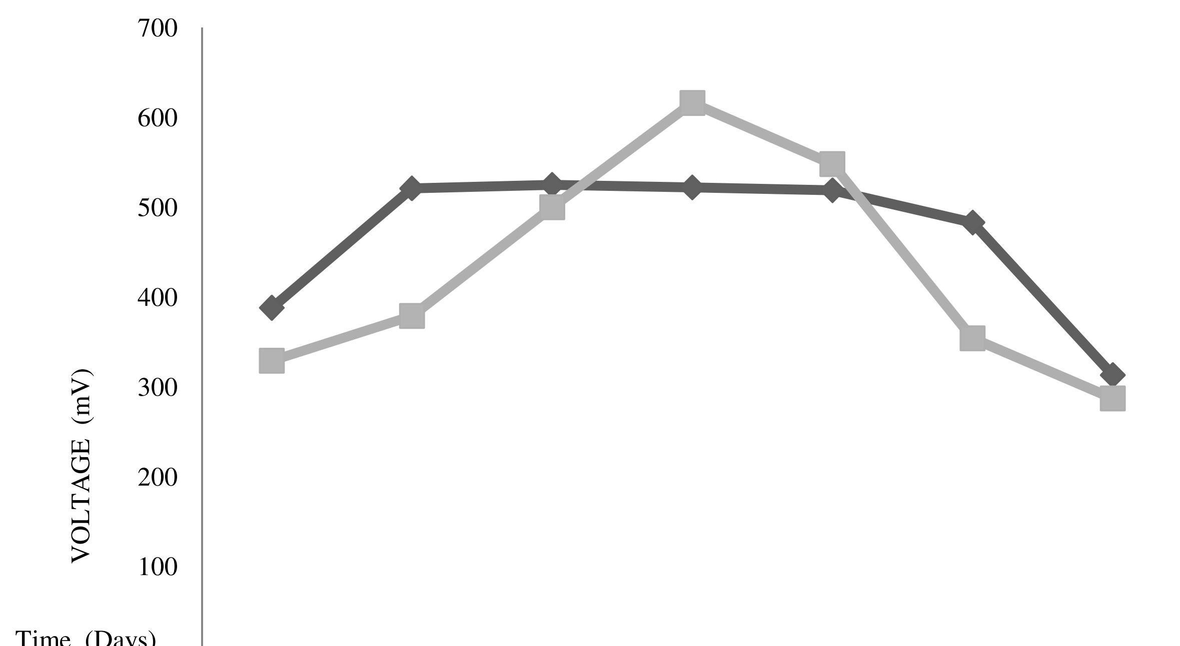
Time (Days)	0	1	2	3	4	5	6
Rotten Tomato juice	452	485	543	505	365	318	301
Whey water	424	455	512	496	372	353	328

Figure-3: Comparison of power generation from whey water and rotten tomato juice by Graphite - Copper electrode.

During the initial stages, the voltage (V) increased sharply and then the increase became gradual till it reached the peak. In the initial phase the microbes got plenty of nutrients and their activity increased rapidly and thus the voltage increased sharply. The peak voltage reaches 525 mV at 2^{nd} day by Graphite - Copper electrode. At this phase, carbon sources are utilized and different end products are produced. The open-circuit voltage peaked to the maximum value after 4^{th} or 5^{th} day of operation and then the OCV remains completely stable for more than 24 hours. Finally, rapid utilization of substrate and buildup of end-products leads to the stationary phase where the cell density and the voltage remain constant. Consequently the decline phase occurs in which the biochemical activity of cells gradually decreases. As a final point, swift utilization of the organic substrate and buildup of end-products leads to the stationary phase where the density of cells and the voltage remains constant. Consequently the decline phase occurs in which the biochemical activity of cells gradually decreases. At this stage, the depletion of lactose of whey water in the anode chamber results in the instability of voltage. In Graphite - Copper electrode Rotten tomato juice produced maximum voltage of 616 mV at 3^{rd} day incubation. Following represent the comparison between whey and rotten tomato juice in Graphite-copper electrode (Figure-4).

Current generation: After designing the experiment, the two chambered Mediator-less MFCs were fed with diverse waste water samples at different conditions to facilitate the development of biomass and the consequent production of electricity. The MFCs were observed throughout the experiment and the Output voltage was recorded after each 24hour interval while inoculation time considered as time 0.

Electricity generation using different wastewaters was observed in the initial experiments. The initial lag phase of about 24 hours followed by increase in cell current was observed after inoculation of different wastewater samples. This initial increase in voltage attribute to the presence of easily utilized components for the microorganisms in wastewater. The exhaustion of these simple nutrients leads to the decrease in the current outputs. But the degradation of complex nutrients sustains the low current generation. One of the advantages of such MFCs is the absence of electrode fouling hence they can be reused without significant activity loss. Hence these characteristics make the MFCs an enormous advantage over the chemical fuel cells that require only purified reactive fuel like hydrogen etc. Hence organic waste treatments coupled with sustainable bioelectricity generation are the chief applications of MFCs. In this paper, current advances of MFC technologies that lay basements for the future MFC development are summarized. Result of current study implies that MFCs will come to the commercial and practical exercise in near future and will turn into the ideal sustainable bioenergy processes.



Time (Days)	0	1	2	3	4	5	6
Rotten Tomato juice	388	521	525	522	519	483	313
Whey water	329	379	500	616	548	354	287

Figure-4: Comparison of power generation from whey water and rotten tomato juice by Graphite - Copper electrode.

Conclusion

Microbial fuel cells are different from the better known conventional cells as promotion of oxidation of electron donors by conventional cells requires expensive catalyst. Due to the complex regulatory distribution of system, high explosion risk and toxicity, they cannot be used at room temperature. Use of environment friendly microorganisms by microbial fuel cells leaves no choice of using some poisonous product and do not have multifarious or keeping up system, hence proving microbial fuel cells to be attractive power source in remote areas. Besides they not only provide electricity but also provide with other by-products like manure, biogas, etc. Also, quite processing with no sound eliminates the risk of noise pollution. With Carbon nanotubes, the MFCs work with enhanced and much more effectiveness using the same substrate and same micro-organism. Therefore the development in the field of microbial fuel cells production and electricity generation, one can see the bright future on earth.

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