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Bioelectricity Production from Microbial Fuel using *Escherichia Coli (*Glucose and Brewery Waste)

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Abstract

Microbial Fuel Cell (MFC) is a technology that utilizes bacterial growth in carbon-containing solutions to generate electricity or hydrogen. In MFC aerobic conditions are maintained at the cathode and anaerobic at the anode. The MFC design can be modified to produce hydrogen at the cathode by applying an additional over potential and omitting oxygen from the cathode. A microbial fuel cell (MFC) is a bioreactor that converts chemical energy in the organic compounds to electrical energy through catalytic reactions of microorganisms under anaerobic conditions. MFC generally makes use of organic matter and hence a large number of waste materials rich in organic content can be subjected to MFC for electricity generation. Power output and Coulomb efficiency are significantly affected by the types of microbes used in the anodic chamber of MFC, configuration of the MFC and operating conditions. The main objective of this work was to utilize waste water and waste products rich in ions or organic content which can be used as a potent source to generate electricity using microbes. The waste used in this study represents syrup industry waste and brewery waste. From this work waste can be used to generate electricity at the same time reduce the damage done to the natural environment.

Keywords: Microbial fuel cell (MFC), anaerobic bacteria, electrical energy, organic content.

Introduction

A fuel cell is comparable to an electrolytic cell or a battery, where chemicals are oxidized or reduced electrochemically to produce electricity. Fuel cells contain two electrodes (anode and cathode) that are either separated by an electrolyte, such as a salt bridge, or a membrane. Half of the reactions take place at the surface of the anode and cathode, and the total sum of the two half reactions is the overall reaction. Fuel cells do not contain stored reactants, but rather the reactants are fed to the cell continuously. The continuous flow of reagents allows for a continuous supply of electrical current as long as the electrodes and a proton exchange membrane stay intact. Microbial fuel cells are a device capable of converting chemical energy into electrical energy[1]. Microorganisms are used to oxidize organic substrates, such as glucose, and to generate electrical power. The generation of power occurs when it utilize substrates to maintain metabolism and reproduction. Within the microbe's cell membrane, the products created from the catabolism of the substrate go through many intermediates that become oxidized and reduced. When oxygen is absent, electrons can be donated from the microorganism to a redox mediator[2]. The redox mediator is then capable of delivering electrons to an electrode via oxidation of the mediator. Once delivered to the electrode, electrons flow from the substrate oxidizing compartment to a compartment which contains a terminal electron acceptor (cathodic compartment)[3]. When a substrate is anaerobically oxidized in the anodic compartment to produce electrons the potential decreases. While at the cathode compartment, reduction takes place. The difference in potential caused by the oxidation of a substrate at the anode and reduction of a substrate at the cathode allows current generation.

A substrate plays significant role in the amount of electrons being generated in a fuel cell. Some substrates are capable of producing a higher coulombic yield than some of the more common sugars used. Even though some microbial fuel cells produced a higher efficiency yield, yet glucose is the most commonly used. As glucose ($C_6H_{12}O_6$) is the most simple sugar (monosaccharide) which is an important carbohydrate and an instantaneous source of fuel (energy) in biological systems, ranging, from bacteria to humans. Use of glucose may be by either in aerobic respiration, anaerobic respiration, or fermentation[4].

Most of the bacteria utilize glucose as source of energy in their environment in order to produce ATP. Many bacteria use glucose, because they possess the enzymes required for the degradation and oxidation of this sugar. Fewer bacteria are unable to use glucose so they use complex carbohydrates like disaccharides (lactose or sucrose) or polysaccharides (starch)[5]. Recently; microbial fuel cells (MFCs) technology has drawn world-wide attention in generating electrical energy from organic matter. MFC is the device that directly converts chemical energy involved in organic matter into electrical energy. Hence a variety of substrates are screened for generation of electrical power. Brewery waste is used as substrate in this is work.

A large amount of brewery wastewater is produced from cooling (e.g. saccharification cooling, fermentation) and washing units in brewery industry on a daily basis which often causes several environmental problems[6-8]. This kind of wastewater is non-toxic and is rich in organic matter as compared to any other industrial waste Beer brewery wastewater might be good source for electricity generation in MFCs due to its nature of high carbohydrate content and low ammonium-nitrogen concentration[9,10]. The composition of brewing effluents might vary depending on various processes, water used, raw material used etc.

The organic components found in a brewery effluent are generally easily biodegradable and mainly consists of sugars, soluble starch, ethanol, volatile fatty acids, etc. due to the high organic content of the brewery effluent it can be uses as a good substrate for MFC. This work focuses on the comparative account between use of glucose and brewery waste water.

Material and Methods

Preparation of salt bridge: About 3% NaCl was added to 100 ml of distilled water and Mixed properly. About 1.6% Agar to the above solution and was boiled for 3 minutes. It was then filled in a PVC pipe sealed with a polythene sheet from one side.

Preparation and sterilization of the substrate: Sterilization of the glucose solution: Glucose was used as the substrate hence it was needed to determine the concentration at which the micro organism shows maximum growth. As higher concentration of glucose has toxic effect on the cells which may lead to the death of the cells. Minimal Inhibitory Concentration (MIC) assay was carried out to determine the concentration of glucose that supports maximum growth.

The glucose solution was sterilized by autoclaving it at 110°C/10psi for 10 min.

Sterilization of brewery waste water: The brewery waste water was autoclaved for 10min at 110°C/10psi.

Culture and Medium: A pure culture of *Escherichia coli* K-805 was used as inoculum in the anode compartment of the MFC. This strain of *Escherichia coli* was taken from NCIM Pune. The culture was sub-cultured on MacConkeys agar slant and was inoculated in LB broth at 37°C for 24 hours until the culture density was reached to 0.8 at 620nm using a colorimeter.

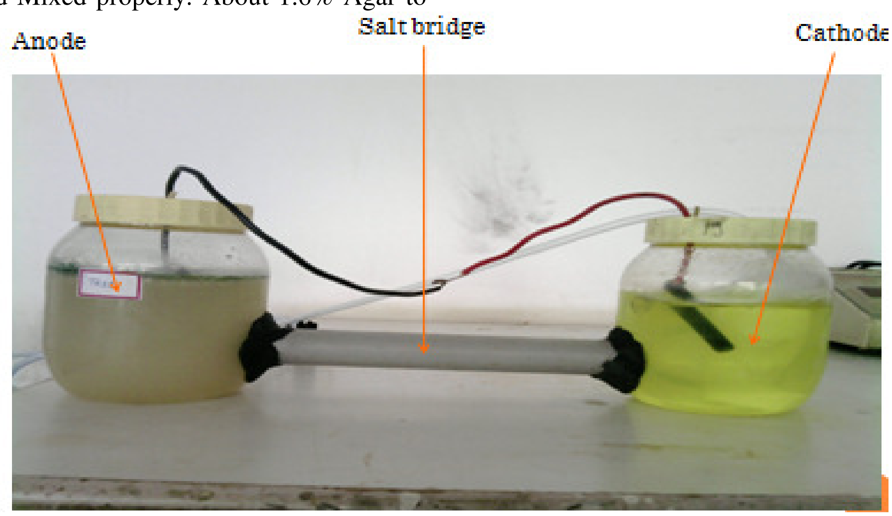

Figure-1
Assembly of the MFC electrolytic apparatus

Table-1
The Anode and Cathode compartment comprises the following solution

Particulars	Anode compartment	Cathode compartment
	400ml of bacterial suspension i.e. (400ml of 15% conc. Onion juice +2ml of *Escherichia coli* K-805 suspension (OD=0.8)) + 100 ml of phosphate buffer saline (pH=7.5) + 5 ml of glucose + 3- 4 drops of methylene blue.	300ml of potassium ferricyanide (3gm in 300ml) +200ml of phosphate buffer saline (pH=7.5)
Electrodes used	Carbon electrode	Carbon electrode

Note: A pinch of NaCl was added to the anode and cathode compartments for initial electrical conductivity.

Procedure for microbial fuel production: For glucose as substrate:

Control: A control was maintained for reference purposes.

The Anode and Cathode compartment comprises of the following solution: For brewery waste water: Same as above protocol

Results and Discussion

Since glucose is a very good source of food for the microorganism, it was decided to check the voltage that the microorganisms generate when glucose was used as the substrate.

Now, since high conc. of glucose can have an inhibitory effects on the microorganisms thus MIC (Minimal Inhibitory Concentration) assay was carried out and it was seen that 4-6% conc. of glucose supports good growth.

The day 1 reading was reported to be 351mV when glucose was used as the substrate. This reading was very close the first reading for the onion juice. However the second day reading i.e. after 48hrs of the process was reported to be much higher (393mV). The third reading but natural showed a drop in the reading i.e.310mV. Thus in this case the highest reading was obtained on the second day i.e. after 48hrs of the process.

The next substrate used was the brewery waste water from a village brewery. Since the brewery water is very much rich in organic contents hence it can prove to be a very good substrate. Also the large amount of water released by the brewery industry can be put to better use using a microbial fuel cell apparatus.

Interestingly the reading obtained when brewery waste water was used as the substrate was 534mV. This value exceeded every other value that was obtained until now. However the second day reading showed a sudden drop i.e.293mV. In this case the microbial death phase probably started after the first day. Also the reading on the third day further dropped down to 263mV.
But even then the brewery waste water allowed the micro organisms to generate a voltage of 534mV or 0.534V.

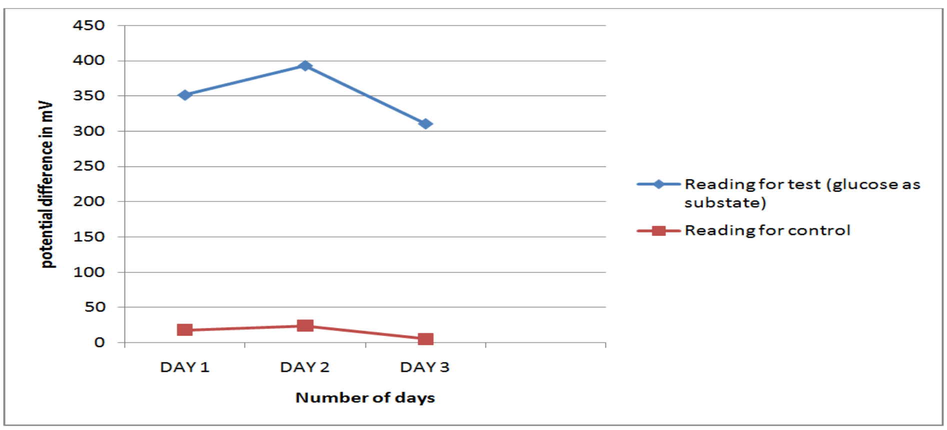

Figure-2
Graph for the glucose as substrate

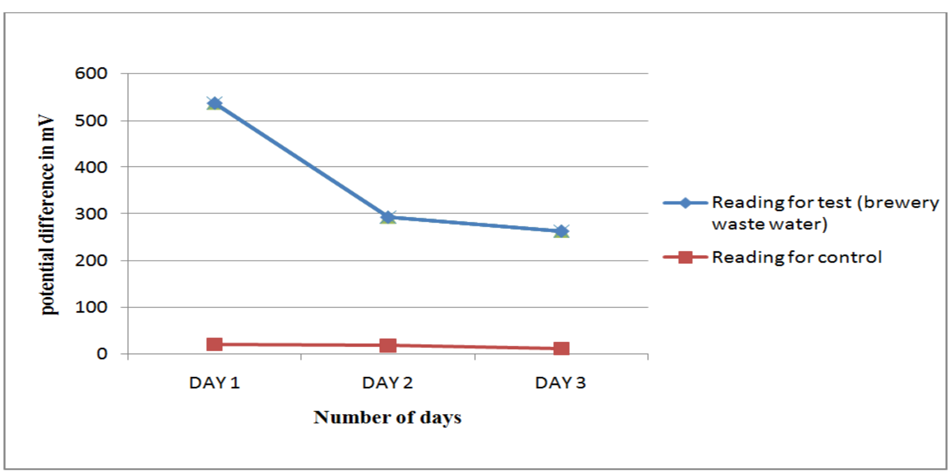

Figure-3
Graph for the brewery waste water as substrate

Table-2
Comparisons of results for the two substrate

Sr.no.	Substrate used	Day 1	Day 2	Day 3
1	Glucose	351mV	393mV	310mV
2	Control	18mV	24mV	5mV
3	Brewery waste water	538mV	293mV	263mV
4	Control	20mV	18mV	12mV

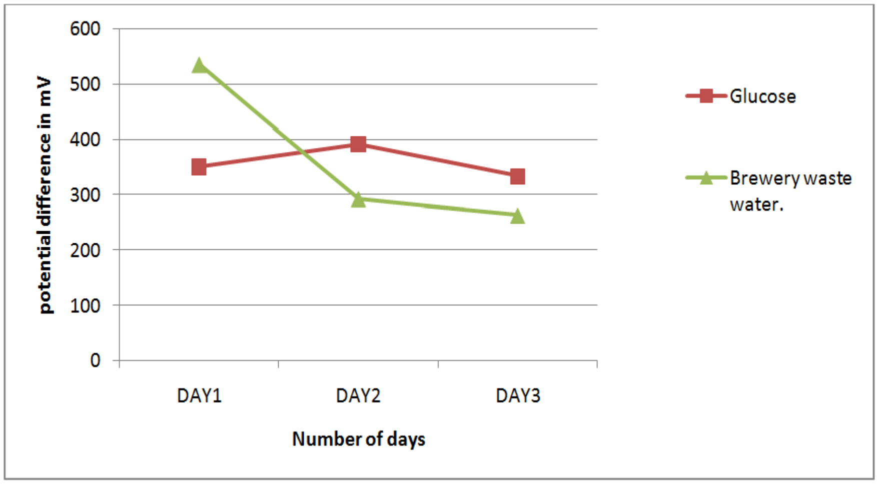

Figure-4
Graph comparing the readings of voltage for 3 days using different substrates.

Discussion: The above results suggest that a potential difference can be generated by the micro organism (*Escherichia coli)* when a suitable substrate is used. The substrate plays an important role as the yield of the voltage is mainly depending on how well the substrate is being utilized. Microorganisms present in the anodic compartment use anaerobic respiration to break down organic substances. During the catabolism of glucose or any other substrates like brewery waste as in this work, NADH is formed and is the starting intermediate for the electron transport chain. Electrons produced by the oxidation of glucose are channelized towards the anode by a redox mediator (Methylene blue). Once the redox mediator is reduced, the electrons are shuttled from the microorganism's membrane to an electrode. The electrons thus liberated are then attracted to carbon electrode from which the electrons pass through the copper wire to the cathode e compartment.

During the entire process it has been seen that a fairly good amount of voltage is being generated in the process. The brewery waste water gave the best results as the output of the voltage was maximum i.e.534mV whereas in case of glucose as a substrate it was found to be 393 mV. Thus it can be stated that the brewery waste water probably consist lot of organic components which provide a source for microbial growth.

If the amount of substrate is increased the net gain of the voltage increases and a measureable amount of the voltage is generated in the process. According to the Ohm's law the current is directly proportional to the potential difference across the two points.

$$I = \frac{V}{R}$$

Hence it can be said that if voltage is produced then the current is also flowing.

Conclusion

In this work, the generation of bio electricity using *Escherichia coli* within a microbial fuel cell was demonstrated. Two substrates used represent various forms of waste that are released in the environment. The first substrate is glucose as many bacteria readily use glucose, since they possess the enzymes required for the degradation and oxidation of this sugar. This waste represents the waste materials/water released from the syrup industry, which is rich in glucose or the other sugars. Glucose gave good result in for generation of electric energy i.e a potential difference of 393 mV. Thus it can be said that other sugars such as sucrose, maltose, etc. can also be used to check their role as the substrate for the fuel cell.

The second substrate used was brewery waste water which was collected from a village brewery. This waste represents the industrial waste which can cause a lot of damage to the environment. This kind of wastewater is non-toxic, but with high in organic content. An organic component in brewery effluent are generally easily biodegradable and mainly consists of sugars, soluble starch, ethanol, volatile fatty acids, etc,. Thus due to this reason it can prove to be a very good substrate for the microbial fuel cell. Also the results obtained for this type of waste was the best i.e.534mV.

Thus we can conclude that since the brewery waste water gave better results when used in a microbial fuel cell, the waste water from the other industry (e.g. tanning industry, dairy industry etc.) rich in organic content can also be subjected to a microbial fuel cell setup.

Thus on a whole it can be stated that all the waste materials rich in organic content can be used to generate electricity thereby reducing the environmental pollution as well as obtaining useful fuel from it. The current thus obtained can be amplified using an amplifier and can be used to light bulb or any low power driven appliances.

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