A sustainable energy source from microbes using microbial fuel cells

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Abstract- A microbial fuel cell (MFC) is a sustainable energy source which hopes to meet the growing demand of energy in the world by providing bioelectricity. The microbes like bacteria help produce current and voltage by metabolizing the carbon source into ions which are accepted at their respective electrodes. The prototype cell designed by our team is novel, cost effective, portable and environment friendly. This microbial fuel cell is a two chambered setup containing four graphite electrodes as the anode and four aluminum electrodes as the cathode. The MFC has been optimized under various experimental condition which includes sugar concentration, surface area of the electrodes, material of the electrodes as well as the effects of reducing salts on the current and voltage.

A pure strain of *Escherichia coli* (DH5α) was used for our experimental setup. This system can be used with a permanent bioreactor post modification to keep the voltage flow constant for a long time and requires low maintenance. The prototype has been able to power up to 4 LED bulbs or the current can even be stored in a rechargeable battery for future use. The main target of this project is to help farmers by using these fuel cells to power small sprinklers in arid lands in areas where there is an energy deficit like in farm lands in and around India by using waste water as the substrate for the cell.

1. Introduction

The ever-increasing demand and consumption of energy in the world has put pressure on the depleting finite fossil fuel reserves. The fossil fuel usage also is responsible for Carbon dioxide emissions which in turn leads to global warming and climate change. In order to balance out this demand, more emphasis needs to be laid on sustainable energy sources. Renewable energy sources are those which get naturally replenished with time, but these technologies like wind, solar and tidal sources are very expensive to implement, require huge amount of area and affect the surrounding ecosystem negatively. Sustainable energy sources are those which can meet the growing demand of today’s people without compromising on the demand of the future [1]. Despite the growing demand, it represents only a small fraction of global energy consumption amounting to about 8.4 % of the total energy production. The advantages of the microbial fuel cell are that it is affordable, accessible and eco-friendly. The microbial fuel cell is a biological fuel cell generating bioelectricity by the help of microbial metabolism which breaks down carbohydrates into ions. The electrochemical activities were measured
by the electric potential and current generation capacity of the microbial fuel cell system. After successfully creating a working prototype, certain factors were varied to create a more efficient and useful fuel cell which has a variety of applications. The research done in the past was mainly based on a single chamber cell and used waste water as the carbon source but produced negligible amount of power and so the results were not promising [2].

The main idea of the cell is that bacteria can easily be grown in cultures of Luria Broth (LB) by providing it with nutrients to help proliferate the number of living bacterial cells. These cells are inoculated into a glucose solution. The bacteria will use the glucose solution as the primary carbon energy source for its metabolism. The microbial fuel cell is a device that converts chemical energy into electrical energy using bacteria as a biocatalyst which requires an energy source like glucose or lactose which it uses under anaerobic or aerobic conditions to produce ions which is accepted by anode and this helps in creation of a potential at the terminals [3]. Initially the MFC was set up by using a consortium of microorganism present in the soil and generated negligible amount of power. The next step was to use a pure strain of bacteria which was isolated and then cultured. This technology has successfully been implemented in some places where algae was used as the biocatalyst instead of bacteria. But, the algae cause’s a lot of problems like formation of biofilms [4] requires a constant temperature to be maintained and so it requires a lot of monitoring and hence bacteria is seen to be a better option since its biofilm can easily be removed by chemical means while algae is difficult to remove[13].

With the advancement of science with time, newer technologies are coming into the world which has helped create many new applications for this fuel cell. This fuel cell functions well even if the small 650 ml prototype cell is used. If a bioreactor is used, much higher power can be generated, and this opens up a huge amount of new applications like-

1) Sprinklers for agriculture- This fuel cell can be placed even in the most isolated areas with the harshest of the conditions and once setup, should be able to power the sprinklers for the farms and hence is a cheap yet efficient energy source for agriculture where electricity is a problem. In turn it will be very beneficial for the farmers facing droughts and water management issues

2) Biosensors- With the growth of biotechnology and nanotechnology, there have been new devices created which are able to monitor many different factors or perform tests in big factories and for this low energy requiring sensors, a small fuel cell will provide adequate energy.

3) Desalination of water- This fuel cell can be used to desalinate the sea water and make it useful for drinking and this can be used even for arid and dry conditions and hence will be an easy and cheap alternative to reduce dependency on fossil fuels for power.

4) Waste water treatment- The main research being conducted is to use the waste water to generate enough energy to treat this water and power the remaining power plant hence reducing the running cost of the plant.

2 Materials
2.1 Microbial Fuel Cell
The microbial fuel cell is constructed in a way in which the electrodes provide maximum surface area for the microorganism to act upon and should be stable and if required portable [5]. The electrodes need to be made of non-reactive materials so that they do not affect the cells half reactions and in turn
should not negatively affect the power generation. There should be no source of contamination in the cell and only the pure culture should power the cell. After assimilation, the cell created has very low maintenance requirements and can be left unmonitored for days. The prototype cell is made using two plastic containers (biodegradable) which are bad conductors of heat and current, which helped keep the wastage of energy at the minimum. Initially the cells must be wiped with 90% ethanol to make the cell sterile and devoid of contaminants. The 1st anode chamber was made airtight and hence was anaerobic, while the 2nd cathode chamber was aerobic. For the circuit to be completed, a salt bridge was used. This was made using a bendable PVC pipe which was tightly packed with cotton which was soaked in 10% KCL to allow free movements of ions between the 2 chambers without allowing the liquids to mix-up and the high concentration of salts also acted as a barrier to plasmolyze unwanted microbes and hence killing them. Copper wires were used to make all the connections and close the circuit. A multimeter was used to detect the changes in the current and voltage and was monitored after a fixed time period.

2.2 Electrodes
Maximum current and voltage was harnessed using non-reactive and non-toxic electrode surfaces with maximum surface area for the bacteria to act upon. This reduces the ohmic losses and enhances the electrode kinetics [6]. The main idea was to keep the cost of the cell low and so the electrodes were varied by using different materials like carbon, aluminum and copper. The problem faced when using these electrodes is that there is a formation of biofilm on the electrodes surface considerably reducing surface area to react and so, *Escherichia coli* was selected which forms a limited biofilm when MFC is running for a lot of days. The electrodes were fitted unto the lid of the MFC in each of the chambers separately.
2.3 Salt bridge
The salt bridge helps in the flow of charge without interfering with the 2 half-cell reactions. The salt bridge for the MFC prototype was made out of a PVC pipe and loaded with 10% concentrated Potassium Chloride (KCl) solution which is a good conductor of charge and then connected to the main cell in such a way that the salt bridge was at the same level in both cells hence providing equilibrium of pressure between the cells and this prevented any cross flow into the other chambers. The salt bridge should be made in such a way that is should never dry up hence requiring very low amount of maintenance.

2.4 Introduction about the bacteria
*Escherichia coli* was selected instead of the other microorganism like yeast or algae as it is considerably easier to grow and quite thermo tolerant. *Escherichia coli* acted as a biocatalyst by helping in the conversion of chemical energy into electrical energy by glucose metabolism [7]. A pure culture of *E. coli* was used which had been isolated from a sample and then grown in an agar plate and slants for future use. This *E coli* grows best at 37 °C and has exponential growth every 20 mins. A non-virulent and non-pathogenic strain was selected for all the experimental studies. This *E coli* was used at optical density of 0.12 which meant that the *E coli* was in the Lag phase of its growth cycle and can adapt easily to changes in sugar source when compared to any other phases of its life cycle. It follows a sigmoidal growth curve having 4 stages. Sub culturing was done carefully and in sterile conditions to avoid contamination.

Luria Broth is a nutritionally rich medium which can easily be modified and is mainly used to grow bacteria. It contains peptides and casein proteins, Vitamins, Trace elements (N, S, Mg), Minerals as well as an energy source. The bacteria needs a regulated pH of 8, and the Broth must be prepared in distilled water and then autoclaved [8]. This Luria Broth is also used with agar to make petri plates, to grow pure culture and stored for later use. Slants were also used to grow the microorganism and later sub culturing was performed.

2.5 The carbon source
Bacteria need a carbon source on which it performs metabolizes to yield the required ions which participate in the half cell reaction. The bacteria use carbohydrates like glucose, maltose, sucrose or lactose as it energy source. But keeping in mind the cost of the cell and the efficiency, it was decided that glucose was a better and a more power efficient energy source when compared to the other carbon sources [9]. LB was not used for the fuel cell, since it has other trace elements which can affect the functioning of the fuel cell and for that reason, it was only used to grow the number of bacterial cells and after which these cells were transferred into glucose which was used as the primary carbon source and was autoclaved and sterile.

The concentration of glucose required for the efficient MFC was optimized by varying the concentration between 2.33% up to 30%. The *E coli* was grown in the Luria Broth and then centrifuged to collect the pellets which were then washed before being loaded into the cell with the
glucose solution of various concentrations. The bacteria has to adapt and start using this sugar and so a lag phase is seen when sugar is just introduced into the cell. Followed by a log or exponential phase in which the *E coli* uses this sugar to produce maximum amount of current and voltage hence this is the most beneficial phase for the bacteria. There is a way to reduce this lag from a lot of hours just to a few hours using certain reducing ferrous salts (FeCl₂, FeSO₄). The cell stops functioning once all the glucose in the solution has been used up or if there is a built of toxic material inside the fuel cell due to release of metabolites from the dead cells causing the drop in pH. The residual glucose concentration was also determined and accordingly changes were made to reduce wastage of sugar.

2.6 Glucose metabolism
The structurally conserved and ubiquitous pathways of central carbon metabolism provide building blocks and cofactors for the biosynthesis of cellular macromolecules. The relative uses of pathways and reactions, however, vary widely among species and depend upon conditions, and some are not used at all. Most bacteria which grow on glucose as the sole carbon source, represent fundamentally different metabolic lifestyles and are phylogenetically distinct [10]. Mainly the Bacteria follow either the Entner-Doudoroff pathway or the Pentose Phosphate pathway to break down the carbohydrates into the ions.

3 Method
3.1 Varying concentrations of glucose-
The samples were used under identical conditions, and the concentration of sugar was varied from 2.33% all the way up till 30% and everything was weight by volume percentages. Each of these glucose solutions were autoclaved and maintained in sterile environment to prevent outside contamination from affecting the readings. Up to 3 experiments were conducted per sample to help maintain accuracy in the readings. The readings were then compared in the form of total power generated per unit area. Each experiment was conducted times to check the accuracy of the readings.

3.2 Varying surface area of electrodes used
Initially, carbon rods were used as the anode for the experiments with a surface area of 22.36 square centimeters. During these experiments, four electrodes were used at once to provide maximum surface area while, keeping the cost of the cell low. The number of electrodes was varied from 1 to 6 and hence the varied the surface area was used to check if any significant change was detected in the power being generated. The anode and cathodes surface area were kept constant and surface area of the electrodes should be maximized, since the bacteria should be exposed to maximum area on the anode which helps in higher bacterial metabolism, in turn providing with better current as well as voltage.

3.3 Effect of Methylene Blue
Experiment using methylene blue was conducted to test its effect on the current as well the voltage. Since methylene blue acts as an electron acceptor, it should create more demand for electrons and hence more ions should flow through the cell making the cell more power efficient. The methylene blue was added along with glucose solution and the bacterial culture in anodic region.

3.4 Effect of Ferrous Salt
There is a lag phase which is seen when we initially inoculate the bacteria into the chamber containing glucose and this phase lasts for about 16 to 18 hours, where there is low production of current as well as voltage. We tried using ferrous salts like FeCl₂ and FeSO₄ to check the effects of it on the reaction and determine the change in power generation.
4 Result and discussion -

4.1 Varying sugar concentration –

Table 1 - Concentration of sugar used and the power generated from it

| Concentration of glucose used (W/V) | Volume used in milliliters (ML) | Average Power generated in milliwatt |
|-------------------------------------|---------------------------------|-------------------------------------|
| 2.33%                               | 650                             | 15                                  |
| 5%                                  | 650                             | 20                                  |
| 10%                                 | 650                             | 31                                  |
| 15%                                 | 650                             | 47                                  |
| 20%                                 | 650                             | 85                                  |
| 25%                                 | 650                             | 114                                 |
| 30%                                 | 650                             | 59                                  |

Graph 1 - Current (blue) and voltage (orange) generated from a 2.33% glucose sample
Graph 2- Current (blue) and voltage (orange) generated from a 5% glucose sample

Graph 3- Current (blue) and voltage (orange) generated from a 15% glucose sample
Graph 4: Current (blue) and voltage (orange) generated from a 20% glucose sample

Graph 5: Current (blue) and voltage (orange) generated from a 25% glucose sample
After running multiple cells by keeping the outside conditions for the cells constant and only varying the sugar concentration, it was noticed that the current increased with increase in glucose concentration until a point after which it drastically fell. While in the case of voltage, it increased linearly with increase in glucose concentration [11]. By comparing the readings and analyzing them, it was decided that 25% is the optimum condition for the *E coli* to grow in and so in the future experiments, the same concentration was used to determine the effect of surface area on the resultant power of the cell. The cell is currently producing current in amperes and voltage in volts while using the prototype fuel cell. The fall in values of current seen in very high concentration of sugars (higher than 28%), which tends to inhibit the growth of the bacteria and causes bacterial death.

4.2 Change in Surface Area-
Initially, it was decided to use carbon rods as the electrodes are cheap and non-reactive. The team started by using a single electrode and went up to 6 electrodes and noted down the change in the power. When surface area is increased, the bacteria gets significantly more area to attach and react with, hence providing higher current and voltage values. But it was observed that there was linear growth in current and voltage until we used 4 carbon electrodes, and after that there was a very slight (negligible) increase in current so hence keeping in mind the cost of the cell, it was decided to use 4 electrodes as the optimum surface area for our fuel cell. The reason for this might be that the number of cells in the chamber weren’t enough to totally occupy all the electrodes and so very small changes are seen in current as well as voltage.

**Graph 6**- Current (blue) and voltage (orange) generated from a 30% glucose sample
Table 2 Table shows comparison between surface area of the electrodes used and power generated for carbon electrodes

| Number of electrodes | Total surface Area in square centimeters(cm²) | Power Generated in milliwatt(mW) | Power Generated per Unit area(cm²/mW) |
|----------------------|-----------------------------------------------|---------------------------------|--------------------------------------|
| 1                    | 5.59                                          | 19                              | 3.39                                 |
| 2                    | 11.18                                         | 39                              | 3.48                                 |
| 3                    | 16.77                                         | 75                              | 4.47                                 |
| 4                    | 22.36                                         | 115                             | 5.14                                 |
| 5                    | 27.95                                         | 119                             | 4.25                                 |
| 6                    | 33.54                                         | 121                             | 3.60                                 |

4.3 Methylene blue as biocatalyst
Experiments using methylene blue were conducted which helped amply the current as well as the voltage, since methylene blue acts as an electron acceptor hence more demand for electrons and hence a more efficient fuel cell. And so, Methylene blue was added in all the future experiments to increase the power generation [12].

4.4 Ferrous salts helped in reducing the lag phase
The ferrous salt FeCl₂ was not very effective and helped reduce the lag phase time by 2-3 hours, and this salt was highly corrosive and ended up degrading the aluminum electrodes being used. The ferrous salt FeSO₄ was highly effective and helped reduce the lag phase time to about 9 hours and this helped keep the fuel cell running for a much longer time, but it did not amplify either the current or voltage marginally. So, it was only effective in reducing the lag phase timings and was also added in all the future experiments.

Conclusion
The microbial fuel cell has the potential to be a sustainable and renewable energy source which is cost effective and yet efficient. The prototype created by our team has a very low cost and even the running costs are low, this fuel cell is made from totally biodegradable material hence it is environmentally friendly. The bacteria used is also a non-virulent strain hence no threat to humans and animals. This energy source is a clean and green energy source which has shown us promising results and if efficiently optimized, this cell can be used in the industries soon.

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