Electricity Generation from Biowaste Based Microbial Fuel Cells

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Abstract

In this article, it has been established that voltage generated in a microbial fuel cell decreases linearly with respect to time. In other words, the first order derivative of voltage generated with respect to time is a negative constant. Thus the rate of change of voltage generated with respect to time has been established to be independent of time. It has been found that a mixture of biowastes can actually result in higher extractable current than any single component although this is not always true in general. Further, it has been found that when a component results in higher voltage production, it ends up reducing the cell life.

Keywords: *Renewable energy technology, conversion of chemical energy to electrical energy.*

1. Introduction

It has been known for almost one hundred years that bacteria could generate electricity [1]**.** But only in the past few years has this capability become more than a laboratory novelty. The microbial fuel cell (MFC) is a new form of renewable energy technology that can generate electricity from what would otherwise be considered waste. The reasons for this recent interest in using bacteria to generate electricity are a combination of the need for new sources of energy, discoveries about microbial physiology related to electron transport, and advancement of fuel cell technologies.

Microbial fuel cells produce electricity from organic matters. Unlike conventional fuel cells, MFCs have certain advantages like high energy-conversion efficiency and mild reaction conditions. In addition, a fuel cell's emissions are well below regulations [2]. MFCs also use energy much more efficiently than standard combustion engines which are limited by the Carnot Cycle. In theory an MFC is capable of energy efficiency far beyond 50%. In fact, using the new microbial fuel cells, conversion of the energy to hydrogen is 8 times as high as conventional hydrogen production technologies [3].

In an MFC, bacteria are separated from a terminal electron acceptor at the cathode so that the only means for respiration is to transfer electrons to the anode. An MFC is thus a bioelectrochemical system that derives electricity by mimicking bacterial interactions found in nature. Microorganisms catabolize compounds such as glucose [4], acetate or wastewater [5]. It is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms [6].

A typical microbial fuel cell consists of anode and cathode compartments. In the anode compartment, fuel is oxidized by microorganisms, generating electrons and protons. Electrons are transferred to the cathode compartment through an external electric circuit, and the

protons are transferred to the cathode compartment through a seperator. Electrons and protons are consumed in the cathode compartment, combining with oxygen to form water.

In general, there can be two types of microbial fuel cells: cells with mediator and cells without mediator. Such biological fuel cells take glucose and methanol from food scraps and convert it into hydrogen and food for the bacteria. The electrons gained from this oxidation are transferred to an anode, where they depart through an electrical circuit before reaching the cathode. Here they are transferred to a high potential electron acceptor such as oxygen. As current now flows over a potential difference, power is generated directly from microbial fuel by the catalytic activity of bacteria [7].

The microorganisms have the ability to produce electrochemically active substances that may be either metabolic intermediaries or final products of anaerobic respiration [8]. When microorganisms consume a substrate such as sugar in aerobic conditions they produce carbon dioxide and water. However when oxygen is not present, they produce carbon dioxide, protons and electrons [9].

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

The cells thereafter are made to use inorganic mediators to tap into the electron transport chain of cells and to accept the electrons that are produced. The mediator crosses the outer cell lipid membranes and plasma wall; it then begins to liberate electrons from the electron transport chain that would normally be taken up by oxygen and other intermediates. The reduced mediator exits the cell laden with electrons that it carries to an electrode where it deposits them. This electrode becomes the electrogeneric anode or negatively charged electrode. The release of the electrons means that the mediator returns to its original oxidized state ready to repeat the process. It is important to note that this can only happen under anaerobic conditions. If oxygen is present, it will collect all the electrons as it has a greater electronegativity than the mediator. A number of mediators have been suggested for use in microbial fuel cells. These include natural red, methylene blue, thionine or resorfuin etc. [10].

This is the principle behind generating a flow of electrons from micro-organisms. In order to turn this into a usable supply of electricity, this process has to be accommodated in a fuel cell. To generate a useful current, it is then necessary to create a complete circuit. The mediator and the micro-organism are mixed together in a solution to which is added a suitable substrate, glucose for example. This mixture is placed in a sealed chamber to stop the entry of oxygen, forcing the micro-organism to use anaerobic respiration thereby. An electrode is placed in the solution, which would then act as the anode. In the second chamber of the MFC, there is placed another solution and an electrode. This electrode, the cathode, is positively charged and is the equivalent of the oxygen sink at the end of the electron transport chain. It is however external to the biological cell. The solution is an oxidizing agent that picks up the electrons at the cathode. Incidentally, this is not particularly practical as it would require large volumes of circulating gas. A more convenient option is to use a solution of a solid oxidizing agent.

Connecting the two electrodes there is a wire or any other electrically conductive path. Completing the circuit and connecting the two chambers there has to be a salt bridge or an ion exchange membrane. This feature allows the protons produced to pass from the anode chamber to the cathode chamber. The reduced mediator carries electrons from the cell to the electrode. Here the mediator is oxidized when it deposits the electrons. The electrons then

flow across the wire to the second electrode, which acts as an electron sink. From here they pass to an oxidising material completing the process.

2. A Survey of the Literature on Microbial Fuel Cells

Water based organic matters that can be used in an MFC can be simple carbohydrates, acetate and butyrate, and complex organic compounds, domestic wastewater, and manure sludge [11].

It is noteworthy that anaerobic digestion is basically goes on inside the MFC. Anaerobic digestion is typically applied in sewage sludge treatment due to its advantages over aerobic systems, such as lower energy consumption, smaller amounts of solids generated, lower nutrient requirement and potential energy recovery from the produced biogas. Sewage sludge is stabilized during anaerobic digestion by converting most organic matter into biogas [12].

Microbial fuel cells use biocatalysts for the conversion of chemical energy to electrical energy ([13], [14], [15]). As most organic substrates undergo combustion with the evolution of energy, the biocatalyzed oxidation of organic substances, by oxygen or other oxidizers, provides a means for the conversion of chemical to electrical energy. Biocatalysts take part in the process of producing electricity in either of the two following ways: the biocatalysts can generate the fuel substrates for the cell by biocatalytic transformations or metabolic processes, or the biocatalysts may participate in the electron transfer chain between the fuel substrates and the electrode surfaces. A variety of electron mediators are used for the electrical contacting of the biocatalyst and the electrode [16].

Microorganisms have the ability to produce electrochemically active substances that may be either the metabolic intermediaries, or the final products of anaerobic respiration. For the purpose of energy generation, these fuel substances can be produced in one place and transported to a microbial fuel cell to be used as fuel. The biocatalytic microbial reactor produces the microbial fuel. The biological part of the device is however not directly integrated with the electrochemical part. It allows the electrochemical part to operate under conditions that are not compatible with the biological part of the device.

In another approach, the microbiological fermentation process proceeds directly in the anodic compartment of the fuel cell, supplying the anode with the fermentation products. In this case, the operational conditions in the anodic compartment are governed by the biological system, and therefore they are significantly different from those in conventional fuel cells. This would be a real microbial fuel cell which is not a simple combination of a bioreactor with a conventional fuel cell. This configuration is also often based on the biological production of hydrogen gas, but the electrochemical oxidation of H_2 is performed in presence of the biological components under mild conditions.

Yet a third approach involves the application of artificial electron transfer relays that can send electrons between the microbial biocatalytic system and the electrode back and forth. The mediator molecules take electrons from the biological electron transport chain of the microorganisms and transport them to the anode of the microbial fuel cell. In this case, the biocatalytic process performed in the microorganisms becomes different from the natural one, because the electron flow goes to the anode and not to a natural acceptor of electrons. Since the natural electron acceptor is expectedly more efficient, it can compete with the desired scheme, and hence it is usually removed from the system. In most of the cases, the microbiological system operates under anaerobic conditions, allowing electron transfer to the artificial electron relays and, finally to the anode.

Various bacteria and algae have been found to be active in hydrogen production under anaerobic conditions [17]. The most effective hydrogen production is observed upon fermentation of glucose in the presence of *Clostridium butyricum* [18]. This conversion of carbohydrate to hydrogen is achieved by a multienzyme system.

Immobilization of the hydrogen-producing bacteria, *Clostridium butyricum* is very important because it stabilizes the unstable hydrogenase system. In order to stabilize the biocatalytic performance, bacteria were in fact introduced into agar gel ([19], [20]) and filter paper [21].

There are many microorganisms producing metabolically reduced sulfur containing compounds such as sulfides and sulfites. Sulfate reducing bacteria form a specialized group of anaerobic microbes that use sulfate as a terminal electron acceptor for their respiration. These microorganisms yield S_2^- while using a substrate, lactate for example, as a source of electrons. This microbiological oxidation of lactate with the formation of sulfide has also been used to drive an anodic process in microbial fuel cells ([22], [23]).

Accumulation of sulfides in the medium results in the inhibition of the metabolic bacteria because they interact with iron containing proteins, blocking the electron transport systems. To prevent the toxic effect of H_2S, the anode should effectively oxidize it. However, many metallic electrodes are corrupted by sulfides because of their strong and irreversible adsorption. Therefore, porous graphite electrodes have also been used ([22], [23]) for the purpose.

Many different organic and organometallic compounds have been tested in combination with bacteria to test the efficiency of mediated electron transfer from the internal bacterial metabolites to the anode of the microbial fuel cells. Thionine has been used extensively as a mediator of electron transport from *Escherichia coli* ([24], [10]).

Ferric complexes have also been successfully used for oxidizing glucose [25]. Since thionine has frequently been used as a mediator in microbial fuel cells, mono and disulfonated derivatives of thionine have been applied to determine the effect of hydrophilic substituents on the mediation of electron transfer [26].

Engineering of the electrochemical cell provides a means of enhancing the electrical contact between a biocatalytic system and an anode, and hence to improve the cell output. The interfacial contact has been found to increase while using a three dimensional packed bed anode [27].

It has been shown that the performance of a microbial fuel cell depends heavily on the primary substrate used in the process of fermentation. The metabolic process in the bacteria is very complex. It involves many enzymes. It may proceed by many different routes. It has been shown that a mixture of nutritional substrates can result even in higher extractable current than any single component [28].

Microbial fuel cells require continuous fermentation of living cells performing numerous physiological processes thus dictating stringent working conditions. In order to overcome this constraint, redox enzymes responsible for the desired processes may be separated and purified from living organisms and applied as biocatalysts in microbial fuel cells ([29], [30]). Enzymes are expensive chemicals. Special ways for their utilization still remains to be established. Methods have however been suggested to electrically contact redox enzymes and electrode supports [31].

The methods of biocatalytic electrodes for oxidation of potential fuel substrates that act as biocatalytic anodes, and reduction of oxidizers that act as biocatalytic cathodes can be of three types. Such electrodes can then be integrated into microbial fuel cell elements. The methods are: anodes for microbial fuel cells based on enzyme catalyzed oxidative reactions,

cathodes for microbial fuel cells based on enzyme catalyzed reductive reactions, and microbial fuel cells based on layered enzyme electrodes.

In the first method, in which anodes for microbial fuel cells based on enzyme catalyzed oxidative reactions are used, the electrochemical oxidation of fuels can be biocatalyzed by enzymes communicating electrically with electrodes. Different classes of oxidative enzymes, oxidases, and dehydrogenases, for example, require the application of different molecular tools to establish this electrical process [16]. The mediator molecules [32] can be adsorbed directly onto electrodes, incorporated into polymer layers, or covalently linked to functional groups on electrode surfaces.

The electrical contacting of redox enzymes that defy direct electrical communication with electrodes can alternatively be established by using synthetic or biologically active charge carriers as intermediates between the redox center and the electrode [33]. The overall electrical efficiency of an enzyme modified electrode depends not only on the electron transport properties of the mediator, but also on the steps of transfer occurring in the assembly. Diffusional electron relays have been utilized to make electrons shuttle between oxidative enzymes and anodes of microbial fuel cells [34], providing the bioelectrocatalyzed oxidation of organic fuels [35], methanol for example [3].

In the second method, cathodes for microbial fuel cells based on enzyme catalyzed reductive reactions are used. The biocatalytic reduction of oxidizers has attracted much less attention than the biocatalytic oxidation of fuels. Nonetheless, in order to construct a microbial fuel cell element, it is essential to design a functional cathode for the reduction of the oxidizer that is coupled with the anode, and allows the electrically balanced current flow. Conventional oxygen reducing cathodes used in fuel cells are usually not compatible with biocatalytic anodes since high temperature and pressure are applied for their operation. Thus, biocatalytic reductive processes at the cathode should be considered as a strategy to design all biomaterial-based functional fuel cells [17]. It has been reported that bioelectrocatalyzed reduction of H_2O_2 has been accomplished in presence of various peroxidases such as horseradish peroxidase [36].

The biocatalytic reduction of oxidizers in nonaqueous solutions immiscible with water is important because it can be coupled to biocatalytic oxidative processes through liquid-liquid interfaces. Some enzymes [37], particularly peroxidases [36] can function in nonaqueous solutions. Horseradish peroxidase electrodes have been tested for biocatalytic reduction of organic peroxides in nonaqueous solvents [38]. The biocatalytic activity of enzymes [39], however, is usually lower in organic solvents than in water.

Biocatalytic systems composed of enzymes and their respective electron transfer mediators such as bilirubin oxidase [40], or fungal lactase [41] are able to biocatalyze the electroreduction of O_2 to H_2O significantly decreasing the potential. These systems, however, are composed of dissolved enzymes and mediators operating through a diffusion path that is not acceptable for technological applications.

Rotating disk electrode experiments have been performed to estimate the electron transfer rate for the overall bioelectrocatalytic process corresponding to the reduction of O_2 [42]. Physical characterization of the systems is essential to optimize the electrode performance. For each of the functional electrodes, the complex sequence of reactions must be resolved kinetically in order to determine the rate limiting step. Once the rate limiting step is identified, biomaterial engineering on the respective redox protein could be undertaken to optimize its electron transfer functionality [17].

The third method is to use microbial fuel cells based on layered enzyme electrodes. The bioelectrocatalyzed reduction of H_2O_2 and the oxidation of glucose allow us to design

microbial fuel cells using H_2O_2 and glucose as cathodic and anodic substrates respectively [43]. The potentials of the electrodes are negatively shifted and positively shifted when the concentrations of the glucose and H_2O_2 are elevated.

The microbial fuel cell voltage and current outputs are oxygen insensitive. This oxygen insensitivity of the bioelectrocatalytic process at the anode originates from the effective electrical contact of the surface with the electrode support, as a result of its alignment ([44], [45]). The stability of the microbial fuel cell was examined at an optimal loading resistance as a function of time [43]. The power decreases by about 50% after 3 hours of cell operation. This loss could originate from depletion of the fuel substrate, leakage of the fuel or the oxidizer into the wrong compartment, or degradation of the biocatalysts. Since the cell voltage appears to be stable, the current produced also decreases by the same factor. Recharging the cell with the fuel substrate and oxidizer could compensate for this component of the decrease in the current output.

Charge transfer processes across the interface between two immiscible electrolyte solutions can provide an additional potential difference between cathodic and anodic reactions due to the potential difference at the liquid-liquid interface. Many different interfacial liquid-liquid systems have been studied using numerous experimental approaches [46]. The reduction of peroxide in dichloromethane, and the oxidation of glucose in aqueous solution, bioelectrocatalyzed by the electrode, enables us to design a liquid-liquid interface microbial fuel cell using peroxide and glucose as cathodic and anodic substrates respectively [47].

3. Objectives

It is needless to stress on the universal fact that there is a lot of biowaste that could actually be considered as stored energy. This waste in liquid form could allow bacteria to convert it to electricity. This aspect is worth researching indeed which is why it was chosen in our work.

We have thus far mentioned that voltage generated in microbial fuel cells decreases with respect to time [48]. We have also come to know that a mixture of nutritional substrates can result even in higher extractable current than any single component [28]. It is therefore important to know in what way the voltage gets decreased. That would be known if we can fit a mathematical model expressing voltage as a function of time. From the model, we would be able to evaluate the rate of change of voltage with respect to time. We would further be able to see whether the rate of change is a constant or is time dependent, and this would give us the mathematical model in terms of a differential equation. From the mathematical model, we would further be able to know regarding how long such a cell stays functioning.

We therefore had the following objectives: to construct microbial fuel cells using cow dung, drain water, rice washing water, and slurry collected from biogas plant, to derive mathematical models to express voltage generated from the cells as a continuous function of time, so as to have an idea practically how long a cell remains functional, to evaluate the rate of change of the generated voltage with respect to time, to extrapolate how long the microbial fuel cells stay functioning, and to observe whether a mixture of biowastes does actually result in higher voltages.

4. Methodology

A salt solution, in our case, of pure NaCl, would be added to each of the biowaste samples to make the mixture electrically conductive. This mixture would be placed in a sealed chamber to stop entering of oxygen, thus forcing the microorganism to use anaerobic respiration. An electrode would then be placed in the solution that would act as the anode.

In the second chamber of the MFC there would be placed another solution and another electrode. This electrode, called the cathode would be positively charged and would be the equivalent of the oxygen sink at the end of the electron transport chain, only now it would be external to the biological cell.

The solution would be an oxidizing agent that would pick up the electrons at the cathode. In our case, we shall use potassium ferricyanide as the oxidizing agent. Potassium ferricyanide is added to the cathode to accept electrons. It is very reactive with the graphite electrode. Ferricyanide has a fairly positive potential compared to the organic matter in the anode and helps to drive the flow of electrons. With the addition of ferricyanide ions, the power can be increased 50-80% over a MFC with dissolved oxygen [49].

Figure 1. An MFC with Drain water and Slurry

Connecting the two electrodes would be a wire and completing the circuit and connecting the two chambers there would be a salt bridge. In place of commercially available electrodes, graphite rods extracted from dry cells would be utilized, the tips of which had been soldered to copper wires traveling from one chamber to the other. The soldering was performed at a melting temperature of 391^{o} C.

For preparation of salt bridges, a water solution containing concentrations of 3% NaCl and 1.6% agar was allowed to boil inside a microwave oven for nearly 3 minutes. The hot solution was poured into sawed PVC pipe sections each of length 4 inches by sealing one end with polythene. The setup was thereafter allowed to cool for nearly 2 hours inside a High Efficiency Performance Air Filter. The salt bridges were thus ready for use.

5. The Experimental Findings

Table 1 below depicts the micro voltmeter readings in millivolts when

i. Cow dung,
ii. Drain water,
iii. Rice washing water,
iv. Cow dung + Slurry,
v. Drain water + Slurry,
vi. Rice washing water + Slurry,
vii. Cow dung + Slurry + Vermicompost,
viii. Drain water + Slurry + Vermicompost,
ix. Rice washing water + Slurry + Vermicompost,
x. Slurry, and
xi. Slurry + Vermicompost,

were used in the cells. These findings have been included in a monograph by Barua [50].

After every experiment was set up, the first datum was taken after 1 complete day. In this way, after 1 complete day every consecutive datum was taken. The values noted were the peak values observed during the process of measurement of the data. This was so done because the electricity produced was intermittent probably due to coarseness of the cells.

Table 1. Voltage Generated (Micro-Volt) Using Different Bio-wastes

Bio-wastes	Day 1	Day 2	Day 3	Day 4	Day 5
cow dung (250 gm)	147	139	128	116	103
drain water (400 ml)	141	135	125	113	101
rice washing water (400 ml)	137	129	118	104	98
cow dung (250 gm) and slurry (5 gm)	189	172	153	138	117
drain water (400 ml) and slurry (5 gm)	185	171	150	134	120
rice washing water (400 ml) and slurry (5 gm)	190	179	161	145	123
cow dung (250 gm) and vermicompost (2 gm)	159	145	136	123	111
drain water (400 ml), slurry (5 gm) and vermicompost (2 gm)	147	138	126	117	109
rice washing water (400 ml), slurry (5 gm) and vermicompost (2 gm)	151	143	136	122	107
slurry (250 gm)	197	181	169	145	133
slurry (250 gm) and vermicompost (2 gm)	192	174	152	143	129

Vermicompost here refers to organic manure produced by earthworms. It is a mixture of worm castings, organic material including humus, live earthworms, their cocoons and other

organisms. Vermicomposting is an appropriate cost effective and efficient recycling technique for the disposal of non-toxic solid and liquid organic wastes. Earthworms constantly tunnel and feed on dead plants and decaying insects during the daylight hours. As the earthworm digests organic matter, the matter is passed out in casts that have large amounts of calcium, bacterial diversity, available nitrogen, magnesium, phosphorus, and potassium. These casts highly improve the compost as the organic material decomposes (Ref. www.cals.cornell.edu, www.erfindia.org).

6. Empirical Analysis of the Experimental Results

The experiments were performed at room temperature. As can be seen from the tables depicted above, we have observed the extent of voltage generation from 4 different biowastes, viz. cow dung, drain waste water, rice washing water, biogas plant slurry, and and some of their combinations.

The first three samples were found to have generated a certain voltage not exceeding 150 millivolts. In the case of cow dung, 147 mV was the reading on the 1st day of observation. In the course of the next 5 days, it decreased to 103 mV. Similarly, in the case of drain water, the reading decreased from 141 mV to 101 mV. Finally, in the case of rice washing water, it was seen to have decreased from 137 mV to 98 mV. It may be noted that the 3 samples were activated on the same day i.e. March 3, 2008 while the readings were taken till March 7, 2008 to avoid any unwanted variations brought about by external agents.

In the next phase of the experimentation, biogas plant slurry was added to all the 3 previously used samples. The fact that had been taken into consideration behind the utilization of biogas slurry was that it contains a highly propagative concentration of micronutrients essential for the survival of bacteria. As such, it becomes imperative that it must contain a higher concentration of decomposing bacteria as well necessary for the MFC.

True to our expectations, the MFC exhibited an increased generation of voltage, compared to the original MFC content devoid of any other additional substance, which in our case, and is slurry. On the 1st day of observation i.e. April 1, 2008, the MFC containing cow dung and slurry showed a reading of 189 mV while it decreased to 117 mV by April 5, 2008. Similarly, in the case of drain water and slurry, it reached down to 120 mV from an initial reading of 185 mV. The sample containing rice washing water and slurry too showed the same signs. In the 1st instance of observation, it was 190 mV while it fell down to 123 mV in the course of the next 5 days.

The 3rd phase of experimentation required the addition of vermicompost as nutrients for the inherent bacteria surviving therein. This time, both the original contents along with slurry were added upon to vermicompost.

The new set of MFC was activated on April 7, 2008 and the readings were being taken consecutively for 5 days till April 11, 2008. In the 1st case i.e. the one comprising cow dung, slurry and vermicompost, reading varied from 159 mV to 111 mV. In the 2nd case involving drain water, it decreased from an initial value of 147 mV to 109 mV. Finally, in the 3rd case containing rice washing water, it was found to decrease from 151 mV to 107 mV.

In the final stage of experimentation, only slurry was decided to be used. Staring from April 21 to April 25, the MFC was found to display a voltage of 197 mV to 133 mV. On addition of vermicompost, it exhibited a similar trend of voltage generation.

Eventually, we have studied 11 different cases. Plotting of the data with respect to time showed nearly linear and trend. One point looked almost certain that the mathematical model expressing voltage generated as a continuous function of time should be of degree 1. We

therefore decided to proceed to fit equations of degree 1, and check statistically whether a hypothesis of linearity in defining voltage generated with respect to time stands. If we can conclude that the hypothesis of linearity is not rejectable statistically, we would not proceed to fit a nonlinear model. Whereas for a nonlinear model the rate of change of voltage generated would be time dependent, for a linear model it would be a constant.

7. Statistical Analysis of the Data

From Table 1, it is empirically evident that the equation expressing voltage generated as a function of time is a straight line. Accordingly, we now proceed to fit first degree equations using the Method of Least Squares from the data that we have gathered.

Table 2. Best Fitted Straight Line Equations of the type $v = a + b\,t$

Bio-wastes used	$v = a + b\,t$	Coefficient of Determination	Value of $\lvert t \rvert$ - statistic for the regression coefficient
cow dung (250 gm)	159.9 – 11.1 t	99.2668 %	20.1541
drain water (400 ml)	153.6 – 10.2 t	98.5227 %	14.1449
rice washing water (400 ml)	148.1 – 10.3 t	98.7067 %	15.1318
cow dung (250 gm) and slurry (5 gm)	207.2 – 17.8 t	99.7984 %	38.5381
drain water (400 ml) and slurry (5 gm)	202.1 – 16.7 t	99.5325 %	25.2721
rice washing water (400 ml) and slurry (5 gm)	210.0 – 16.8 t	98.8512 %	16.0669
cow dung (250 gm) and vermicompost (2 gm)	170.2 – 11.8 t	99.6850 %	30.8117
drain water (400 ml), slurry (5 gm) and vermicompost (2 gm)	156.5 – 9.7 t	99.5451 %	25.6211
rice washing water (400 ml), slurry (5 gm) and vermicompost (2 gm)	164.5 – 10.9 t	97.1623 %	10.135
slurry (250 gm)	214.2 – 16.4 t	98.8824 %	16.2917
slurry (250 gm) and vermicompost (2 gm)	205.1 – 15.7 t	98.0469 %	12.2721

Accordingly, our mathematical model describing the voltage (V) generated, in terms of mV, is of the type:

$$v = a + b\,t,$$

t standing for time, whatever be the biowaste used.

As a consequence,

$$dv / dt = -11.1,$$

when cow dung was used in the microbial fuel cell. The fitted linear relations have been found to be very satisfactory. This means

$$dv / dt = \text{constant}.$$

This then is our required mathematical model. It was known that voltage decreases with respect to time. We have established statistically analyzing the experimental data that the decrement is indeed linear, and thus the rate of decrement is not time dependent.

Regarding testing for possible statistical rejectability of the linear models, we have proceeded in the following way. After the parameters *a* and *b* were estimated in each of the eleven cases from the observed data, the coefficients of determination were found out as follows. It is known that the total sum of squared deviations from the mean can be separated into two parts, the sum of squares of the errors and the sum of squares due to regression. Symbolically,

$$\sum (v_{\text{observed}} - v_{\text{mean}})^2 = \sum (v_{\text{observed}} - v_{\text{estimated}})^2 + \sum (v_{\text{estimated}} - v_{\text{mean}})^2.$$

In statistical terminology,

Total sum of squares (TSS)
= error sum of squares (ESS) + regression sum of squares (RSS).

It is known further that

$$\text{ESS / TSS} = \sum (v_{\text{observed}} - v_{\text{estimated}})^2 / \sum (v_{\text{observed}} - v_{\text{mean}})^2 = (1 - r^2),$$

where r is the concerned coefficient of correlation. 100 r^2 % would give us the coefficient of determination in every case. For example, for the data taken from Table 1, the coefficient of determination was found to be 99.2668 %. This means 99.2668 % of the relationship between time and the observed values of voltage (*V)* generated could be mathematically determinable, leaving only the rest 0.7332 % to errors. In other words, the mathematical model concerned has been found to be very good. For the data from the other tables also, we can see that the models have been found to be very good.

We now proceed to test the statistical validity our statement that the mathematical models are indeed very good. To test the null hypothesis $H_0 : \rho = 0$, against the two sided alternative hypothesis $H_1 : \rho \neq 0$, where ρ is the population correlation coefficient, we would use the statistic [51; page 366]

$$t = r \sqrt{(n-2)} / \sqrt{(1 - r^2)},$$

where $(n - 2)$ is the degrees of freedom, n being the number of observations. In our case, for all eleven tables $n = 5$, and therefore $(n - 2) = 3$ in every case. Now if the absolute value of such a calculated t is greater than the theoretical value of t for 3 degrees of freedom, for any specified level of significance, we would conclude that the population correlation coefficient is significantly different from zero, and in that case we would reject the null hypothesis and conclude that perhaps the population correlation coefficient is different from zero. However if the calculated value of t is less than the theoretical value, we shall conclude that there is no reason to reject the null hypothesis, and in that case the data would be accepted to reflect that the linear relationship between voltage and time is statistically invalid.

The value of $t_{0.025}$ for 3 degrees of freedom is 3.182 [51; page 533]. This means, 3.182 is our theoretical value of t at 5% level of significance. We would consider $t_{0.025}$ because we are testing a two sided alternative hypothesis that $\rho \neq 0$.

It can be seen that the calculated value of t for the data for cow dung (250 gm) is 20.1541 which is much greater than 3.182. Therefore we conclude that the null hypothesis

may be rejected at 5% level of significance. In fact, $t_{0.005}$ for 3 degrees of freedom is just 5.841 which too is very much smaller than 20.1541. This shows that the linear relationship between voltage and time is indeed very highly acceptable.

It can be further seen that for all other bio-wastes too, the conclusion is the same. Hence, it can be concluded that voltage generated is indeed a linear function of time. Again we have found that dv / dt is negative. Thus, voltage reduces linearly with time.

After establishing that voltage generated is linear in time with a negative slope, we may proceed to extrapolate how long it should take for the cells to stop functioning. Indeed, when voltage v reduces to zero,

$$\alpha + \beta t = 0.$$

This means, by the time $t = \tau$, where

$$\tau = - \alpha / \beta, \beta < 0,$$

v *reduces* to zero. In Table 3, we are showing an analysis of intercepts with the values of τ in fractional number of days for various biowastes used in the experiments.

Table 3. Intercept Analysis

Biowastes used	$v = a + b t$	τ
Cow dung	159.9 – 11.1 t	14.4
Drain water	153.6 – 10.2 t	15.0
Rice washing water	148.1 – 10.3 t	14.4
Cow dung + Slurry	207.2 – 17.8 t	11.6
Drain water + Slurry	202.1 – 16.7 t	12.1
Rice washing water + Slurry	210.0 – 16.8 t	12.5
Cow dung + Slurry + Vermicompost	170.2 – 11.8 t	14.4
Drain water + Slurry + Vermicompost	156.5 – 9.7 t	16.1
Rice washing water + Slurry + Vermicompost	164.5 – 10.9 t	15.1
Slurry	214.2 – 16.4 t	13.1
Slurry + Vermicompost	205.1 – 15.7 t	13.1

It can be seen that the cells stay functioning for a minimum of 11 to a maximum of sixteen days. Addition of slurry to cow dung, drain water and rice washing water results in reducing the life of the cell from 14.4, 15.0 and 14.4 days respectively to 11.6, 12.1 and 12.5 days respectively. Addition of vermicompost to the mixture of slurry with cow dung, drain water and rice washing water respectively increases the cell life from 11.6, 12.1 and 12.5 days respectively to 14.4, 16.1 and 15.1 days respectively. For slurry, the cell life is 13.1 days, and addition of vermicompost does not really change this.

We now proceed to analyze the effect of using combinations of biowastes instead of using any single component to generate electricity. It was expected that a combination of biowastes may result in higher voltage generation.

A scrutiny of the values of the intercepts of the straight lines fitted would throw light in this matter (Table 3). In fact, here is a rare case where the intercept has been found to be an important parameter for analysis. In the straight line

$$v = a + b\,t,$$

a is the intercept.

As can be clearly seen, while in the cases of cow dung, drain water and rice washing water the intercepts are 159.9, 153.6 and 148.1 respectively, addition of a small amount of slurry increased the intercepts to 207.2, 202.1 and 210.0 respectively. It is obvious that addition of slurry increases the amount of voltage generated considerably.

We have however found that while addition of a small amount of slurry looks very positive, the same is not the case when vermicompost was added. To the combination of cow dung and slurry, when vermicompost was added, the intercept actually came down from 207.2 to 170.2. To drain water and slurry, addition of vermicompost reduced the intercept from 202.1 to 156.5. Similarly, to rice washing water and slurry, addition of vermicompost resulted in reducing the intercept from 210.0 to 164.5. It may be noted that the reductions are very significant in all these three cases.

Increment in the values of intercepts after addition of slurry to cow dung, drain water and rice washing water was an indication that use of slurry may be highly effective for electricity generation in a microbial fuel cell. Indeed, it was found that the intercept in the case of using slurry happens to be 214.2. Addition of vermicompost reduced the intercept to 205.1.

From the values of the intercepts, it is evident that voltage generation is considerably high when we use slurry (intercept =214.2) in comparison to cow dung, drain water and rice washing water (intercepts are 159.9, 153.6 and 148.1) in that order.

We have further found that addition of slurry to cow dung for example, increases the voltage at the initial stage from 159.9 to 207.2. However, this reduces cell life from 14.4 days to 11.6 days. In other words, if we opt for high initial voltage, the cell would not last long enough, and if the cell has to have a long life, we would have to opt for low initial voltage.

It is known that bacteria will grow as long as there is an abundant supply of nutrients. Previous studies have shown that the rate of bacterial metabolism at the anode increases when the electrical potential of the anode increases. It was recognized that the electrical potential is equivalent to the concentration of electrons; and the electrons are precisely what the bacteria transfer to the anode [52]. Accordingly, to get high voltage, we have to use wastes such slurry, and we would have to keep on replenishing the waste at regular intervals.

In other words, the phenomenon of voltage drop in a MFC should not be regarded as a mere natural electrical phenomenon. It should be appreciated from the perspective that voltage drop is taking place owing to the metabolism of bacteria inside the anaerobic zone of a MFC. Indeed, the more biowaste or in other words, the more nutrients the bacteria get, the more current generation would take place. After a particular period of time, when the nutrient supply would start getting depleted, electricity generation would subside too which would thus translate into a drop in electric potential.

8. Conclusions

From the analysis of the data, we can conclude that:

(i) Both individually and in combinations, cow dung, drain water, rice washing water, and slurry, can be used in microbial fuel cells to generate electricity.

(ii) Voltage generated decreases *linearly* with respect to time. It means, the first order derivative of voltage generated with respect to time is a negative constant.

(iii) The decrease in all of the cases is very highly *steep*, the straight lines concerned making obtuse angles lying between 93.21^0 (slope = -17.8, for cow dung + slurry, Table – 3) and 95.88^0 (slope = -9.7, for drain water + slurry + vermicompost , Table - 3) with the positive direction of the time axis.

(iv) True to our expectations, sometimes a mixture of biowastes can actually result even in higher extractable current than any single component. But this is not always true in general. Addition of slurry increases the voltage generated to a very high extent. However addition of vermicompost does actually have a negative effect.

(v) Cell life decreases after addition of slurry, but it increases after addition of vermicompost. In other words, when a component results in higher voltage production, it ends up reducing the cell life. This is an important finding we would like to note.

(vi) Accordingly, so as to attain comparatively high voltage while cell life is not reduced, proper bio-wastes need to be replenished at regular intervals of time. Therefore, the phenomenon of voltage drop is obviously less critical in microbial fuel cells in comparison to the other conventional cells, because replenishment of biowaste is possible in an MFC.

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