Generation of Electricity Using Microorganisms Isolated from Dump Site Soil

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Abstract— **Microbial fuel cell (MFC), as a new technology for energy generation, has gained a lot of attention in converting a wide range of organic and inorganic substrates to bioelectricity in recent years. This research work involved the isolation of electricigens (organisms that produce electrons) for use in a microbial fuel cell (MFC). The soil sample used served as the anode and showed a high microbial count. The bacterial counts ranged from 3.0 x 10^6 cfu /ml to 5.0 x 10^6 cfu /ml, while the fungal count ranged from 2.0 x 10^6 cfu/ ml to 3.0 x 10^6 cfu/ml. The microorganisms isolated were *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Aspergillus niger, Penicillium niger, Aspergillus fumigatus and Candida spp.* The voltage readings from the MFC peaked at Day 4 (1.100V) under the effect of sparging, and dropped gradually to 0.670V after the 12th Day. Addition of oxygen and reduction in MFC temperature to 24^{0}C lead to an increase in electricity production (1.235V and 1.260V respectively). When the MFCs were connected in series, a bulb was lighted with a current of 0.33mA and with a total voltage of 1.710V.**

Keywords— ***Bioelectricity,*** **electricigens,** ***Microbial Fuel Cell (MFC), Electron Transfer*** **Introduction (*HEADING 1*)**

INTRODUCTION

The need for cheap and consistent energy in the world continues to increase and in an effort to aid Energy independence and to generate electricity that poses no danger to the environment, research initiatives are focused on alternative, affordable, accessible and eco-friendly energy that would help in economic growth if achieved and the need for alternative energy sources are in high demand due to different growing economies facing the problem of energy crisis (1,2).The production of electrical energy from microorganisms' interaction with electrodes using electrons which are either removed or supplied through an electrical circuit. This system is called the Bio electrochemical System (BESs), which is a recent technological invention with the most described type being the Microbial Fuel Cell

(MFC). MFCs are bio-electrical or bio-electrical devices which convert the energy in present in chemical bonds of organic compounds into electrical energy under anaerobic conditions, through catalytic activity. These microorganisms within the MFC breakdown the sugars and other nutrients in their environment and release energy contained within that food in the form of electricity (3). Challenges experienced by people in the rural areas are too numerous to mention, starting from minimal electricity supply to power handsets or electrical lamps or the presence of large tones of wastes in areas like India coupled with the deteriorating Ozone layer which exposes the Earth to the harsh ultraviolet rays, to ameliorate the problem of electricity supply being limited, the need for a cheap and readily available source of electric power is needed which can be supplied by a MFC which provides clean, safe, quiet performance with zero to minimal use hydrocarbons (4,5) and uses less energy, cost effective and is easy to operate among other advantages. The use of MFC should be encouraged because it can be used to clean up the environment asides its main function of electricity generation, it can also be used in a brewery factory for purification of the sludge gotten during the course of alcohol production. MFC can solve the problem of replacing batteries of underwater surveillances devices which are placed in remote areas of the world and it can also be serve as a means of producing hydrogen.

MFC technology makes use of bacteria for generation of bioelectricity by oxidation of organic waste and renewable biomass (6) represents the future of mass electrical power in a cheap and affordable way. Electrigenic microorganisms "breathe" metal compounds much like humans and other organisms breathe oxygen which MFCs take advantage of. A MFC provides these electrigenic microorganisms with a certain configuration of two inert, carbon-based electrodes placed in environments with different amounts of oxygen and substrate concentration (7). Electrons are transferred to the anode by three basic means which are, by electron mediators or shuttles (8, 9), direct membrane associated electron transfer (1) or by connective appendages otherwise known as nanowires produced by bacteria (10, 11, 12) or maybe by other unknown means (13). The transfer of electrons from microorganisms in the anode of the fuel cell to the electrode is facilitated by mediators such as thionine, methyl blue, neutral red and humic acid (14), these mediators allow for electricity production by microorganisms unable to use the electrode. MFCs of this nature are called Mediator MFC, in situations where no mediator is added the MFC is termed a Mediator-less MFC. In many cases, the electrons that reach the cathode combine to protons that diffuse through the Proton Exchange Membrane and oxygen provided as a result of sparging resulting in production of water. (15). MFC operated using mixed cultures achieved greater power frequencies than those with pure culture (8,9) although recent findings have shown that a MFC operated using a pure culture generated power at high densities but the same device was not tested using acclimatized mixed cultures and the cells were grown externally (16). The analyses of the community of microorganisms that are present in MFCs have so far showed a great diversity in composition (8, 11, 17, 18, and 19).

The amount of free energy produced either by normal microbial metabolism or by microbial fuel cell systems is determined mainly by the potential difference (ΔE) between the electron donor and the acceptor according to the following equation:

$$-\Delta G = nF\Delta E,$$

where ΔG is the variation in free energy, n is the number of electron moles, and F is the Faraday constant (96,487 J/V) (20).

Different MFCs can be constructed using a variety of methods and material which would operate under the same principle with a wide range of conditions such as temperature, pH, electrode surface areas, and substrate used. The ranges of condition and in some cases a lack of important data like the internal types of MFCs provide information on construction materials.

The aim of this work was to produce a biological system, where microbes will be used as a power source to drive the circuit and enhance the flow of current, via the production of electrons as a medium for energy generation.

Materials and Method

Sample Collection

Soil sample was collected from at a waste dumpsite located at Aguwan Biri, Minna, Niger State. The sample was transported to the Microbiology Laboratory for analysis, which was carried out immediately after the collection of the sample.

Isolation and Characterization of Micro-organisms

Bacteria was isolated and identified using cultural identification, morphological identification using Gram staining reaction and other biochemical test, as described by Cheesbough (21).

Construction of fuel cells

Three single fuel cells made up of single glass chambers were prepared in the laboratory using galvanized zinc as the anode and carbon rod as the cathode, and autoclaved seawater as the electrolyte. All glassware, plastic ware, and electrodes were autoclaved before use for 20 min at 120 °C. The containers were drilled to make a hole that fits exactly into the PCV pipes, a hot gum was used to ensure fitting without leaking. The PEM was made by soaking wool with 60 grams dissolved in 40 ml of water and heated to boil, and then it was sterilized using the hot air oven at 70^{o}C for 30 min. The essence of doing this was to allow the passage of H^+ to the cathode leading to the formation of water when it reacts whit oxygen. Sterility was ensured by using 70 % ethanol for decontamination. 100% cotton cloth was burnt in a closed thin container and inserted into the galvanized zinc, this was done to provide a source of carbon for the microbes and make them attracted to the anode. All materials were put in place and the sterility was ensured. Each cell was constructed from a 5-L cylindrical flask equipped with a sidearm ending in a 60-mm Schott flange tooled to accept an o-ring (Ace Glass; custom design); the Nafion membrane was pressed against the cathode side with a CAPFE o-ring, and the joint held in place with a quick release clamp. The top of each chamber was equipped with a 150-mm Schott flange, sealed with a silicone o-ring, and each cap was equipped with seven 24/40 ground glass female inlet ports. The anode chamber was fitted with a galvanized zinc anode, a pH combination electrode (Microelectrodes Inc., Bedford, NH, USA), and a polyfluorallomer (PFA) tube [Cole Parmer, (Vernon Hills, IL, USA) 1/ 4%% od] for N2 bubbling. All apparatus and electrodes were sterilized by autoclaving or by rinsing in denatured alcohol (22, 23).

Plate 1. Assembled Microbial Fuel Cells of two different sizes

Effect of non- sparging and sparging on Voltage Readings

The contents of both cathode and anode chambers were mixed continuously with magnetic stirrers and purged with air. Air was passed through separate 0.3 mm-pore-size HEPAVENT filters (Whatman, Middlesex, UK) prior to entering the fuel cell. The cell voltage was controlled using a Model DLK60 potentiostat (Analytical Instrument Systems Inc., Flemington, NJ, USA), while whole-cell potential, anode potential (vs. Ag/AgCl) and current were recorded using a multimeter (Agilent 34970 A data acquisition unit with a 20-channel multiplexer module, 34901 A; Agilent Technologies, Palo Alto, CA, USA). Readings were taken at different temperatures ranging from 24 ^{o}C to 28 ^{o}C for a period of 12 days.

Effect of Substrate Concentration on Voltage Readings

The substrate used in the MFC was dry sugar cane shaft (bagasse). The substrate was added in varying concentration of 2g, 4g and 6g into three (3) different MFCs labelled X, Y and Z. The readings were taken for a period of 7 days using a millimetre.

Results

The soil sample used, which served as the anode, showed high microbial count. The bacterial counts ranged from 3.0 x 10^6 cfu /ml to 5.0 x 10^6 cfu /ml. The fungal count ranged from 2.0 x 10^6 cfu/ ml to 3.0 x 10^6 cfu/ml respectively.

The result Biochemical reaction and culture characterization bacterial soil isolates is shown in Table 1

TABLE 1: Biochemical reaction and cultural characteristics of bacterial soil isolates.

C	Gram Reaction	Shape	Citrate	Urease	Indole	Methvl Red	Voges Proskaeur	Oxidase	Catalase	Coagulase	Mannitol Salt	H2S Production	Starch hvdrolvsis	Organisms
A	-	R	-	+	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
B	+	C	-	-	-	-	-	+	+	-	-	-	-	*Micrococcus spp*
C	-	R	-	-	+	-	-	-	+	-	-	-	-	*Escherichia coli*
D	+	C	-	-	-	-	-	-	+	-	-	-	-	*Staphylococcus epidermis*
E	-	R	+	+	-	-	+	-	+	-	-	-	-	*Klebsiella pneumonia*
F	+	C	-	-	-	-	-	+	+	-	-	-	-	*Micrococcus spp*
G	-	R	+	-	-	_	+	+	+	-	+	-	+	*Pseudomonas aeruginosa*
H	+	C	-	-	-	-	-	-	+	-	-	-	-	*Staphylococcus epidermis*

The electron producing microorganisms isolated were *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Aspergillus niger, Penicillium niger, Aspergillus fumigatus and Candida species.*

Effect of non sparging and sparging on voltage readings

The effect of sparging and non-sparging on microbial fuel cell performance is shown in Fig. 1. From the results obtained, the highest amount of electrical energy in volts was 1.20v on day 5 and the lowest was 0.55v on the first day of the experiment. The readings in this table were taken at temperatures ranging from 24 to 28 ^{o}C, the reason for using the non sparging and sparging as variables resulted due to the fact that oxygen has been termed as the best electron acceptor for it is readily available, inexpensive, produces no waste products and leads to an increase in voltage readings by stimulating the production of more electrons and this also results in reduced proton losses as reported by Gill *et al, (*29). The increase in voltage values is seen in the table. The initial voltage readings were low subsequently the values began to rise with the highest values being 1.270V as a result of sparging and 1.135V when the MFC was not sparged. The readings were taken for a period of 12 days, on days 11 and 12, a decline in voltage readings occurred depicting a typical batch culture system since there was no addition of substrate to the MFC (22).

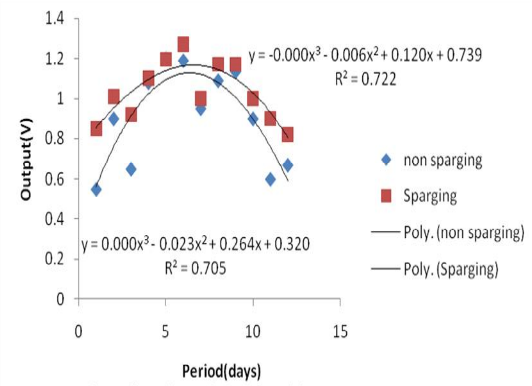

Fig. 1 Effect of sparging on electricity output

Effect of Substrate Concentration on Voltage Readings

The concentration, type and properties of the substrates used in a MFC affects the power output and also the microorganisms present. The substrate used in the MFC was dry sugar cane shaft (Bagasse). The substrate was added in varying concentrations of 2g, 4g and 6g into three (3) different MFCs labelled X, Y and Z. The readings were taken for a period of 7 days. The first day had the highest voltage readings, with MFCX- 0.625V, MFCY- 0.550V and MFCZ-0.535V. On the second day, a rapid decline in voltage readings occurred in MFCX-

0.370V, MFCY-0.400V and MFCZ-0.600V and this change continued till the experiment came to an end. It was observed that instead of the voltage readings to increase, a decrease occurred with the final voltage readings being 0.280V for MFCX, MFCY-0.280V and MFCZ-0.290V. The decrease in voltage readings could be attributed to low substrate concentration or high solution resistance (Park *et al.,* 2003). The over lapping of the graph as seen in Figure 2 as a result of the close proximity of the voltage values. The voltage readings decreased with increase in substrate concentration for all the substrate concentrations up to the 7th day.

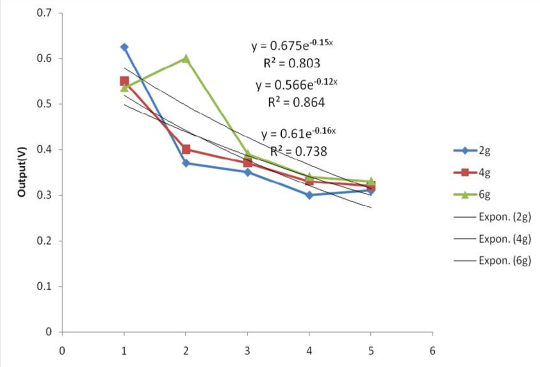

Fig.2 Effect of substrate concentration on electricity out put

Discussion

This research was carried out to determine the presence of microorganisms that are capable of producing electrons and also to determine their optimum function conditions, the research involved the production of a microbial fuel cell (MFC), which produced electricity indicating the presence of ELECTRICIGENS (organisms that produce electrons). The electron producing microorganisms isolated were *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Aspergillus niger, Penicillium niger, Aspergillus fumigatus and Candida species.* Similar microorganisms were also isolated by Ishwar (24) and lovely (25) from abattoir waste water. Microorganisms in the MFC metabolize organic substrates and transfer electrons to an electrode surface. The oxidation of the organic material produces both electrons and protons from the substrate being oxidized. Electrons are transferred to the anode and then to the cathode through a network with microorganisms acting as catalyst on the anode surface as catalysts. The above mentioned microorganisms isolated in the MFC are not true catalysts since they obtain energy from the oxidation of the substrate to support their own growth and create an energy loss which would result in a MFC producing electricity indefinitely, if the anode-associated microorganisms are maintained under favourable conditions (26).

Pumping air to the cathode chamber and its effect on the microbial fuel cell performance was detected. It was observed that increasing oxygen concentration has a positive value on the cell performance which is attributed to the growing of cultures that are nourished by oxygen in the cell. The resultant values obtained differed because sparging stimulates the production of more electrons thereby leading to an increase in the voltage values (Fig 1). Increase in Voltage values was observed when the cathode was shaken or when the water present in the cathode was exposed to air. The start-up temperature of the MFC (28^{o}C) also contributed to the values measured during the course of the research. Generally, the performance of the cell was better in case of air sparging compared to the when the MFC was not sparged. Similar results were also obtained by Lovely(27) who observed that air sparging resulted to increase in electricity generation. The highest amount of electrical energy in volts was 1.20v on day 5 and the lowest was 0.55v on the first day of the experiment. This was in contrast to results obtained by Hussein (28) who recorded 0.58v as the highest. This could be as a result the substrate used, the nature of the electrode, and the operating environment, which significantly affected the metabolism of the cells in the MFC. The internal resistance in a single chambered MFC is reduced due to the high voltage reading of 1.270V which is due to the close proximity of the anode and cathode, also adequate mixing which occurs during sparging leads to reduced proton losses as also observed by Gil et al (29).

Temperature affects the growth and metabolism of microorganisms and hence increased initial formation of exoelectrigenic biofilm, although some microorganisms are known to function at higher temperatures. Low voltage readings were recorded at the beginning of the experiment, depicting the lag phase of a batch culture, after which the voltage values increased with the highest value, 1.270 V obtained at 24^{o} C indicating optimum temperature for high voltage generation.

Another factor that affect the start-up time is substrate addition. The effect of different concentrations of dry sugar cane bagasse (which consists of 45-50% of cellulose) was determined. Addition of the different concentrations of the substrate lead to increase in voltage values for a period of 7 days. The decline in voltage with time in the 3 MFCs used suggests that the carbon source in use had an inhibitory effect on electricity. The decrease in voltage readings could be attributed to low substrate concentration or high solution resistance (26). The values from Fig.2 over lapped each other due to the close proximity of the voltage values. The results were in agreement with those obtained by . Zhang et al (30), who stated that an increase in the substrate concentrations promotes the substrate degradation and consequently results in a higher power generation. These results have important implications for the use of MFCs for electricity generation and wastewater treatment. MFCs can operate over a wide range of temperatures, as stated above but it is also evident that set up procedures can dictate the system performance.

CONCLUSION

Microbial fuel cells are evolving to become a simple, robust technology that can be used to generate power. Some of the conditions for the workability of a MFC include- Oxygen provision at the cathode, adequate substrate and ambient temperature (24-30^{0}C) with the highest voltage reading by 1.27V.When the MFCs were connected in series, a bulb was lit with the total amount of voltage being 1.710V and the current being 0.33mA. With the production of enough energy to power a bulb, this shows that a MFC can serve as a cheap source of power gotten from soil microbe. Six set of MFC were also connected in series and this connection was able to produce 6.000 Volts, this voltage amount can be used to power a mobile phone battery.

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