

Potentials of Mixed and Axenic Microbial Fuel Cells for Electricity Generation

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Abstract: The study was aimed at investigating the potentials of mixed and axenic microbial fuel cells for electricity generation. The study was carried out within a period of six months. 500 mls of domestic kitchen waste water was collected from various locations in Calabar Metropolis, into tightly corked plastic containers and transported to the laboratory for analysis. Microbial fuel cell was designed using plastic containers, graphite electrodes, salt bridge and domestic waste water. Microorganisms from the domestic waste water were identified using standard microbiological techniques. Results from the study showed that a higher mean voltage (1.58v) was recorded by microbial fuel cells with mixed consortium (stacked waste water microbial fuel cell), as compared to that obtained from microbial fuel cell with axenic culture. Among the bacteria cells identified from the waste water, *Salmonella* had the highest voltage production (0.68v) as compared to other isolates. The comparative analysis of voltage density of the fuel cells showed that the stacked waste water microbial fuel cells had the highest voltage density (0.072v/m²) compared to others. However, this study has shown that microbial fuel cell technology could serve as a new core technology for conversion of waste to electricity in future.

Keywords: axenic microbial fuel cells, microbiological techniques, domestic waste water.

1. INTRODUCTION

Energy sources such as solar, wind, hydroelectricity, geothermal among others has been over depended on, to satisfy the growing energy concerns for today and tomorrow, and this has made the search for other options which may involve the exploitation of microbiology and microorganisms as a major stakeholder in energy production of the new age. In the future, renewable energy will constitute a greater portion of the world's energy consumption and production (Potter, 2003; Allen & Bennetto, 2004; Rabaey & Verstraete, 2003; Davis & Higson, 2003; Ieropoulos *et al*, 2006; Park & Zerkus, 2000; Tender *et al*, 2008). Recent predictions for the global energy have lead to the quest for alternative energy resources (Lovley, 2003; Kim *et al*, 1993; Kim *et al*, 2003; Bond & Lovley, 2003; Ringeisen *et al* 2006; He *et al*, 2005; Niessen *et al* 2004; Davis and Higson, 2007). The rate of reduction in non renewable sources of energy implies that there is urgent need for highly efficient energy transformation technologies and ways to use alternative renewable energy sources (Rosenbaum *et al*, 2006). Microbial fuel cells (MFC) technology represents a new path of energy production by harvesting electricity from what would have been regarded as a waste material (Alterman *et al*, 2006). This technology utilizes mostly anaerobic bacteria which may be present in the waste water and works as catalysts to produce electricity while treating waste water (Moon *et al*, 2006). Microorganisms have the capacity to generate electricity from a wide range of organic waste while oxidizing the waste to less harmful forms (Logan, 2006). Although MFCs generate small amount of power than hydrogen fuel cells, a blend of both electricity generation and waste water treatment could reduce the cost of primary treatment of effluent waste water (Rabaey *et al*, 2005). Currently, studies performed on MFCs are concerned with how to increase the power density of the system with regards to the peripheral anode surface area, while little investigation has been carried out on determining the influence of voltage output in contrast to the varying fuel cell components. The main aspect of fuel cell research is to reduce the treatment costs and simplify process implementation conditions (Min *et al*, 2005). Many of the current relevant research are focused on the development of the ways and means to convert chemical energy stored in biomass to electricity (Kim *et al*, 2008). The energy transformation from burning of biomass- chemical to heat, and subsequent utilization to heat for different purposes is less efficient (Kim *et al*, 2008). Since most rural population depend on subsidized yet scarce electricity supply, a technology such as MFCs can convert the energy stored in organic wastes via enzymatic reactions associated with the activity of microorganisms (Min *et al*, 2005; Rabaey & Versaete, 2003).

2. MATERIALS AND METHODS

MATERIALS:

The microbial fuel cell used in this study was constructed with the following materials; Plastic containers with lid to serve as the anode and cathode, PVC pipe for use as the salt bridge, Graphite rods from dry cell battery to serve as electrodes, Other materials included: PVC glue, agar-agar, salt (Sodium chloride), insulating masking tape, copper wires, measuring cylinder, petri dishes, pipette, beakers, funnel, conical flasks, microscope, test tubes, syringes, digital multimeter, etc.

SAMPLE COLLECTION:

500ml of Domestic kitchen waste water was collected from various locations in Calabar metropolis. The samples were dispensed into tightly cork plastic containers and transported to the University of Calabar Microbiology laboratory for analysis.

MEDIA:

The media used for the study were Nutrient agar and MacConkey agar.

METHODS:

PREPARATION OF A MIXED CULTURE MICROBIAL FUEL CELL FROM WASTE WATER:

The plastic containers used were washed and air dried, the graphite electrodes were also washed and dried to remove any salt and acid residue. Copper wires were attached to the electrodes with the means of soldering iron so as to enable the transmission of electrons between the compartments. A known volume of 150ml of domestic kitchen waste water was added into the container that was to serve as the anode and sealed to ensure that the microorganisms contained are forced into anaerobic condition. Equal volume of sterile distilled water was put into the container to serve as the cathode and a salt bridge was used as the proton exchange membrane (PEM) for the transfer of protons across compartments (Delaney et al 2008).

The following method was employed in preparing the salt bridge; the agar and salt mixture was to be mixed in the ratio of 3:1 to the required volume of distilled water and heated in an autoclave at 121°C for 15 minutes at 15 PSI which is aseptically transferred into a sawed PVC pipe with diameter of 2 inches, that was sealed at one end to hold the agar and is left to cool and solidify (Deka and Barua, 2010)

The anode, cathode and salt bridge is joined together in a H-shaped configuration as described by Logan *et al.*, 2006. Holes were bored into the lids to pass wire already soldered to the electrodes and all were connected to a multimeter to detect the passage of electric current measured in milliamperes (mA) and the voltage in millivolts (mV)



PLATE 1: photograph of the graphite electrodes



PLATE 2: photograph of double chambered waste water microbial fuel cell

SAMPLE PREPARATION FOR MIXED CULTURE MICROBIAL FUEL CELL:

Sample of the waste water for bacteriological isolation was drawn by washing the anodic electrode in 9.0ml of sterile distilled water to rinse the biofilm already accumulated on the electrode thereafter 1.0 ml is taken for serial dilution.

PLATING PROCEDURES:

1.0 ml of waste water is aseptically withdrawn and is diluted with 9.0 ml of distilled water and mixed thoroughly, then, 1.0 ml of mixture is withdrawn and mixed with another 9.0 ml of distilled water. Decimal dilutions of 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} were made and 10^{-3} , 10^{-5} , 10^{-7} dilutions as well as the undiluted sample were plated out using pour plate method on nutrient agar and MacConkey agar and incubated at room temperature of 30°C

PURIFICATION OF ISOLATES:

At the end of 24 hrs incubation period, the various colony types were noted and were sub-cultured onto nutrient agar plates. Agar slants were prepared for the storage of the bacterial colonies.

PREPARATION OF MICROBIAL FUEL CELL USING AXENIC CULTURE SYSTEM:

Axenic culture can be described as, a bacteria culture which is free from contamination from other microorganisms; therefore axenic culture is what microbiologists refer to as a “pure culture”- which contains a single bacterium species from a single colony.

For the purpose of microbial fuel cells, the broth type axenic culture was used. The underlisted steps were used in order to achieve my aim of comparing the voltage of the various organisms isolated from the waste water.

fifteen grams of peptone medium was mixed in 1000ml of water to make peptone water which was sterilized using the normal technique of 121°C at 15PSI for 15 minutes.

Microbial fuel cells were prepared using the ‘H’ shaped method described by Logan *et al.*, 2006 which was the design built in section 3.4 with the exception of the source of the electrolyte.

The electrolyte for the axenic culture MFC was obtained by mixing 10ml of bacterial suspension into the anodic chamber containing peptone water while the cathode chamber contained sterile distilled water.

The setup was left to stand for 10 days and voltage readings were taken using a multimeter (DT380D) digital multimeter.

METHOD FOR PREPARING A TEN (10) STACKED MICROBIAL FUEL CELL USING DOMESTIC WASTEWATER:

Ten (10) individual fuel cells are prepared as stated using the H-shaped double chambered technique as described by Logan *et al.*, 2006. The anodic electrolyte is maintained as waste water while the catholyte was changed from sterile distilled water to an oxidizing agent-Potassium ferricyanide. These ten cells were connected in series using flexible copper wires and the single positive and negative terminals to the multimeter for measurement of the voltage. The setup was left to stand for 10 days after which records of results stopped.



PLATE 3: Axenic culture microbial fuel cell



PLATE 4: Axenic culture microbial fuel cell

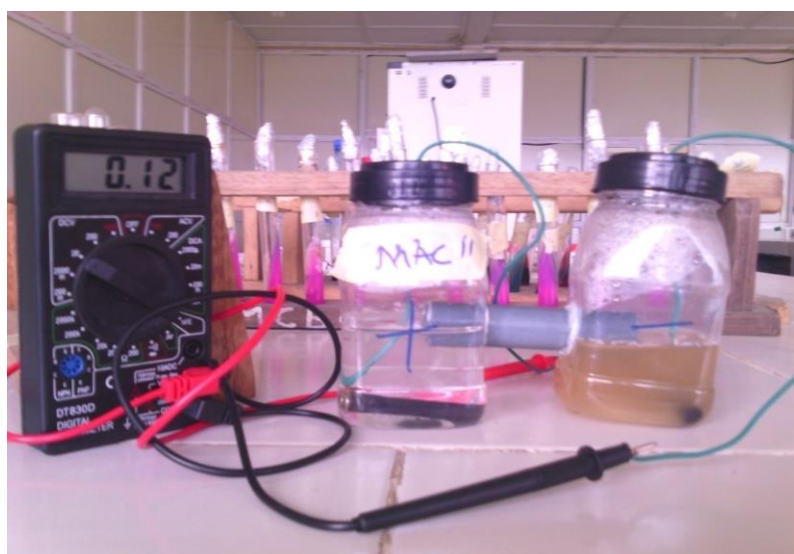


PLATE 5: Axenic type microbial fuel cell



PLATE 6: Axenic culture microbial fuel cell



PLATE 7: Stacked wastewater microbial fuel cell

3. RESULTS

WASTEWATER MICROBIAL FUEL CELL:

The project results come in two parts; the first part is the results gotten from the incubation of the waste water. The wastewater was gotten from the domestic kitchen i.e. water which has been used for washing various items in the house. The waste water was subjected to the treatment using the microbial fuel cell technique to see how much electricity it generates. The microbial fuel cell was observed daily for ten (10) days and the results were recorded to show an increase or decrease in voltage and current according to the growth phase of the microbes in the water sample-lag, log, stationary and decline phases. The results obtained and recorded are gotten from a batch type culture. The MFC was also allowed to stand for about a month without replacing the waste water for about a month until it ran out of electrical power.

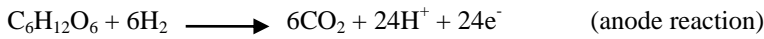
The double chambered microbial fuel cell as the name implies, consists of two parts or chambers the cathode and the anode separated by a proton exchange membrane (salt bridge). According to Logan 2006, which states that the H-shape systems are acceptable for basic parameter research, such as examining power production using new materials, or types of microbial communities that arise during the degradation of specific compounds, but they typically produce low power densities. The H-shape system was also found easy to build and manage had has been adopted by various researchers in their work Jang *et al*, 2004, park & Zeikus 2003 etc thus conforming to the statement of Logan 2006. Thus the reason for using this system for the determination of electrical energy generated by the waste water notwithstanding the many disadvantages that arises in using the system.

The anode compartment is negatively charged using the interactions of the microbes to generate protons and electrons. The protons are transported across the PEM to the positively charged cathode chamber where it is oxidized either by the oxygen in air or by an oxidizing agent.

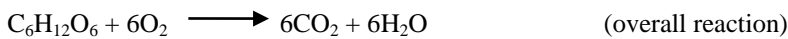
The equation for the cathode reaction is given below:



The reaction for the anode compartment is given as follows:



The general equation for both anode and cathode is given as:



The fuel cell was setup with the anode containing the wastewater and the cathode sterile distilled water the amount of voltage observed was within the voltage density range of the research works done by various authors/ researchers for waste water and microbial fuel cell in general which is about 0.1-1.5mV and a voltage density of 0.06 (Wang et al, 2009a). The results are shown in Table 1.

TABLE 1: Domestic kitchen wastewater microbial fuel cells voltage records

	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	TOTAL	MEAN	D=V/A
WWMFC 1	0	0.05	0.1	0.3	0.3	0.5	0.5	0.8	0.8	1.1	1.1	5.55	0.505	0.039
WWMFC 2	0	0.1	0.2	0.4	0.4	0.6	0.8	0.8	1.2	1.2	0.8	6.5	0.591	0.046
STACKED WWMFC	0	0.4	0.8	1	0.8	1.5	1.9	2.02	2.5	3	3.5	17.42	1.584	0.072

Legend: WWMFC= wastewater microbial fuel cell.

$$D=V/A$$

Where

D= voltage density

V=voltage

A=area of electrode (v/m²)

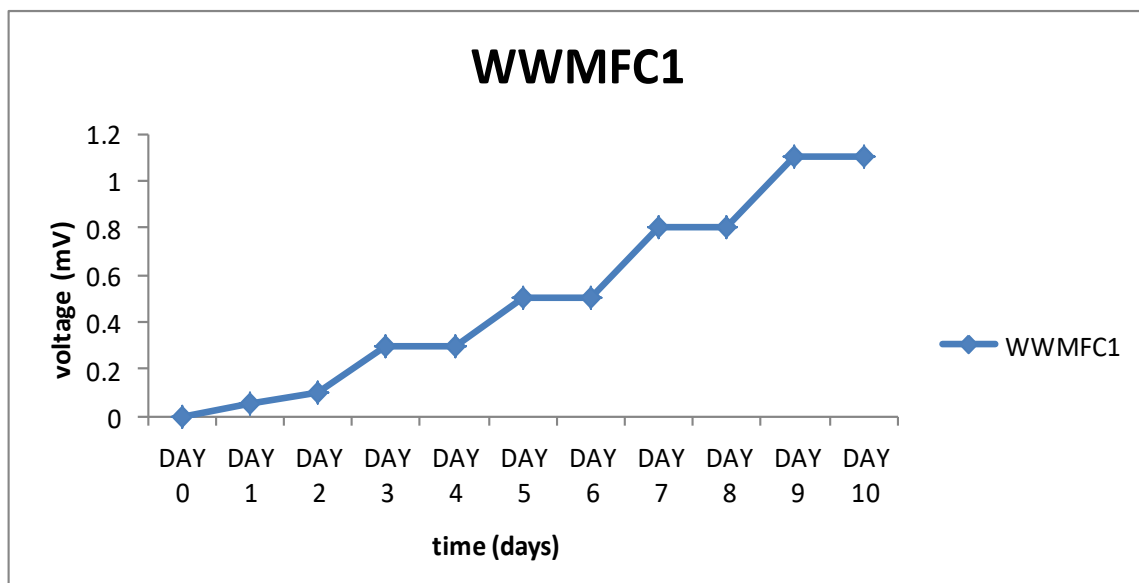


FIGURE 1

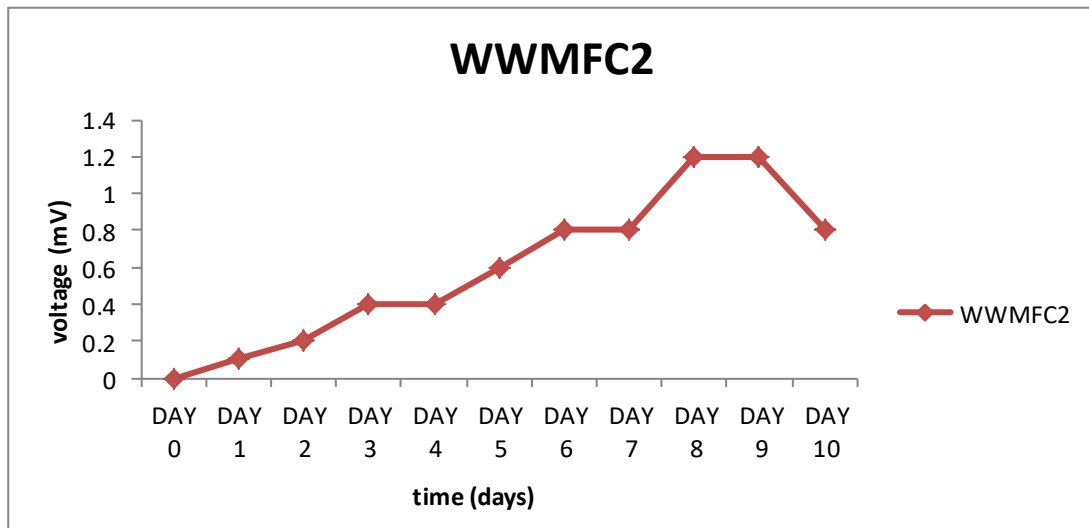


FIGURE 2: Graph of voltage (mV) against time (days) for wastewater fuel cell 2

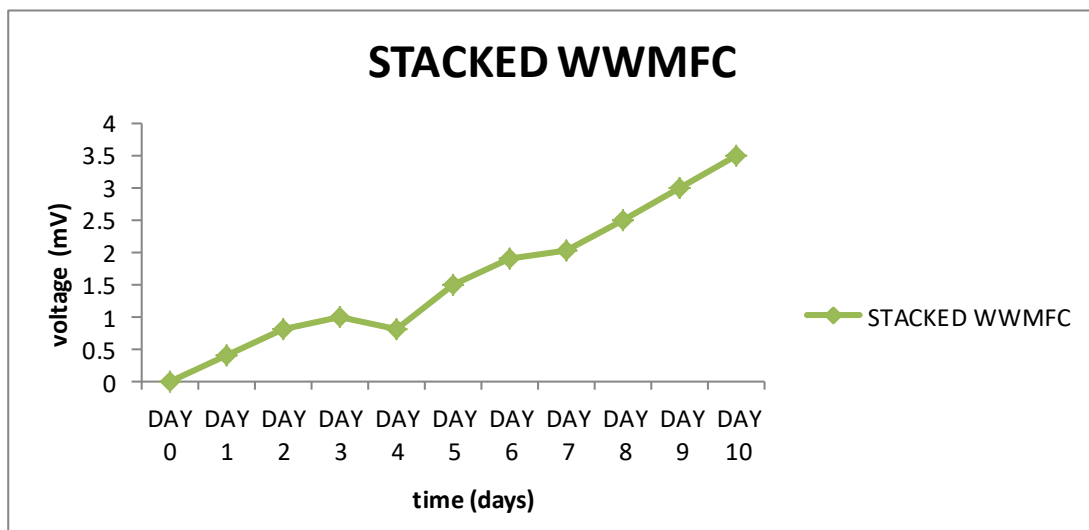


FIGURE 3: Graph of voltage (mV) against time (days) for stacked wastewater fuel cell

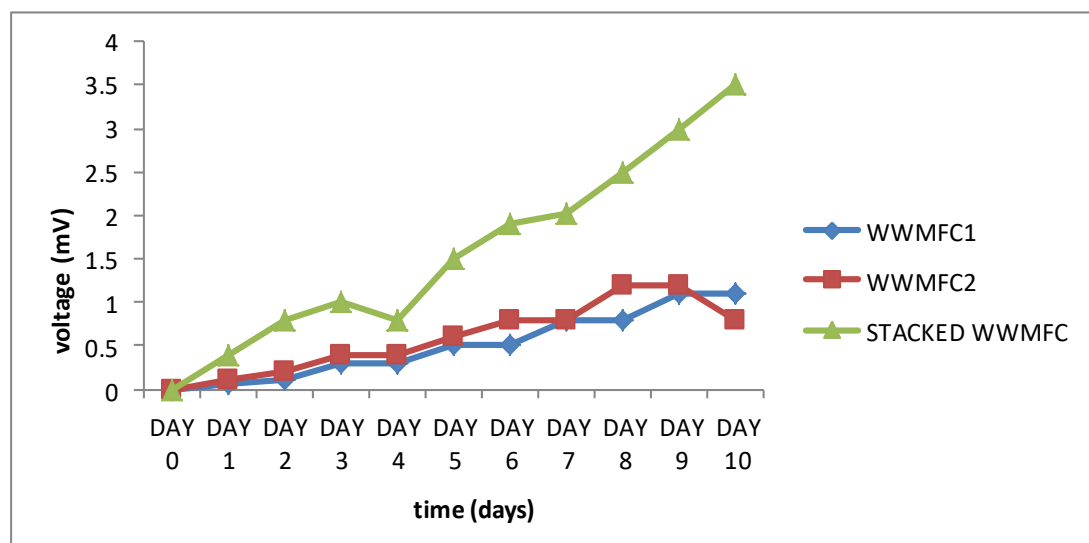


FIGURE 4: combined for all fuel cells; Graph of voltage (mV) against time (days)

LEGEND:

Y-axis = voltage reading (mV)

X-axis = time (days)

PLATE COUNT AND CULTURAL MORPHOLOGY:

The plates were incubated at 37⁰C for 24hrs in triplicates and discrete colonies were enumerated.

BIOCHEMICAL ANALYSIS:

Purified isolates were subjected to various biochemical tests and were identified based on morphological and biochemical characterization.

TABLE 2: Total bacterial count from domestic kitchen wastewater

	NUMBER OF COLONIES	DILUTION FACTOR	PLATING FACTOR	COLONY FORMING UNIT (CFU)
NA ISOLATES				
10⁻³	TNTC	10 ⁻³	0.1	TNTC
	TNTC	10 ⁻³	0.1	TNTC
10⁻⁵	80	10 ⁻⁵	0.1	8.0 X 10 ⁵
	110	10 ⁻⁵	0.1	1.1 X 10 ⁴
	130	10 ⁻⁵	0.1	1.3 X 10 ⁴
10⁻⁷	50	10 ⁻⁷	0.1	5.0 X 10 ⁷
MAC ISOLATES				
10⁻³	69	10 ⁻³	0.1	6.9 X 10 ³
	120	10 ⁻³	0.1	1.2 X10 ²
	60	10 ⁻³	0.1	6.0 X 10 ³
10⁻⁵	80	10 ⁻⁵	0.1	8.0 X 10 ⁵
	40	10 ⁻⁵	0.1	4.0 X 10 ⁵
	40	10 ⁻⁵	0.1	4.0 X 10 ⁵
10⁻⁷	30	10 ⁻⁷	0.1	3.0 X 10 ⁷
	42	10 ⁻⁷	0.1	4.2 X 10 ⁷
	36	10 ⁻⁷	0.1	3.6 X 10 ⁷

LEGEND:TTNC= Too Numerous To Count; NA isolates= nutrient agar isolates; MAC isolates = macConkey agar isolates

AXENIC CULTURE MFC ANALYSIS:

11 microbial fuel cells (MFCs) were designed using the method described by Logan 2006 that is the H-shape configuration. In the cathode compartment was sterile distilled water and in the anode, sterile peptone broth was inoculated with the various isolates gotten from the initial sample the waste water and the setup is allowed to stand for 10 days in a dust free area in the laboratory with good ventilation and at ambient temperature and pressure and results are taken 3 times daily and the average daily voltage increase was recorded and the results are shown in Table 3.

TABLE 3: Table of values for the axenic culture microbial fuel cell

	DAY0	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7	DAY8	DAY9	DAY10	TOTAL	MEAN	D=V/A
NA1	0.01	0.26	0.32	0.35	0.36	0.41	0.44	0.35	0.33	0.37	0.43	3.63	0.33	0.026
NA2	0.01	0.11	0.19	0.15	0.17	0.21	0.24	0.25	0.26	0.31	0.36	2.26	0.21	0.016
NA3	0.01	0.24	0.31	0.35	0.35	0.49	0.62	0.65	0.66	0.66	0.61	4.95	0.45	0.035
NA4	0.01	0.04	0.12	0.25	0.29	0.32	0.35	0.3	0.25	0.12	0.04	2.09	0.19	0.015
NA5	0.01	0.13	0.12	0.09	0.08	0.07	0.07	0.07	0.08	0.08	0.09	0.89	0.08	0.006
NA6	0.01	0.11	0.15	0.17	0.19	0.17	0.19	0.2	0.23	0.23	0.3	1.95	0.18	0.014
NA7	0.01	0.14	0.15	0.3	0.31	0.32	0.3	0.29	0.2	0.15	0.14	2.31	0.21	0.009
MAC1	0.01	0.06	0.09	0.06	0.08	0.1	0.14	0.12	0.08	0.06	0.04	0.84	0.08	0.006
MAC2	0.01	0.07	0.13	0.15	0.16	0.14	0.17	0.19	0.44	0.63	0.62	2.71	0.25	0.019
MAC3	0.01	0.16	0.23	0.25	0.24	0.19	0.19	0.2	0.21	0.19	0.19	2.06	0.19	0.014
MAC4	0.01	0.04	0.12	0.25	0.29	0.32	0.35	0.3	0.25	0.12	0.4	2.45	0.41	0.032

LEGEND:

NA= Nutrient agar isolates

MAC- macConkey agar isolates

D=V/A

Where

D= voltage density

V=voltage

A=area of electrode (v/m^2)

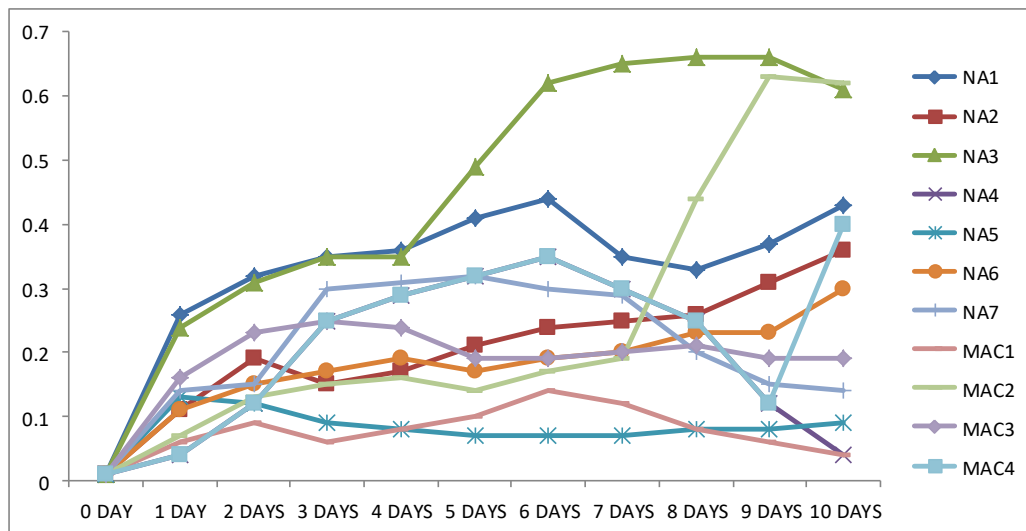


FIGURE 5: axenic cultures MFC compared; Graph of voltage (mV) against time (days)

VOLTAGE DENSITY:

Voltage density is a property of microbial fuel cell used to calculate the efficiency of the cells. It is calculated by measuring the area of the electrode used and dividing it by the voltage. It is measured in voltage per unit area.

Formular for calculating voltage density is given by:

$$\text{Voltage Density} = \text{Voltage} \div \text{Area Of Electrode}$$

$$\text{V.D.} = \text{V/A (mVM}^{-2}\text{)}$$

WHERE:

V.D. = Voltage Density

V = Voltage Of Fuel Cell

A = Area Of Electrode (12.8648)

TABLE 4: VOLTAGE DENSITY OF FUEL CELLS BOTH MIXED CULTURE AND AXENIC CULTURES

	V.D
WWMFC1	0.038
WWMFC2	0.046
STACKED WWMFC	0.072
NA1	0.026
NA2	0.016
NA3	0.035
NA4	0.015

NA5	0.006
NA6	0.014
NA7	0.009
MAC1	0.006
MAC2	0.019
MAC3	0.014
MAC4	0.032

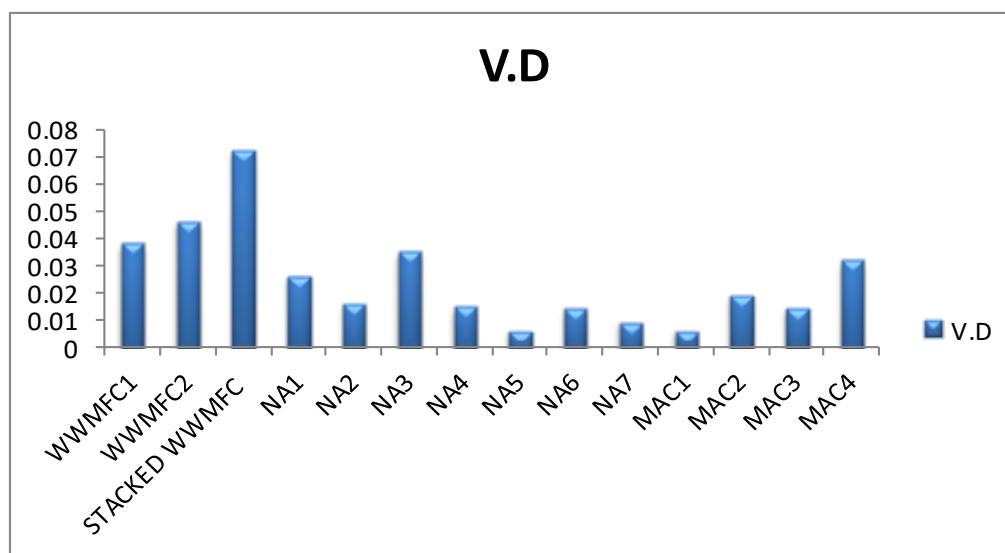


FIGURE 6: voltage densities of both the mixed and axenic cultures

4. DISCUSSION

Recently, great attentions have been paid to microbial fuel cells (MFCs) due to their mild operating conditions and using variety of biodegradable substrates as fuel. In this rasearch, a bioelectrochemical device that is capable of producing bioelectricity by the driect metabolic activities of microbes while degrading waste was designed. This is probably the last frontier in the role of microorganisms in our environment as they will directly and/or indirectly improve life in the society by generating electricity instead of its predominant economic importance which is to cause disease.

In line with the objectives of the research project, a low cost Microbial fuel cell using readily available materials- plastic containers, PVC pipes, graphite electrodes, domestic wastewater among others which are virtually inexpensive was designed. It has been able to generate electricity from both a consortium of microbes and from single genera isolated from the parent stock-wastewater. In the first scenario of study with Wastewater microbial fuel cells (WWMFC) 1, 2 and the stacked configuration which contained various microorganisms from diverse species. After months of modelling, trials and modifications, the H-shaped system was used for the purpose of the experiment and it proved most effective and efficient due to its easy of assembly and maintenance.

Wastewater microbial fuel cell 1 (WWMFC1) after a careful 10 day observation and recording, it showed a voltage of 0 volts on preparation thus conforming to the lag phase of a batch culture, which is the time needed for microorganism in a new system to acclimatize into the new environment. The zero reading of the multimeter also signifies that the system is still in the aerobic state which is less preferred to the anaerobic condition needed for the optimum performance of the fuel cell as it displaces oxygen as the terminal electron acceptor to enable the transfer of those electrons through the anode into the cathode for oxidation. A considerable increase was observed after 24hrs of incubation from 0 to 0.05volts and from 0.05 to 0.1volts. After 48hrs, the system showed a graceful increase, as the system slowly achieved its ideal anaerobic state and consequently the complete displacement of oxygen as the terminating electron acceptor in the anode and so an increase in the density of electrons in the electrode and hence the conductance through the copper wire attached. The voltage progressively increased until about 10 days when a peak voltage of 1.1volt was realized and this was maintained for 4 more days this implies that the configuration has achieved a stationary state after which it was terminated. A total voltage of 5.5volts was observed and the mean was 0.5 volts with a voltage density of 0.039v/m².

After the success of WWMFC1, a second fuel cell was constructed to further confirm the results obtained from the first experiment, this system performed comparably to WWMFC1 by showing a gradual increase from 0volts preparation to 0.1 volts on the first day and to about 1.2volts by the 9th and 10th day after which a slow decline was observed. Its total voltage was 6.5volts, with a mean average of 0.59 and a voltage density of 0.046v/m².

A total of eleven (11) isolates were gotten from the process of incubating, purifying and identifying the organisms present at the anodic electrode as biofilm. These organisms were of the following genera- *Shigella spp*, *Serratia marcescens*, *Salmonella spp*, *Bacillus spp*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Staphylococcus aureus*, and *Escherichia coli*. This is in accordance with Akshay *et al*, 2016 who reported *Proteus vulgaris*, *Erwinia dissolvens*, *Shewanella putrifaciens*, *Pseudomonas aeruginosa* and *Escherichia coli*. These isolates were further subjected to MFC treatment using sterile peptone water as the anodic electrolyte.

NA1 which is *Shigella spp* recorded a voltage of 0.01 on preparation, 0.26volts after 24hrs and at termination after 10days, a voltage of 0.43volts was observed. *Shigella spp* also recorded a total voltage of 3.63volts and average voltage of 0.33volts and a voltage density of 0.026v/m²

NA2 was observed to be *Serratia marcescens*, at preparation gave a voltage of 0.01 and after 24hrs the voltage rose to 0.15volts and on the 10th day, a voltage of 0.36volts was observed. Its total voltage was 2.26volts, an average of 0.21volts and a voltage density of 0.016v/m²

NA3- *Salmonella spp* showed a voltage of 0.24 after 24hrs and at the end of the 10th day it increased to 0.61volts; with a grand voltage of 4.95 and a mean average of 0.45 volts, its voltage density totalled 0.035v/m².

NA4 which is *Shigella spp* showed a voltage of 0.01 on preparation, after 24hrs, a voltage of 0.04 was observed and at termination after 10days, a voltage of 0.04volts was observed. It also recorded a total voltage of 2.09volts and average voltage of 0.19volts and a voltage density of 0.015v/m²

NA5 which was identified as *Bacillus spp* in the first 24hrs after setup showed a voltage of 0.13, this was fairly steady and so by the end of the 10th day, its voltage dropped slightly to 0.09volts. A total voltage of 0.89 was computed and its mean voltage was 0.08 with a voltage density of 0.006v/m².

Pseudomonas aeruginosa was the next isolate with a voltage of 0.11 after 24hrs of preparation, and on day 10, a voltage of 0.30 was observed, a total of 1.95 volts and a mean of 0.19 volts was computed; its voltage density was 0.014v/m².

Streptococcus faecalis was also subjected to the same treatment and the following results were obtained: 0.14volts was observed after 24hrs, and increased to 0.32 volts on the 5th day, after which the voltage fell back to 0.14 on the 10th day. A total of 2.31 volts was calculated with a mean average of 0.21 volts and its density was 0.009v/m²

Finally, the result for *Escherichia coli* was gotten as 0.04volts after 24hrs, and by the 10th day, a voltage of 0.4volts was recorded. Its mean total was 2.45 volts with a mean volts of 0.41v and a voltage density of 0.032v/m². This observation was in agreement to that reported by Mohan and Das (2008), who observed *Enterobacter cloacae* 11T-BT 08 in MYG medium to have generated 0.4v.

By means of a series connection, a configuration known as the stacked microbial fuel cell was constructed. The stacked system consisted of 7 single domestic waste water fuel cells connected in series. The result of this system is thus; after 24hrs a voltage of 0.4 volts was obtained and this increased over a 10 day period to 3.5volts. a total of 17.42volts was recorded with an average voltage of 1.5volts and a voltage density of 0.072v/m². Microorganisms actively catabolize substrates and bioelectricities are generated (Mostafa and Sang 2015). They play important roles in anode chamber and generated electrons. These generated electrons are utilized to reduce electron acceptors in the cathode once they pass through external circuit (Rahimnejad *et al.*, 2011), and this follows logically from what has been reported that this process leads to electrical power and organic waste removal contemporarily (Park *et al.*, 2014).

Voltage density (V.D) is a property used to measure the efficiency of the fuel cell. It is defined as the voltage passing through a unit area (m²) of an electrode. This property was used in the study to compare the production of electricity between the consortium and the axenic systems. And after computation of the various V.Ds, it was observed that none of the single bacterial species could match up to the voltage density of the consortium. The voltage density of the wastewater MFCs was calculated as 0.038 and 0.046v/m² for WWMFC 1 and 2 respectively, while the highest voltage density for the axenic cultures *Salmonella spp*, stood at 0.035 making it the bacteria of choice in an axenic culture. *Escherichia coli* had a V.D of 0.032 and a *Bacillus spp* microbial fuel cell had the least voltage density of 0.006v/m².

5. CONCLUSION

Microbial fuel cells are evolving to become a simpler and more robust technology. It is quite possible that in the future, microbial fuel cells can be improved by increasing the electrode surface area, the bacterial cell mass, the electron mediator type and concentration and by identifying better microbial strains.

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