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Isolation And Identification of Bacteria Associated with Bioelectricity Generation from Fruit Wastes and Gutter Sludge using Microbial Fuel Cells (Mfc) in Kano Metropolis, Kano State, Nigeria.

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ABSTRACT

Bioelectricity generation is another environmentally friendly mean of waste management, commonly carried out using microbial fuel cell (MFC) technology. The present work aims to generate bioelectricity from the fruit wastes using microbial fuel cells (MFC) technology and to isolate and identify bacteria during bioelectricity production. Fruit samples of production from different fruit wastes of banana, lemon, orange, water melon and pawpaw wastes were collected from Naibawa-Yanlemo fruit market's dump site in Kano metropolis, Kano State Nigeria. Physicochemical parameters such as biological oxygen demand (BOD), chemical oxygen demand (COD), total carbon (TC), total nitrogen (TN), organic matter (OM), Temperature and pH of slurries of individual and mixed fruit wastes were determined. Biochemical tests such as voges-proskauer test, oxidase test, catalase test, citrate test and sugar fermentation (TSI) test were also carried out using standard procedures. Generation of bioelectricity was monitored and recorded within a constructed seven dual chambered microbial fuel cells (MFCs) with wasted fruit as readily available materials. Current, current density, power and power density produced from different waste samples for twenty days was also recorded as bioelectricity generated from microbial fuel cells. Bacterial counts at the beginning and end of bioelectricity generation was also recorded from different fruits waste samples for microbial fuel cells (MFCs). The result of pH mean value of slurries of individual fruits is recorded to be all acidic with lemon having the highest acidity recorded as 3.45±0.62. The pH mean value of the mixed fruit was recorded as 7.1±0.23. Waste pawpaw fruit had 6±0.62 % the percentage of organic matter among all the individual waste fruits while the mixed fruit combined had 10±0.62 % mean value of percentage organic matter (OM). Two (2)

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bacterial genera were biochemically identified as *Klebsiella pneumoniae* and *Eshericha coli.* The highest generation of voltage (mV) from microbial fuel cells was achieved from gutter, mixed fruit and orange as 650, 400 and 390 mV respectively. The peak highest current produced was achieved from orange waste samples as 0.7mA/m^2 at day fourteen. Waste lemon had the highest colony count among all the fruits at the beginning and end of the bioelectricity generation of 2.2×10^3 and 4.12×10^2 respectively. Mixed fruits had the colony count of 2.10×10^5 and 7.20×10^3 at the beginning and end respectively.

Keywords: Bioelectricity, microbial fuel cells (MFC), waste fruit, mixed fruits, current

INTRODUCTION

Microbial fuel cell (MFC) is a process that uses an active microorganism as a biocatalyst in an anaerobic anode compartment for production of bioelectricity (Tardast *et al.,* 2012). MFCs were considered as promising technology that serves two main purposes, electricity generation and waste management. It can be further defined as a device which converts chemical energy to electrical energy during substrate oxidation with the help of microorganisms (Sabat *et al.,* 2013). Earlier, Torres (2009), defined it as a system in which microbes convert chemical energy produced by the oxidation of organic and inorganic compound into ATP by sequential reaction in which electrons are transferred to a terminal electron acceptor to generate electric current.

A typical microbial fuel cell comprises of anode and cathode compartment which are separated by a membrane. Microbes' duel permanently in the anode compartment which are separated by a membrane. Microbes' duel permanently in the anode compartment metabolizes organic compounds to generate electron and proton. The electrons generated are then transferred to the anode surface, from the anode the electron moves to the cathode through electric circuit, while the proton is transferred to cathode through membrane. Electron and proton are consumed in the cathode compartment combining with oxygen to form water (Nwokocha *et al.,* 2012; Das and Mangwani, 2010).

In today's fast-growing world, the rate of energy consumption is rising at unexpected rates with each passing day (Dahunsi *et al.,* 2015). In any economic development there's need for efficient source of energy, and Nigeria isn't left out among the countries with greatest demand for energy. To meet its growing energy requirements, the country has been investing hugely in developing its hydroelectric power generating capacity from water source like the Kainji power station in Kainji Niger state, the Jebba power station in Jebba Niger State, along with the Shiroro power stations in Shiroro, Niger State and in the aspect of liquefied natural gas (LNG) (Ali *et al.,* 2017).

Development of renewable and sustainable energy sources is an alternative environmentally friendly means to meet up the country's energy demands. Surely, productions of renewable energy from materials that are readily and locally available are extremely advantageous and reduce the cost of its production. Many municipal waste management programs do attempt to harness organic waste energy through combustion in waste-to-energy plants and methane collection from microorganism activity in landfills (Nwabunwanne *et al.,* 2020). While such systems make use of the energy available in food waste, they do not directly benefit the individuals who produce the waste, and may also involve additional costs to those individuals

related to collection (Hassan *et al.,* 2015). The amount of solid waste generated in Nigerian cities is steadily increasing as a result of population explosion and the continual growth of industries and agricultural practices (Muazu *et al.,*2020). Among the most generated wastes in Nigeria urban and rural cities are fruit wastes. Governments and industries are on the lookout for technologies that will allow for more efficient and cost-effective treatment of these wastes. The economic prospect of biogas technology in states that generated large tonnes of fruit wastes in Nigeria such as Kano is great because of the large availability of raw materials. Studies have explored fruits wastes to produce fertilizer (Muazu *et al.,* 2020), biogas (Budiyono *et al.,* 2018), and electricity (Washington *et al*., 2015). Biogas is a type of renewable energy that are produced not only in fruit wastes but also from many animal and plant wastes through decomposition. Biogas production from organic waste materials consists of four main stages which include hydrolysis, acidogenesis, acetogenesis and methanogenesis. The stages are interdependent on one another in such a way that the product from one stage is a precursor for the next stages and each stage involves different types of microorganisms (Sagagi *et al.,*2009). The resulting gas mixture consists primarily of methane (50-75%), carbon dioxide (25-50%), quantities of hydrogen, hydrogen sulphide ,ammonia and other trace gases (Peter,2009).

Bioelectricity generation is another environmentally friendly mean of waste management, commonly carried out in microbial fuel cell (MFC) technology. The MFC device uses electrochemically active microorganisms to generate electricity from different wastes such as feather (Chaturvedi and Verma, 2016; Yusuf *et al*., 2020), sludge (Rasel *et al*.,2018) and different amount of voltage are generated depending on the microorganisms involved, condition and type of substrate used (Washington *et al.,*2015). This is aimed to isolate and identify bacteria associated with bioelectricity generation from fruit wastes and gutter sludge using microbial fuel cells (MFC) in Kano metropolis, Kano State, Nigeria.

MATERIALS AND METHODS

Sampling

Sampling Sites

Fruit samples of banana, lemon, orange, watermelon and pawpaw wastes were collected from Naibawa Yanlemo fruit market's dump site. Yanlemo market was located in Kumbotso local government Kano (Longitude 8.55^0E and Latitude 11.927^0N). Sludge from gutter was collected from gutters in residential area of Dorayi Chiranchi in Kumbotso Local Government Kano (Longitude 8.476^0E and Latitude 11.953^0N).

Plate 1: Large Volume of Spoiled Orange Disposed at Yan Lemo Market

Sample Collection

Samples of respective fruit wastes were picked by means of hand picking and placed into a clean polythene bag after which the bags were tightly closed. Sludge from gutter was collected from gutters in residential area of Dorayi Chiranchi in Kumbotso Local Government Area Kano, about 500g of gutter sludge was collected using a portable hand metal shovel and placed into a clean plastic container the container was tightly closed. The samples were placed into a clean plastic container and covered. All the samples were transported for analysis to the Postgraduate Microbiology Research laboratory of Department of Microbiology, Bayero University Kano, Nigeria.

Processing of Samples

Two hundred Gram of each fruit wastes (orange, lemon, pawpaw, watermelon, and banana) was weighed and homogenized to produce slurry with the aid of an electronic blender.

Physicochemical Analysis of the Waste Samples

Physicochemical parameters of the waste samples (fruit wastes, gutter sludge and cow dung) which include, pH, Temperature, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (BOD), Total Carbon (TN), Total Nitrogen (TN) and Organic Matter (OM) were analyzed.

pH

This was determined using a digital pH meter. The slurry sample was poured into 50ml beaker and the meter probe was dipped into it for a minute. The pH reading was taken directly from the meter (Lawal *et al*., 2011).

Temperature

Temperature was determined using mercury –in –glass thermometer. The thermometer was dipped into the slurry, and the reading was taken after some minutes in degree centigrade (Lawal *et al*., 2011).

Biological Oxygen Demand (BOD)

BOD was determined by measuring the amount of oxygen consumed after incubating the sample in the dark at 20^0C, for five days. The oxygen consumption was determined from the difference between the dissolved oxygen concentration in the sample before and after the incubation period (Ogundele, 2010).

Chemical Oxygen Demand (COD)

The titrimetric method was used to determine the chemical oxygen demand of the various waste samples. Four grams (4g) of H_2SO_4 was placed in a refluxing flask and 20mL of the diluted samples was added and mixed thoroughly. Ten milliliters (10mL) of standard potassium dichromate ($K_2Cr_2O_7$) solution were added with the addition of several beads which was preheated at 600^0C for 1hr, the flask was then attached to the reflux condenser. Thirty milliliters (30mls) sulfuric acid (H_2SO_4) containing silver sulphate (Ag_2SO_4) was added through open end of the condenser and mixed thoroughly by swirling. This was refluxed for 1hr, then allowed to cool and the condenser was washed with 25mls distilled water. The mixture was diluted with 100mls of distilled water and cooled down at room temperature. Three drops of ferroin indicator was added, a blank solution was prepared in the same way with same volume of distilled water replacing the sample. This was titrated with FAS (ferrous ammonium sulphate Fe $(NH_4)_2$ $(SO_4)_2$) to an end point of reddish brown from blue green (Kashani, 2009).

Total Nitrogen

The total nitrogen was determined using DR/2010 manual. The pour-thru cell procedure was used, the cell was clean by pouring a few sodium thiosulfate pentahydrate crystals into the cell funnel. It was then flush through the funnel and cell with enough deionized water in order to dissolve it, then 25mL of the sample was filled in the mixing graduated cylinder, another 25mL of the sample was filled in the mixing graduated cylinder with deionized water. Three drops of mineral stabilizer was added to each cylinder, it was then inverted after several times to mixed, another three drops of polyvinyl alcohol dispersing agent was added to each cylinder, it was then inverted several times to mix. Pipet 1.0mL of Nessler reagent was added into each cylinder, it was then inverted several times to mix, each solution was poured into a sample cell.

Organic Matter

The organic matter was determined using dichromate method, 1.00g of the waste sample was weighed and transfer to a 250mL erlenmeyer flask, then 10.0mL of 1.00 N potassium dichromate solution was pipetted into the 250mL Erlenmeyer flask. A blank was prepared by pipetting 10.0mL of 1.000 N potassium dichromate solution into an empty 250mL Erlenmeyer, then a 20.0mL of concentrated sulfuric acid was pipetted into each flask, each

flask was then covered with an inverted 50mL erlenmeyer flask, it was then swirled gently to mixed and then placed on cooling pads. It was then allowed for 10 minutes reaction period to begin, when the display shows the percentage of organics then the graduated cylinder was used immediately to add 100mL of deionized water to each flask it was then swirled briskly to mixed, 25mL of the samples was then filter into a 50mL erlynmeyer flask, then 25mL of the blank was poured into a sample cell, the blank was then placed into the cell holder the light shield was then closed, 25mL of the filtered samples was added into a sample cell (the prepared sample), the prepared sample was then placed into the cell holder, the light shield was then closed (Umoh *et al*., 2019).

Total Carbon

Total carbon was determined as described from the DR/2010 manual, the blank was filled with 10mL of the sample, the blue ampul cap was then filled with the sample. A high range dissolved oxygen accuvacampul was filled with the sample, the tip immersed was kept while the ampul was filled completely. Without inverting the ampul, the ampul cap that has been filled with sample was immediately placed securely over the tip of the ampul and shaked for 30 seconds (the cap prevents contamination with atmospheric oxygen). The accuvac vial adapter was placed into the cell holder. When the display showed the readings of the total oxygen the ampul was then shaked for 30 seconds. The blank was then placed into the cell holder and the light shield was closed. The accuvacampul was then placed into the cell holder and the light shield was closed and waited for 30 seconds after 30 seconds the air bubbles dispersed from the light path, then the result in mg/L was displayed (Umoh *et al*., 2019).

Bacterial Analysis of the Fruit Wastes Samples

Bacterial analysis was carried out at the point of feeding, at the peak of production and at the end of retention time according to the method of Cheesbrough (2006).

Determination of Bacterial Count

This was carried out according to the method of FAO (2010). For all the waste samples, 25g of each sample was weighed and homogenized in 225ml of sterile distill water to formed a stock solution, then 1ml was withdraw from the stock solution and introduced into a test tube containing 9 ml of sterile distill water which give one in ten dilutions (10^{-1} dilution). This was then serially diluted using 9ml of sterile distill water up to (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} 10^{6}, 10^{7}, 10^{8} and 10^{9}), dilutions. Using a sterile syringe, 1ml each from10^{-1} 10^{-2}, 10^{-3}, 10^{-4}, $10^{-5}10^{6}$, 10^{7}, 10^{8} and 10^{9} was carefully and aseptically inoculated into fresh nutrients agar (prepare according to manufacturer instructions) in duplicate by pour plate method. All the plate was incubated at 37^{o}C for 24hrs, after incubation, plates containing 30-300 colonies was selected and colonies counted. The average was taken and the number obtained was multiplied by the inverse of dilution factor. This gave the number of colony forming units per gram of a sample (cfu/g).

Isolation and Identification of Bacteria

This was carried out according to the method of FAO (2010). For all the waste samples, 25g of each sample was weighed and homogenized in 225ml of sterile distill water to formed a

stock solution/homogenate. Loopful of homogenates was inoculated onto appropriate media such as Nutrient Agar, Mac-Conkey Agar, Eosin Methylene Blue (EMB), and incubated at 37^{o}C for 24hrs. Colonies from different culture media was examined physically for their characteristic appearance which include colony size, shape, pigmentation, odor and reaction to gram staining to reveal their morphology microscopically. Biochemical tests such as Voges-Proskauer test, Oxidase test, Catalase test, Citrate test and Sugar fermentation (TSI) Test was equally carried out.

Biochemical Characterization of the Isolates

Citrate Utilizing Test

Simmon's citrate agar was prepared in slants in bijou bottles as recommended by the manufacturer. using a straight wire, the media was first streak with the test organism and then stabbed to the butt of the bottle. This was then incubated at 37^{o}c for 24-48 hours. A blue color formation indicates a positive result while no color changes indicate a negative result (Cheesbrough, 2006).

Triple Sugar Iron Test

A tube of kligleriron agar was inoculated using a sterile straight wire, first the butt was stabbed then the slope was streaked and incubated at 35-37^{0}C for 18-24hours. Lactose fermenting bacteria appeared as yellow butt and yellow slope, glucose fermenting bacteria appeared as red butt and red slope, blackening in the media indicated hydrogen sulphide production and cracks in the medium was due to gas production (Cheesbrough, 2006).

Oxidase Test

Isolated microorganisms were grown in nutrient agar medium for 24-48 hours at 37^{o}C. A filter paper was placed into a Petri dish and a few drops of dilute1% solution of oxidase reagent (tetramethyl-phenylenediamine-dihydrochloride) which was prepared by standard procedure were added. One large colony will be taken with a loop and tapped lightly onto the wet filter paper. Formation of a blue-purple colour by the cells within 30 seconds indicates a positive oxidase result (Cheesbrough, 2006).

Voges-Proskauer Test

Some bacteria in the fermentation of glucose, produce other products such as ethanol and 2,3-butanediol rather than large amounts of acid as does *E. coli.* A test for acethyl methyl carbinol (a precursor of 2,3-butanediol that appears in the growth medium) is performed. A pink color developing after a few minutes indicates the presence of acetyl methyl carbinol. MR-VP broth was inoculated for 48hours at 37^{o}c. Twelve drops of 5% solution of alpha-naphthol and 4 drops of 40% potassium hydroxide (KOH) was added. The mixture was agitated vigorously and left to stand. Positive test resulted in a red or pink colour (Cheesbrough, 2006).

Catalase Test

Isolates will be grown in nutrient Agar Medium for 24-48 hours at 37^{o}C. After incubation a

thick smear of the organism was made on clean slide and 3% hydrogen peroxide was poured onto the colonies. Formation of air bubbles indicates the positive result. And absence of bubble indicated negative result (Cheesbrough, 2006).

Generation of Bioelectricity (Mfc)

Construction of Microbial Fuel Cell (MFC) and Assembling

The microbial fuel cells were constructed as described by Chaturvedi and Verma (2016) with some slightly modification. A total of seven dual chambered MFCs (Microbial Fuel Cells) setup were made in this experiment with readily available materials. The dual chambered were designed consisting of two identical plastic containers (750ml each with an operating volume of 700ml and a head space of 50ml). The cathode and anode chambers were connected by a salt bridge. The salt bridges were prepared by adding 1g of NaCl into 100ml of distilled water and mixed properly. Then, 6g of nutrient agar was added to the salt solution and was boiled for 3 min. It was then filled into a half inch PVC (poly vinyl chloride) pipe sealed with an aluminium foil paper from one side and attached to the chambers using electric adhesive gum to make them leak proof. Two identical carbon rod electrodes with an apparent height (h) of 5.5cm and 0.794 radius were used as electrode material in both cathode and anode chambers, to which a 10cm copper wire was connected and fixed with PVC gum, and the copper wires were passed through a thin hole on the plastic container cover, respectively. The anode chambers were fed with different substrate (fruit wastes), while the cathode chambers were fed with distilled water. Mixture of fruit wastes containing80g each of water melon, banana, lemon, orange and pawpaw were chopped, minced with knife and blended using kitchen blender. A total of 400g of each fruit wastes were added to the anode chamber, followed by the addition of 300ml of water. In addition, 400g of sludge was taken and mixed with 300ml of water and used as control for the experiment. The current and voltage emanating from the cells were calculated using digital multimeter.

Current Density, Power and Power Density

The current density was calculated by dividing current and area, where area h…….i

Where,

= 22/7……ii

h = 5.5cm……..iii

r = 0.794……....iv

While the power was calculated by multiplying voltage and current and the power density was calculated by dividing power by area.

RESULTS

The result of the physicochemical analysis biological oxygen demand, Chemical oxygen demand, pH, temperature, total carbon, total nitrogen and organic matter tested for different waste samples (Sludge, Mixed fruits, Orange, Banana, Lemon, Water melon and Paw-paw) showed that gutter sludge had the highest BOD, COD, OM, TN, TC values. However, lemon and orange were observed to have lowest BOD, COD, OM, TN, TC content. Biological oxygen demand sludge was observed to have the highest value of 34.8 mg/L, while orange

have the least value of 5.2 mg/L respectively. The same sludge was observed to have the highest chemical oxygen demand of 166.4 mg/L value, while lemon exhibited the lowest value of 20 Mg/L. The highest value of 1200 mg/L for total carbon was observed in mixed fruit and 16.0% for organic matter was observed in gutter sludge, while orange was observed to have the lowest total carbon of 118 mg/L and least value of 1.18% for organic matter. The same mixed fruit have the highest total nitrogen of 1.6 mg/L content, while lemon have the least value of 0.12 mg/L for total nitrogen. This result is presented in Table 1.

Table 1: Physicochemical Parameters of the Various Waste Samples

Parameters	Sludge	Mixed fruit	Orange	Banana	Lemon	Watermelon	Paw-paw
BOD(mg/L)	34.8	17.6	5.2	18	10	24	31.2
COD(mg/L)	166.4	24	21.4	22	20	35.6	27.2
pH	8.9	7.1	3.98	4.58	3.45	4.78	4.65
Temp	33	28.9	32	30	34	34	33.4
TC(mg/L)	164	1200	118	640	458	802	602
TN(mg/L)	0.50	1.6	0.45	0.52	0.12	0.52	1.56
OM(%)	16.0	10.2	1.18	6.4	4.02	4.58	6.02

Keys:
BOD: Biological oxygen demand, COD: Chemical oxygen demand, TC: Total carbon,
TN: Total nitrogen, OM: Organic matter.

Figure 1 showes the recorded voltage for 20 days. Gutter sludge was observed to have the highest voltage with 657mV at day 9, while banana has the least voltage of 8mV. Upon comparison of the voltage of various samples, no significant difference was observed among the various waste samples (P=3.88). The highest voltage recorded in pawpaw, lemon, water melon, orange, banana, sludge and mixed fruits were 264mV, 389mV, 401mV, 380mV, 150mV, 654mV and 409mV respectively. The lowest voltage recorded in all the aforementioned samples are 8mV, 25mV, 111mV, 170mV, 12 mV, 98mV and 15.9mV respectively.

Figure 2 revealed that gutter sludge produced the highest current, while banana has the least. The same trend was observed for power, current density and power density. In all the figures listed below gutter sludge was observed to have the highest current, power, current density and power density while banana has the least. These results were presented in figures 3, 4 and 5 respectively.

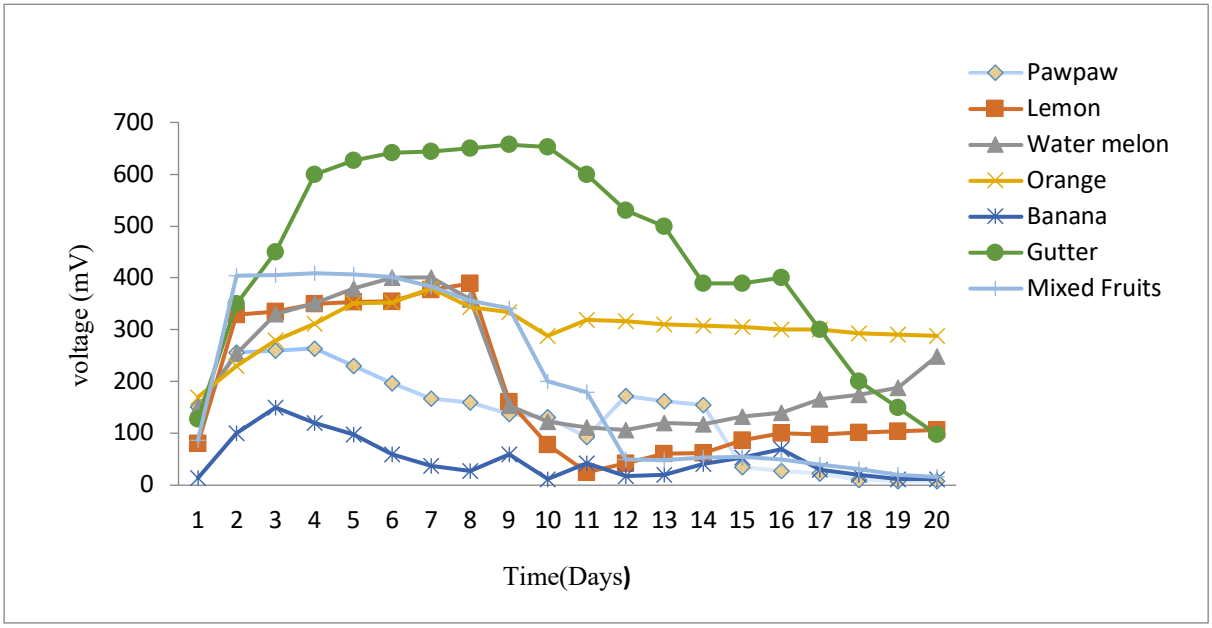

Figure 1: Generation of Voltage (mV) from Microbial Fuel Cell of Different Waste Samples Versus Time

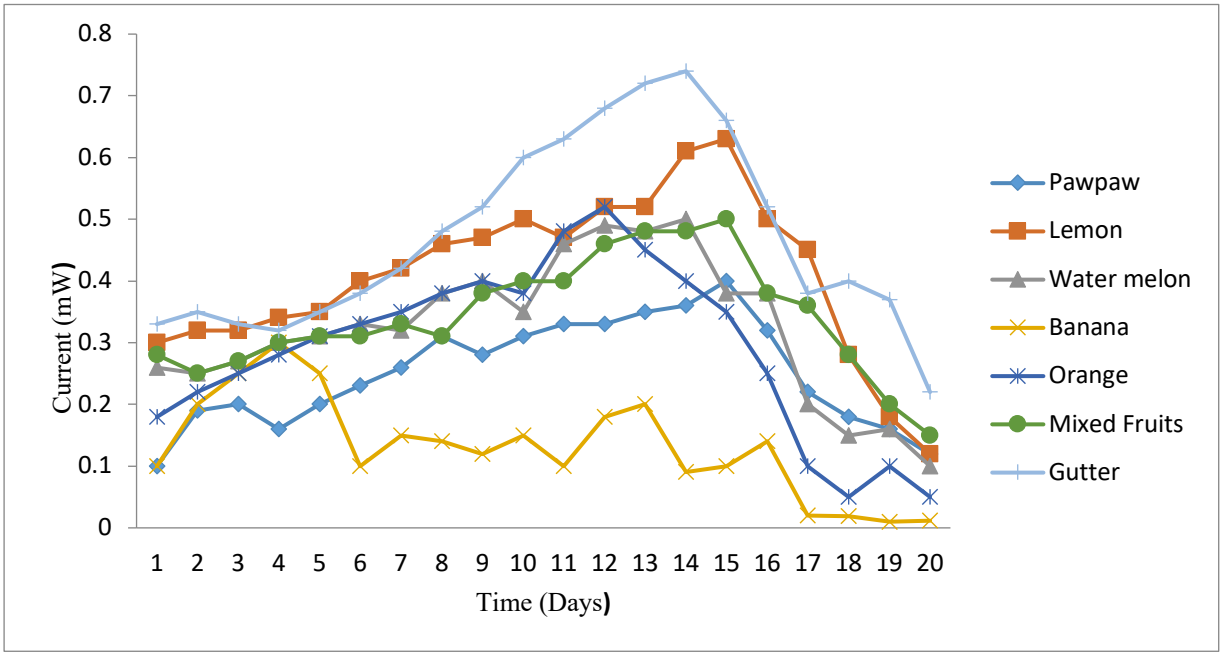

Figure 2: Current (mW) Produced from Microbial Fuel Cell of Different Waste Samples Versus Time

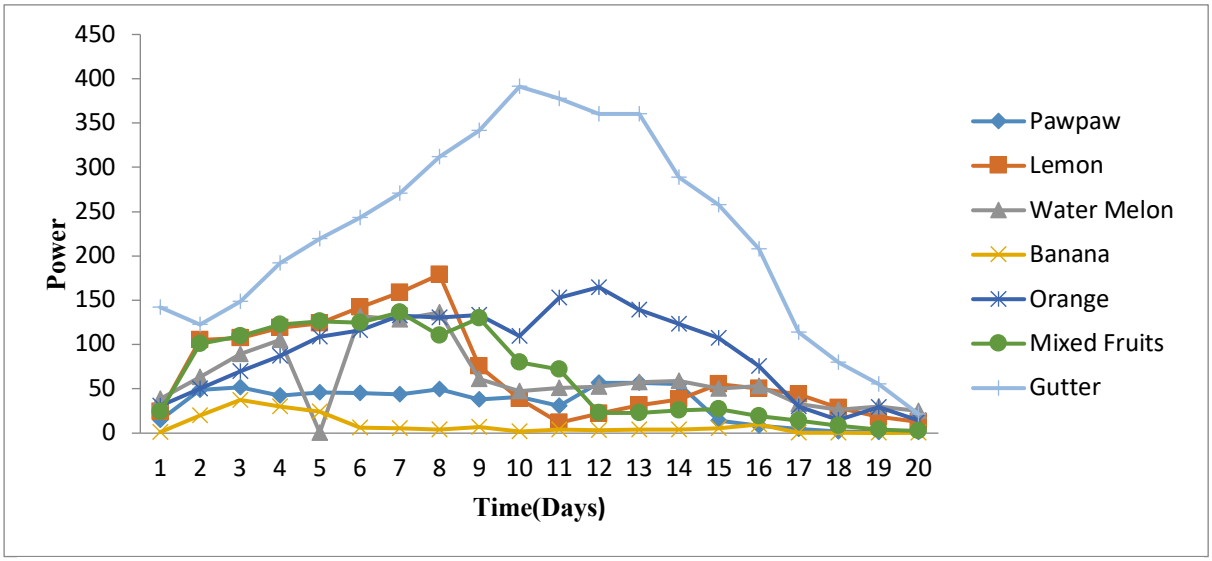

Figure 3: Power Produced by Microbial Fuel Cell of Different Waste Samples Versus Time

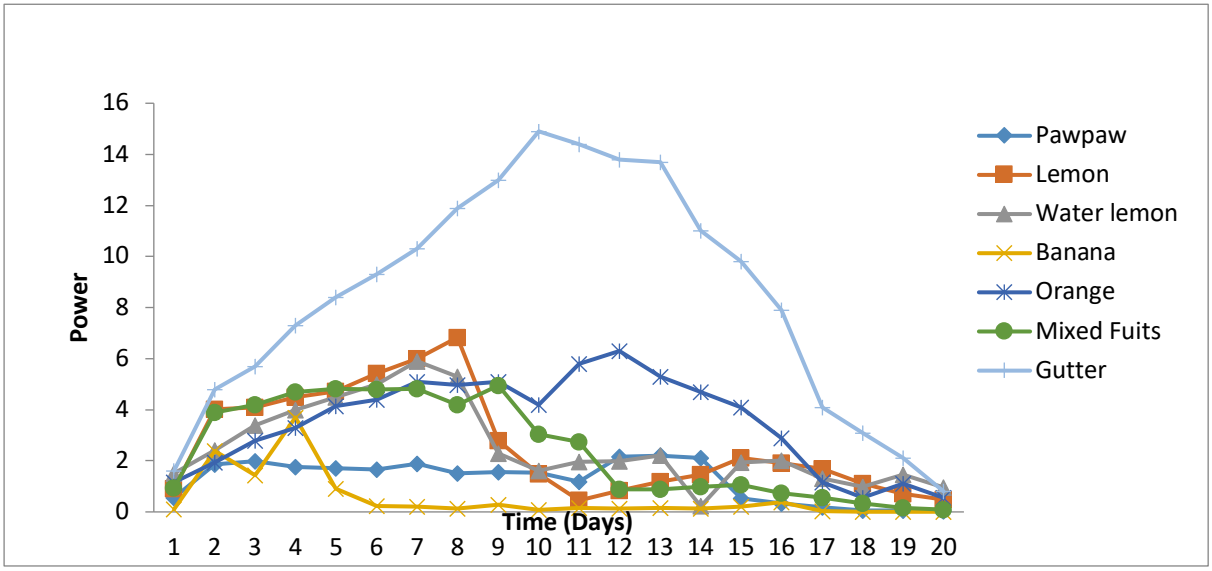

Figure 4: Current Density Produced from Microbial Fuel Cell of Different Waste Samples Versus Time

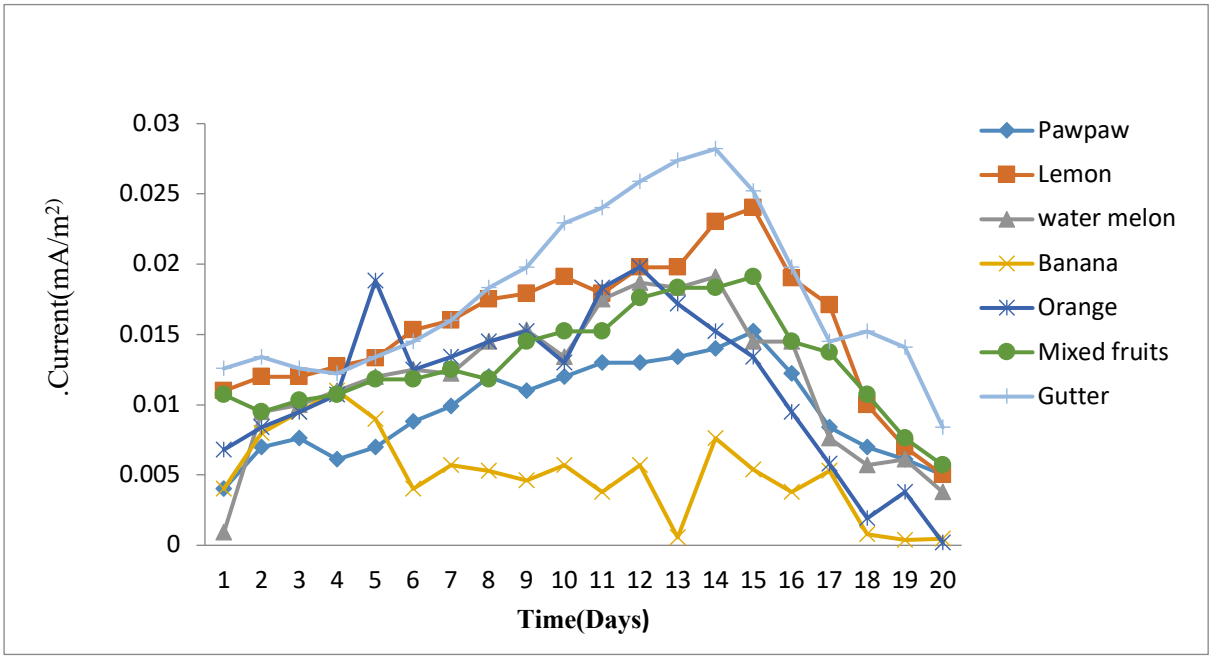

Figure 5: Power Density Produced from Microbial Fuel Cell of Different Waste Samples Versus Time

Escherichia coli and *Klebsiella* were found to be the persistent organisms throughout bioelectricity generation process. Table 3 presents the results of the various biochemical tests conducted along with their morphology. The two isolates were observed to be oxidase and H_2S negative and positive for catalase. The isolates were further observed to produce gas with *E. coli* showing positive for citrate and Voges-Proskauer as *K. pneumonia* showed negative

Table 2: Bacterial Analysis of Different Waste Samples for Microbial Fuel Cells (MFCs)

	Microbial Count (cfu/g)	
Substrate	**Beginning**	**End**
Gutter sludge	2.88×10^{8}	8.68×10^{4}
Banana	2.22×10^{4}	6.92×10^{2}
Orange	1.12×10^{4}	4.28×10^{2}
Lemon	2.2×10^{3}	4.12×10^{2}
Pawpaw	1.38×10^{5}	6.22×10^{3}
Watermelon	1.11×10^{5}	5.18×10^{2}
Mixed fruits	2.10×10^{5}	7.20×10^{3}

Table 3: Biochemical, Gram Staining and Microscopic Characteristics of Test Isolates.

Category of test	**Type of test**	***Klebsiella pneumoniae***	***E. coli***
Biochemical test	Indole	--	+
	Gas production	+	+
	H_2S production	--	--
	Motility	--	+
	Citrate	+	--
	Oxidase	--	--
	Glucose	+	+
	Lactose	+	+
	Voges-Proskauer	+	--
	Catalase	+	+
Microscopy	Morphology	Rod	Rod
	Gram reaction	Gram negative	Gram negative

Keys: + = Positive, -- = Negative, H_2S = Hydrogen Sulphide

DISCUSSION

Bioelectricity through MFCs enables recovery of renewable energy and of nutrients from various organic waste materials and is thus highly important for the transition to a more sustainable society. The performance and stability of the bioelectricity generation process is highly dependent on an array of different microbial groups, and their networks and functions are in turn influenced by substrate characteristic and operating parameters. The result of the physicochemical analysis which analyzed the BOD, COD, pH, Temperature, TC, TN and OM tested on each sample revealed that lemon and orange have lower BOD and COD which indicate lesser bacterial activities this could be as a result of low pH in lemon and orange which rather support fungal growth instead of bacteria as suggested by the findings of Jiang-Zhong (2015).

Electricity generation in MFC containing gutter sludge was monitored for 20 days. Highest voltage of 657mV was recorded on day 9, after which slight decrease in voltage was observed this result supported the findings of Anand (2015) who reported that there's increment in voltage from day 3 up to day nine, after day nine there was a slight change in voltage with time as compared to the earlier times. Generally, voltage for all the cells are divided into three phases; the voltage rise, stationary and fall phase. The voltage rise phase consists of two stages that is the early stage (days 0-3) and advanced voltage rise stage (days 4-9). The early stage has lower voltage change gradients than advanced voltage. Accordingly, Kovács *et al*., (2013) stated that during the early stage, voltage growth is slow because of three reasons: low bacteria population and the bacteria are trying to acclimatize to the cell environment; presence of high concentration of electron acceptors; limited colonization of the anode surface by bacteria.

For this reason, during the early stage, every time agitation was done on the cells a foul smell was expelled from the cells. The degree of the stench decreased with time from day 0-3. On day 4, there was no more smell which meant that there was a low concentration of sulphates in sewage. During the advanced voltage rise stage, there was an increase in the bacteria community responsible for the generation of voltage. The steady increase is a factor which is also increased by the bacterial community forming a film/colonizing the anode surface. The stationary phase is of maximum constant voltage. Since the voltage developed entirely depends on bacteria community responsible for voltage development between the anode and cathode; the voltage at this phase is constant since the bacterial community responsible for voltage development is at a constant population.

The constant population signifies that the number of bacteria dying is equal to the number that is being reproduced. During this stage, the food available can only sustain a constant bacterial population. The constant population was the maximum achievable population by the bacterial community in correspondence with the available food. The last stage of voltage change is the voltage fall phase, this was due to the fall in food for the bacteria, this would have led to a great fall in bacteria community due to decrease in food quantity (Chang *et al*., 2005). It also agrees with the findings of Rasel *et al.* (2018), who reported sewage sludge have the highest voltage of 960mV at the 13th day from the microbial fuel cell. While, banana has the lowest voltage 8mV at day 20, this result is in line with the findings of Kamau *et al.*

(2018), who reported that banana has the lowest voltage with 0.021V to 0.23V on day 5 and 12. Upon comparison of the voltage of various samples, no significant difference was observed (P= 3.88). However, the highest voltage recorded in pawpaw, lemon, water melon, orange, banana, and mixed fruits were 264mV, 389mV, 401mV, 380mV, 150mV, and 409mV respectively, this result shows that mixed fruits have the highest voltage among all the fruit wastes this is because the device contained more concentration of fruits, and it seems like proportionated better conditions for oxide- reduction reactions in the bioelectrogenic process, this agreed with findings of Washington *et al.* (2015), who reported highest voltage of 330mV in single microbial fuel cell which contained a blend of fruits and vegetables. In this study, the microbiological analysis during anaerobic digestions of the different waste samples revealed high microbial count at the beginning of the experiment with gutter sludge having the highest count of $2.98x10^8$ while banana and watermelon has the lowest count of $1.14x10^4$ each. Reduction in microbial count was observed at the end of the digestion across all the substrates (wastes). *Escherichia coli* and *Klebsiella pneumonia* were found to be the persistent bacteria throughout the digestion process.

CONCLUSION

In conclusion, the findings of this study revealed that the physicochemical parameters of the various substrates used in gutter sludge have the highest value for biological oxygen demand, chemical oxygen demand, total carbon, total nitrogen and organic matter. Voltage, current, power, likewise current density and power density generated were observed to be higher when gutter sludge were used for microbial fuel cells while the lowest values were obtained from banana sludge. *Escherichia coli* and *Klebsiella pneumoniae* were found to be the persistent bacteria throughout the bioelectricity generation process.

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