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Electricity Generation Potential of Cow Paunch from Abattoirs in Akure, Ondo State, Nigeria

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ABSTRACT

The potential for electricity generation from cow paunch was investigated using microbial fuel cell. The cow paunch was collected from two (2) abattoirs; BOD, COD, Total Solids, Total Volatile Solids, Total Organic Carbon, pH, temperature, and electrical conductivity were evaluated. Isolation and identification of microorganisms from the cow's paunch was done. Cow paunch was used as the substrate in the anode chamber while potassium ferricyanide was used in the cathode chamber as the electron acceptor. The current and voltage generated in the set up were monitored for 21 days using a digital multimeter. Results from this experiment revealed that the highest bacterial population of 5.11 × 108 cfu/g for aerobic bacteria and 3.09 × 108 cfu/g for anaerobic bacteria was observed in Topland cow paunch. Bola cow paunch had an aerobic bacteria population of 1.01 × 107 cfu/g and a 9.83 × 106 anaerobic bacteria population. There were spontaneous increases and decreases in the voltage and current readings during the experiment. The Topland cow paunch generated the highest voltage of 942mV on day 2 and a current of 4.63mA on day 1 of the experiment. The Bola cow paunch generated its highest electric voltage of 901mV on day 9 evening of the experiment, generating its highest current of 3.04mA on day 3 afternoon of the experiment. It is concluded from the findings of this research that cow paunch has a high potential to produce electric current and voltage due to the diversity of electogenic microorganisms in them, and concurrently reduce the harmful effect of direct disposal of cow paunch on the environment.

Keywords: *Microbial Fuel Cell (MFC); Cow Paunch; Current; Voltage.*

1.0 Introduction

Energy has been considered for a long time as a crucial element in fulfilling human fundamental needs, which is required in various forms during human activities. It is viewed as a major factor in the development and support of a nation's economic growth and in upgrading the standard of living [1]. Due to human civilization, urbanization, and progress, energy demands are increasing on a daily basis all over the world. Renewable and non-renewable energy sources are the two major categories of energy sources [2]. Fossil fuel is one of the most overly exploited energy source in the world; tremendous depletion, atmospheric pollution, ecological imbalances, global warming and other unusual environmental conditions are arising due to the overexploitation of fossil fuels. The fast depletion of non-renewable energy sources has led scientists to the investigations of alternate renewable sources of energy generation that are cheap and eco-friendly [3].

For most of human history, renewable energy has been the primary source of energy. Plant biomass was the primary energy source for most of human history; it was burned for heat, to feed animals, used for transportation and plowing. Renewable energy are from energy sources that are naturally replenished [4]. Sources of renewable energy include: Solar energy - from the sun, Geo-thermal energy - from heat inside the earth, Wind energy, Biomass - from plants and Hydropower from flowing water [4]. Biotechnology in recent times has tried to develop a mechanism whereby sustainable

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electricity can be generated by the activity of microorganisms on waste and renewable biomass in a device called microbial fuel cell (MFC) [5]. Rapid microbial decomposition of organic wastes occurs mostly by anaerobic digestion, in which microorganisms break down biodegradable components in organic wastes in the absence of oxygen. The interaction of microorganisms with electrodes in the cell is maintained by electrons flowing through an electrical circuit. A cogent feature of this renewable source of energy is its ability to generate electricity while concurrently treating the waste [6].

An abattoir, despite its crucial role of slaughtering, processing, preservation and storage of meat for human consumption; remains a major source of waste generation in Nigeria [7]. The high content of putrescible organic materials, faecal and pathogenic microorganisms in these wastes is alarming, as it can lead to several health conditions if ingested by human [8],[9].

Wastes generated during the slaughtering and processing of farm animals in abattoirs include liquid and solid wastes such as blood, horns, bones, reject meat, fat spent-water, faecal components, slurry of suspended solids and paunch manure are among others [8]. Poor waste disposal and some human activities in abattoirs are widely recognized around the world as polluting the environment in so many ways, which include; water pollution, air pollution, land pollution, odor and degrading the environment [10].

Paunch (the contents of the rumen in ruminants) has been recorded as a substantial slaughterhouse waste in some Nigerian municipal abattoirs in terms of volume. It was therefore found to be one of the major abattoir wastes that require proper management and treatment in most abattoirs in the country. There is therefore need to explore and established the possible uses and treatment of cattle paunch manure [11].

2.0 Materials and Method

2.1 Sample collection

Cow paunch was collected into sterile plastic bags aseptically from Bola and Topland Meat, Akure, Ondo State, Nigeria for microbiological and physicochemical analysis after the cows were slaughtered and transported to the Microbiology Laboratory, Federal University of Technology, Akure (FUTA) immediately. Microbiological analysis was done within 24 hours of sample collection.

2.2 Steriliation of materials

All glasswares were sterilized in the hot air oven at 180^0C for 3 hours. Inoculating loops and needles were flamed to redness in Bunsen burner flame and allowed to cool down close to the Bunsen burner before and after each use. Spatula was dipped in 70% ethanol and flamed before and after use. The workbench was always disinfected using 70% ethanol before and after each day work and in the occasion of spillage on the workbench.

2.3 Preparation of culture media

Nutrient Agar (NA), Eosin Methylene Blue Agar (EMBA), MacConkey Agar (MA), *Salmonella-Shigella* Agar (SSA) and Mannitol Salt Agar (MSA) were used to cultivate and isolate bacteria while Sabouraud Dextrose Agar (SDA) was used to isolate fungi from the cow paunch. Each of the medium was prepared according to the Manufacturer's instructions, these was properly corked with cotton wool and sterilized in the autoclave for 15mins at 121°C and left to cool down to about 50°C before pouring. Pour plates method was used to cultivate the microorganisms. All processes were done under aseptic techniques, beside an open flame of a Bunsen burner.

2.4 Serial dilution

The bacterial and fungal counts of the samples were determined; serial dilution of cow paunch samples were done up to 10^{-7} dilutions. 9 mls of distilled water were dispensed into each clean test tubes and was properly corked with cotton and foil plugs. This was then sterilized in the autoclave for 15 minutes at 121°C and left to cool down to room temperature. One gram of each of the cow paunch samples was added in 9 mls of sterilized distilled water which is shaken to make 10^{-1} and sterile syringe was used to add 1 ml of the 10^{-1} into another test tube containing 9 mls of sterilized distilled water to make it 10^{-2} and continued until dilution 10^{-7} [12].

2.5 Isolation of bacteria and fungi isolates from the cow paunch

For bacterial and fungal isolation, dilutions 10^{-3}, 10^{-5} and 10^{-7} were used for each of the cow

paunch. Using pour plate method, 1 ml of each sample was pipette with the aid of a sterile syringe into sterile Petri dishes. Molten agar was poured on it and swirled gently for even distribution of microorganisms in the plate. The agar was set still and allowed to gel before incubation. The agar plates were incubated in an inverted position at 35 ± 2°C for 24 hours for both aerobic and anaerobic bacteria and 25 ± 2°C for 48 hours for Fungi in duplicates. Distinct colonies were counted and sub-cultured on freshly prepared agar plates in duplicates, [12].

2.6 Identification of isolates

Identification of fungal isolates was done microscopically using Lactophenol Cotton Blue (LPCB) staining reagent and culturally according to the methods of [13]. Bacterial isolates was identified microscopically and biochemically according to the methods described by [14]; [12].

3.0 Building the Microbial Fuel Cell

3.1 Making the salt bridge

The salt bridge used NaCl as an electrolyte for proton exchange.. The salt bridge was prepared using 5%Sodium Chloride (NaCl) and 10%agar-agar. Five grams of NaCl was weighed into an empty pot with 10 grams of agar-agar. 100mls of distilled water was added and boiled to dissolve the agar-agar. The agar was allowed to cool down to about 50^{o}C before pouring into the PVC pipes of about 10 cm long and 4 cm in diameter, which were covered at one end with nylon and rubber bands. Once the pipes were filled, they were left to stabilize and solidify for about 10 mins [15].

3.2 Building of the anode and cathode containers

The anode and cathode containers were built using 1.2 litre plastic containers. Holes were drilled on one sides of two containers, making sure that the holes were exactly opposite each other. After that, a short PVC pipe containing the proton exchange membrane was inserted into the holes of the two containers and epoxy gum was then used to hold the pipe and the container together to avoid leakage. Holes which were two millimetres (mm) in diameter were drilled on top of the two plastic container lids to allow passage of flexible wires into the chambers. This set-up was filled with water past the holes/joints and allowed to stay overnight to check for leakages and water tightness [16].

3.3 Composition of anodic and cathodic chamber

Graphite rods from discarded dry cell batteries were used as electrodes; these were prepared in 100% acetone for 30 minutes. One end of the electrodes was attached to 1 mm copper wire by coiling one end of the wire around the electrode. These were introduced into each of the anode and cathode chambers by passing through the bored hole on the top of the plastic lid. 1200 mls of cow paunch was introduced into the anode chambers and the cathode chambers were filled with 1.2 litres of 1 M Potassium ferricyanide. For the control, sterilized cow paunch was used as the substrate.

3.4 Circuit assembly

The already prepared electrodes were each inserted into the anode chambers containing cow paunch and into the cathode chamber containing potassium ferricyanide solution for the microbial fuel cells. Both chambers were connected by an agar-salt bridge, wire from both the anode and cathode chambers were connected to a digital multimeter as described by the method of Prakash (2016a) [17].

3.5 Testing the fuel cell

The PVC pipes containing the salt-agar mixture were fixed between the two containers using epoxy material. The constructed MFC chambers were then sterilized with 70% ethanol. The set up was then tested for any form of leakages by pouring water in the containers and checking for leakages.

4.0 Results and Discussion

Bola cow paunch was made up of 98% green plants and 2% grains with green colouration. Topland cow paunch is a sludge which contains debris and is a dark brown colour, also with a foul odour. The difference in the physical appearance of samples helps to ascertain the diversity of forage fed to the ruminants. The feeding pattern affects the microbial population in the rumen as well as the physicochemical, proximate, and mineral constituents of the cow paunch.

4.1 Isolation of microorganisms

The total aerobic and anaerobic bacteria counts for the cow paunch are shown in tables 1 and 2; Topland cow paunch had a higher bacteria load of 5.11×10^8 cfu/g for aerobic bacteria and 3.09×10^8 cfu/g for anaerobic bacteria. Bola cow paunch had an aerobic bacteria load of 1.01×10^7 cfu/g and 9.83×10^6 cfu/g. Topland cow paunch had a higher fungal count of 7.0×10^4 sfu/g, while Bola cow paunch had a fungal count of 2.0×10^4 sfu/g as shown in table 3.

The bacterial and fungal count revealed that bacteria are more abundant in the rumen than fungi, this agrees with the review of Matthews *et al*. (2019) [18]; Where he stated that bacteria are the most abundant microbes in the foregut of ruminant animals, with approximately $10^{10} - 10^{11}$ cells/ml and over 200 species. Due to the insignificant difference in the aerobic and anaerobic bacteria population from each sample, most of the isolates obtained are considered facultative anaerobes, this could be due to the anaerobic nature of the ruminant environment; this is consistent with the findings of Khattab *et al*. (2017) [19], where he isolated anaerobic bacteria from the rumen and concluded that anaerobic bacteria diversity in the rumen could be one of the keys of enhancing ruminant performance and productivity. The higher bacterial count in Topland cow paunch could be due to its higher moisture content and the environmental hygiene in the abattoir as compared to cow paunch from Bola meat.

Table 1: Total Aerobic Bacterial Count

Cow Paunch	Bacteria count (cfu/g)
Topland Meat	5.11×10^8
Bola Meat	1.01×10^7

Key- cfu/g; colony forming unit per gram

Table 2: Total Anaerobic Bacterial Count

Cow Paunch	Bacteria count (cfu/g)
Toplad Meat	3.09×10^8
Bola Meat	9.83×10^6

Key- cfu/g; colony forming unit per gram

Table 3: Total Fungal Count

Cow Paunch	Bacteria count (cfu/g)
Topland Meat	7.0×10^4
Bola Meat	2.0×10^4

Key- cfu/g; colony forming unit per gram

Table 4: Biochemical Characteristics of Bacterial Isolates

Isolate Code	Gram Reaction	Cell Shape	Colony Colour	Citrate	Oxidase	Motility	H2s	Gas	Urease	Indole
Be6	-	Rod	Yellowish	-	+	+	-	-	-	+
Ms1	+	Rod	Cream	+	-	-	-	-	-	-
Be3	-	Rod	Cream	+	-	+	-	+	-	-
E3	-	Rod	Cream	-	-	-	-	+	-	-
Bm2	-	Rod	Cream	+	-	+	-	+	-	-
E7	-	Rod	Off-White	+	-	-	-	+	+	-
E8	-	Rod	Greenish	+	+	+	-	-	-	-
M5	-	Rod	Cream	+	-	+	+	+	+	+
S4	-	Rod	Cream	-	-	-	-	+	-	-
Ms7	+	Cocci	Cream	+	-	-	-	-	-	-
Ms4	+	Cocci	Cream	-	-	-	-	-	-	-
S6	-	Rod	Cream	-	-	+	+	-	-	-
Ams2	-	Spiral	Cream	+	-	-	+	+	+	-
E5	-	Rod	Cream	-	-	-	-	-	+	+

Glucose	Sucrose	Lactose	Starch Hydrolysis	Catalase	Mannitol	Maltose	Arabinose	Xylose	Galactose	Mr	Vp	Probable Organism
+			+	+	+	-				-	-	Burkholderia Ultramatica
+	+	+	+	+	-	+	+	-	+	-	-	Bacillus Sp
+	+	-	-	+	+	+	+	+	+	-	+	Enterobacter Cloacae
+	-	+	-	-	-	+	-	-	-	+	-	Escherichia Coli
+	-	+	+	+	+	+	+	+	-	-	-	Kosakonia Cowanii
+	+	+	-	+	+	+	+	+	+	-	+	Klebsiella Pneumonia
+	-	-	-	+	-	+	-	-	-	-	-	Pseudomonas Aeruginosa
+	-	-	+	+	-	+	-	+	-	+	-	Proteus Vulgaris
-	-	-	-	+	+	-	-	-	+	+	-	Shigella Sp
+	+	+	+	+	+	+	-	-	-	+	+	Staphylococcus Aureus
+	+	+	+	-	-	-	-	-	-	+	-	Streptococcus Sp
+	-	-	-	+	-	-	-	+	-	+	-	Salmonella Sp
+	-	-	+	+	+	+	-	-	-	-	-	Treponema Sp
-	+	-	-	+	+	+	+	+	-	-	-	Yersinia Enterolytica

4.2 Biochemical characterization of bacterial isolates

The biochemical characteristics of the isolates obtained are shown in table 4. The probable identity of the isolates based on their biochemical characteristics are; *Bacillus* sp, Burkholderia ultramafica, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Kosakonia cowanii, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella sp, Shigella sp, , Staphylococcus aureus, Streptococcus sp, Treponema sp and Yersinia enterocolitica.

4.3 Morphological and microscopic identification of fungal isolates

Table 5: Morhpolohical and Microscopic Characteriation of Fungal Isolates

Isolate code	Morphological and Microscopic Characteristics	Probable organism
SDA 1	Green surrounded by white, powdery and spreading. Reverse is pale yellow. Conidiophores stipes are hyaline and coarsely roughened, often more noticeable near the vesicle, conidial heads globose to subglobose, conspicuously echinulate.	Aspergillus flavus
SDA 6	Colonies are black with a slight yellow reverse. Conidiophores arising from long, broad thick-walled, sometimes branched foot cells. It has tall conidiophores ad conidia are large with radiating heads.	Aspergillus niger
SDA 7	Cream bacteria-like colonies. Candida albicans showing spherical to subspherical budding yeast-like cells or blastoconidia. The cells are arranged in chains.	Candida sp
SDA 8	Colonies are rapid growing, filamentous, greyish, wooly branching multinucleated hyphae that fill s up entire petri dish. Turning black after a few days. Sporangia are globose with a flattened base, columellae and apophysis together are globose. Sporangiospores are angular, subglobose to ellipsoidal, with ridges on the surface.	Rhizopus sp
SDA 9	Rapid growth, texture is woolly to cottony, white with cream centre. Hyphae are septate and hyaline.	Piromyces sp

Fungal isolates identified from the cow paunch are shown in table 5 as Aspergillus flavus, Aspergillus niger, Candida sp, Rhizopus sp and Piromyces sp. Aspergillus niger, Candida sp and Rhizopus sp are common to the cow paunch.

4.4 Occurrence of microorganisms

Table 6 and 7 shows the frequency and percentage of occurrence of bacterial and fungal isolates in the cow paunch. Bacillus sp, Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus sp, Staphylococcus aureus are bacteria isolates common to the cow paunch. Burkholderia ultramafica, Kosakonia cowanii and Treponema sp were only identified from Bola cow paunch while Salmonella sp, Shigella sp, Proteus vulgaris, and Yersinia enterocolitica were peculiar to Topland cow paunch. Aspergillus is the most abundant fungi genera in the cow paunch. The distribution of microorganisms in each cow paunch could either be due to the cow's feeding diet or abattoir hygiene. This is buttressed in the work of Zhao et al. (2018) [20], where he studied the response of rumen bacterial diversity and fermentation parameters in beef cattle to diets containing supplemental daidzein and discovered that the feed given to ruminants affects ruminant microbial diversity.

Table 6: Frequency of Occurrence of Bacteria Isolates

Isolate	Topland Cow Paunch (%)	Bola Cow Paunch (%)
Burkholderia ultramatica	-	8%
Bacillus sp	10%	16%
Enterobacter cloacae	15%	13%
Escherichia coli	26%	23%
Kosakonia cowanii	-	6%
Klebsiella pneumonia	4%	5%
Pseudomonas aeruginosa	7%	5%
Proteus vulgaris	3%	-
Shigella sp	6%	-
Staphylococcus aureus	9%	9%
Streptococcus sp	6%	5%
Salmonella sp	10%	-
Treponema sp	-	10%
Yersinia enterolytica	4%	-

Table 7: Percentage of Occurrence of Fungal Isolates

Isolate	Topland cow paunch (%)	Bola cow paunch (%)
Aspergillus flavus	29%	20%
Aspergillus niger	34%	42%
Candida sp	13%	17%
Rhizopus sp	20%	21%
Piromyces sp	4%	-

4.5 Proximate and physicochemical composition of cow paunch

The moisture and carbohydrate contents were relatively high in both cow paunch samples. Topland Meat however has a higher Moisture and carbohydrate content. Bola meat has a higher Nitrogen and Fibre content while the Ash and Fat content is considerably low for the cow paunch.

Figure 1: Proximate Composition of Cow Paunch from Bola and Topland Meat

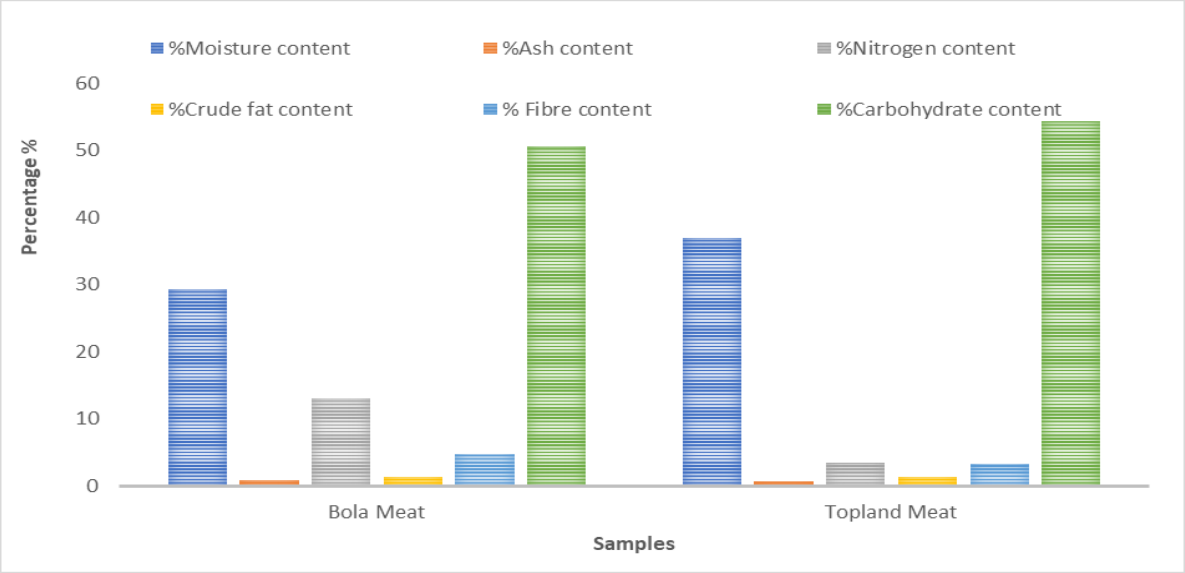


Figure 2: Electric Voltage Generation from Sample Obtained from Bola Paunch, Akure

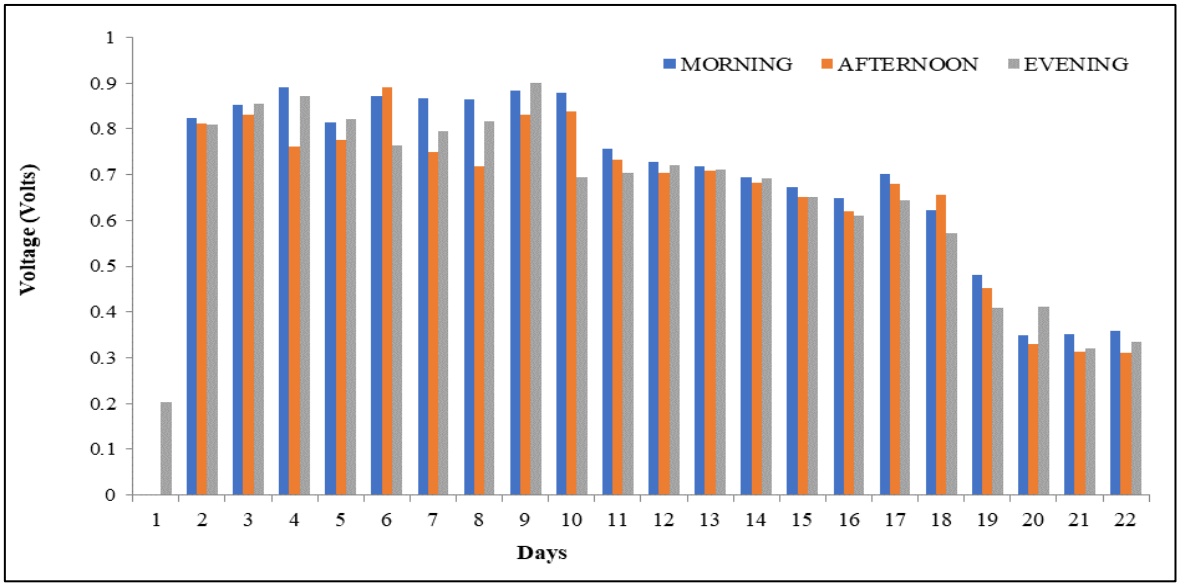
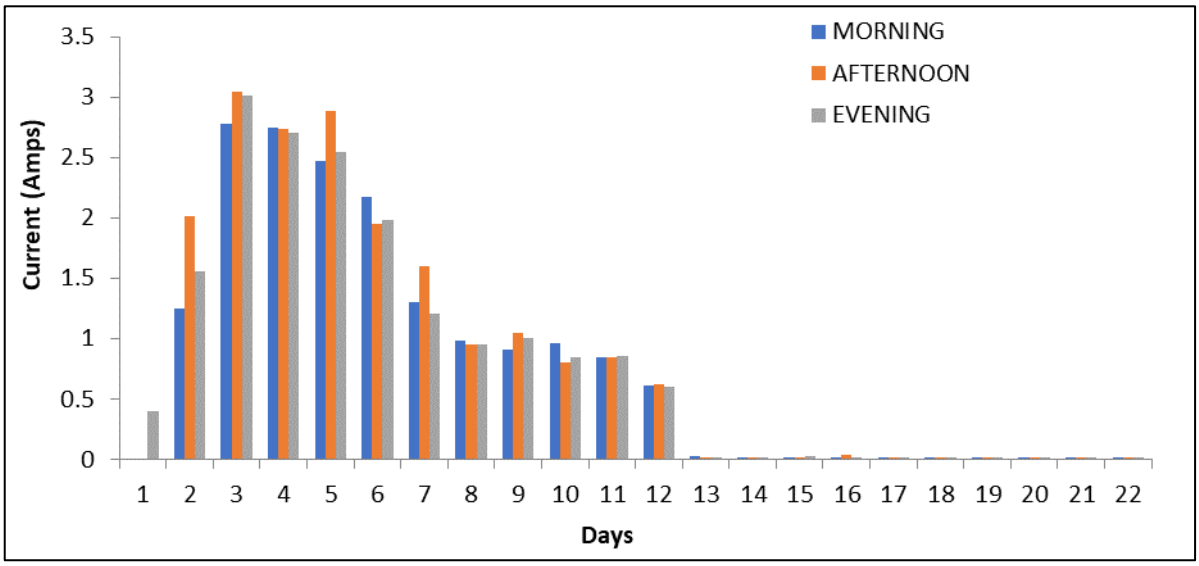


Figure 3: Electric Current Generation from Sample Obtained from Bola Paunch, Akure



It is important to note that the proximate composition of cow paunch influence the performance of the microbial fuel cell. pH of cow pauch is in consistent with the reports of Franzolin et al. (2010) [21] where he reported that rumen pH can vary from 5.5 to 7.5. The BOD and COD in the samples were generally high due to the nature of the cow rumen.

Table 8: Physicochemical Composition of Cow Paunch

Physicochemical properties (mg/l)	Bola Cow paunch	Topland Cow paunch
pH	6.9	7.1
COD	2840	2120
BOD	1.1	3.4
TS	5.1	9
TVS	3.2	7.1
TOC	3.55836	4.5806
EC	1668	1776
TN	2.1	0.56
PO4	6.584224	9.81648
NH3	6.72	3

PH – Hydrogen potential; COD – Chemical Oxygen Demand; BOD – Biochemical Oxygen Demand; TS – Total Solids; TVS – Total Volatile Solids; TOC – Total Organic Carbon; EC – Electrical Conductivity; TN – Total Nitrogen; PO_4 – Phosphate; NH_3 – Ammonia.

For Bola cow paunch; Moisture content is 29.30%; Fat content is 1.32%; Ash content is 0.8743%; Fibre content is 4.76%; Nitrogen content is 13.12%; Carbohydrate content is 50.63% Topland cow paunch has Moisture content of 36.90%; Fat content is 1.32%; Ash content is 0.76%; Fibre content is 3.29%; Nitrogen content – 3.50%; Carbohydrate content – 54.24%.

4.6 Electricity generation from cow paunch using microbial fuel cell

Voltage generated from the cow paunch was relatively high throughout the experimental days. Cow paunch from Bola Meat generated its highest electric voltage of 901mV on day 9 (evening) of the experiment and generated its highest current of 3.04mA on day 3 (afternoon) of the experiment. Topland Meat sample generated its highest voltage of 942mV on day 2 (evening) of the experiment and its corresponding high current of 4.63mA on day 1 of the experiment. For cow paunch from Bola meat, the electric current gradually increased within the first few days of the experiment and then declined; Cow paunch from Topland Meat generated a high current in the first day of the experiment, which declined subsequently and further shows spontaneous increase and decrease. This result is similar to the findings of Faloni and Adegunloye (2020) [22] who experienced spontaneous increase and decrease in the generation time when generating electricity from pig dung but disagrees with the reports of Adegunloye and Ojo

(2019) [23] who reported a progressive increase in the generation period (14 days), when decayed wood was used for current and voltage generation. This could be due to the similarity in the composition and microbial population of pig dung and cow paunch.

7.0 Conclusions

It is important to note from the electric current and voltage generation that cow paunch has a great potantial to generate electricity using microbial fuel cell through the activities of microorganisms. This could serve as an alternative for electricity generation and a safe from the erratic electricity supply.

8.0 Acknowledgment

This work was carried out by both authors. D. V. Adegunloye supervised the research work and the experimental work was carried out by E. Omopariola. All authors read and approved the final manuscript.

Figure 4: Electric Voltage Generation from Sample Obtained from Topland Paunch, Akure

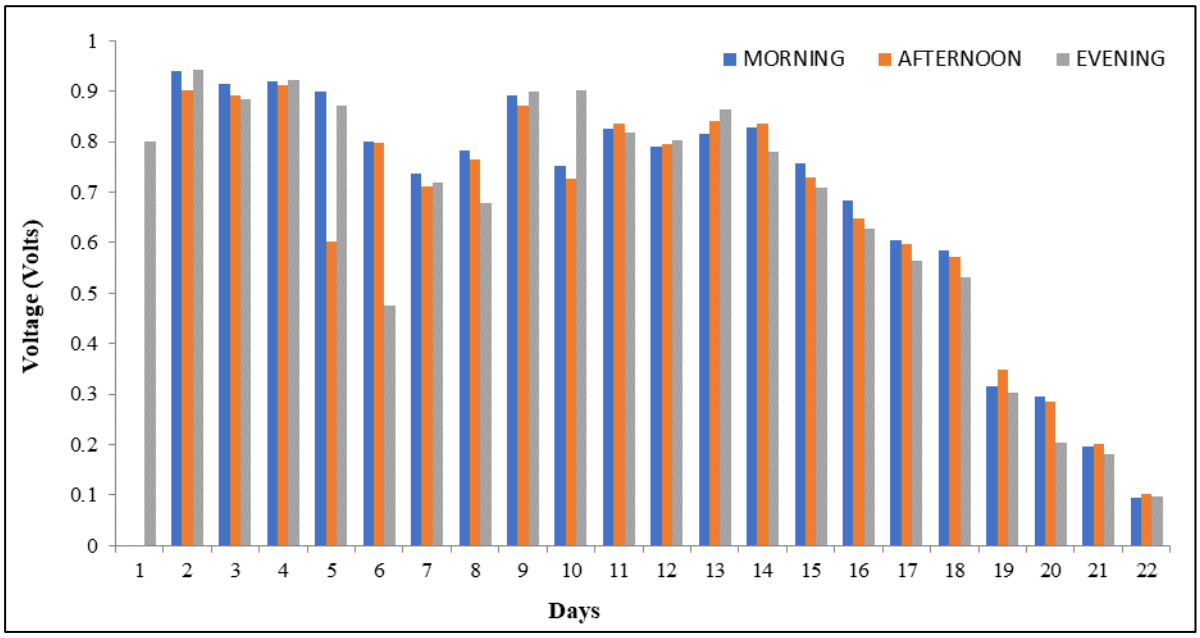
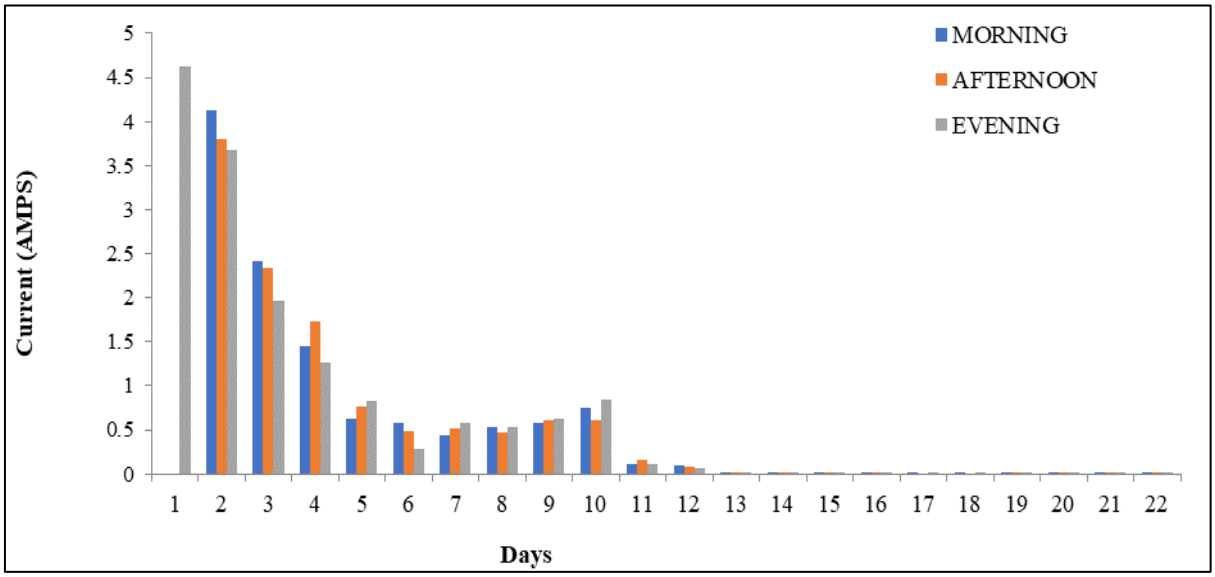


Figure 5: Electric Current Generation from Sample Obtained from Topland Paunch, Akure



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