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Enhancing Electricity Generation with the use of $KMnO_4$ as an electron acceptor in Microbial Fuel Cell

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Abstract

The use of potassium permanganate ($KMnO_4$) to enhance electricity generation in a microbial fuel cell (MFC) was evaluated. Proximate compositions of pig dung were determined. Microorganisms were isolated and identified using conventional and molecular methods. Double chamber types of MFCs were constructed. The anode chamber contained the pig dung sample, while the cathode chamber contained 0.1 M $KMnO_4$. Current cum voltage were observed three times daily for a period of 40 days. Nine bacteria and five fungi were identified. *Bacillus mycoides* of the phylum Firmicutes were dominant. Before electricity generation, the highest bacterial and fungal load was from Apatapiti (2.71×10^5 cfu/g) and FUTA (2.93×10^4 sfu/g) pig dung respectively. After, the bacterial and fungal load was highest in Apatapiti (1.35×10^5 cfu/g) and South Gate (1.60×10^4 sfu/g) pig dung respectively. Generally, the highest voltage and current were from FUTA (1301 mV) and Apatapiti (4.5020 mA) pig dung, respectively. Findings revealed for the first time that pig dung yielded an output voltage as high as 3003 mV, which powered low voltage appliances conveniently. Hence, pig dung is a potential renewable electricity generation source and its use would curb environmental toxics and health hazards.

Keywords: Microbial fuel cell, pig dung, $KMnO_4$, current, voltage.

1. Introduction

Energy is an integral factor for the socioeconomic growth, progress, and development of any nation. Globally, the usage of fossil fuel to generate electricity, automobile mobility and in many industrial establishments has recently recorded an alarming increasing rate, and this elicits global energy crisis simultaneously (EIA, 2013). It has been predicted from studies that fossil fuel will govern the world's energy supply and will amount to about eighty percent by 2040 (EIA, 2013).

Over the years, the demand for more animal protein to maintain an exponentially expanding population has given birth to a conspicuous and alarming increase in livestock production with sundry start-up of diverse animal farms. Simultaneously, the disposal of animal dung into the environment has been on a colossal increase, inadvertently creating a challenge in waste management unresolved (Iregbu *et al.*, 2014). Pig dung as an MFC substrate would be an advantageous alternative in electricity generation, this will thereby lead to its reduction in the environment. Also, it would abate the spurious challenges that accompany its reckless disposal and thereby generate wealth and employment for the nation.

The discovery that the metabolic activities of microorganisms could generate energy in the form of electrical current and voltage has led to a raised concentration in MFC technology (Potter, 1911). These new technologies can be adopted with promising prospects to provide energy in a sustainable mode. However, major improvements are essential for its applications on a large scale to become feasible.

An important alternative research area in the recent approaches to electricity generation was from carbon-free renewable energy and additional power systems (Fan and Xue, 2016). Researches over the years on energy solutions revealed fossil fuels as irreplaceable completely because no solution is sufficient. With respect to this, alternatives should be sorted and sourced in mitigating this vast challenge (assuredly certain that dependence on fossil fuels cannot be sustainable due to finite supplies and pollution) (Franks and Nevin, 2010). MFC is renowned technological advancement with the capacity to meet the goals (dual) of energy production and waste management (Fan and Xue, 2016). MFC has been on the call of all, even till date with its projected relevance in recovering energy; especially electrical energy (Cao, 2019).

MFCs has been limited by quite a lot of parameters some of which include; the rate of oxidation and electron transfer to the anodes by microorganisms, nature of sample substrate, nature of proton exchange membrane (PEM), nature of the electron acceptor (catholyte) used among others (Ginkel *et al*., 2005). The use of oxygen (aqueous or gaseous state) as an electron acceptor at the cathode chamber has been of wide acceptability owing to availability and its high redox potential (Clauwert *et al*., 2008). Nevertheless, its poor contact with the cathode electrode in addition to the slowness in its oxygen reduction rate on the carbon electrode surface has been a major denigration that impedes its usage in MFCs (Li *et al.*, 2009). This downside of oxygen can be circumvented by enabling an increase in the amount of liquefied oxygen

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in the cathode with the use of electron acceptors with high oxidizing potential. The present study explores the usage of $KMnO_4$ in enhancing current and voltage generation from pig dung in a microbial fuel cell.

2. Materials and Methods

2.1. Sample Collection

The used pig dung was collected from pig farms in FUTA, South Gate, and Apatapiti area of Akure, Ondo state. The samples were collected into sterile bags, labelled and conveyed straightaway into the laboratory for immediate analyses.

2.2. Proximate Analysis

Proximate composition of the moisture content, total ash, crude fat, crude fiber, protein content, and carbohydrate content of the pig dung was determined. Moisture Content was determined according to the method description of AOAC (2002). Ash content was analysed using the gravimetric method, according to AOAC (2005). The fat content was determined using the Soxhlet type of the direct solvent extraction method, according to AOAC (2010). The protein content of the sample was determined by the Micro-Kjeldahl method, according to AOAC (2005). The total carbohydrate content of each sample was estimated by "difference," according to the method description of AOAC (2010).

2.3. Isolation of Bacteria and Fungi from pig Dung

The bacteria isolates were subjected to various biochemical tests and with the use of ABIS online software, while the fungi isolates were identified by viewing under a microscope (Olympus CH) (Samson, 2007). The isolates were further identified using molecular methods to ascertain their identities.

2.4. Identification of Isolates at Molecular Level

Bacteria and fungi with the highest percentage frequency of occurrence from each sample were identified to the molecular level. This was carried out using the sequencing techniques according to the method description of Akinyemi and Oyelakin, (2014). The DNA in each isolate was extracted using the procedure of the Zymo bacterial DNA Mini-prep kit. The quality of DNA was ascertained with the use of agarose gel electrophoresis by size fractionation on 1.0 % agarose gel. The extracted genomic DNA was stored at 4ºC. PCR was performed using Hi-Media Taq polymerase (500 U), Hi-media 50 mM $MgCl_{12}$ and Hi-media 10X buffer (500 U) and QIAGEN dNTPs (10 mM each). Universal 16S rRNA forward primer was used. PCR amplifications were performed with an Applied Biosystems Veriti Thermal cycler according to the method of Akinyemi and Oyelakin, 2014. Nine µl of Hi Di Formamide with 1 µl of the amplified DNA totaling 10 µl was loaded on the machine, and the data were expressed as A, C, T, and G on the computer system. The obtained sequence was analyzed with BLAST in the National Centre for Biotechnology Information (NCBI) database. Moreover, the phylogenetic relationship of the bacteria species were compared using their existing sequences obtained from NCBI GenBank (Oyetayo, 2014).

2.5. Construction of Microbial Fuel Cell

Double chamber MFC was built according to the work of Adegunloye and Faloni (2020). The double chambers include; anode and cathode chambers with an occupying volume of one thousand and two hundred milliliters in each of the chamber. The chambers were bridged with a proton exchange membrane (PEM) contained in polyvinyl chloride pipes with dimension 14 cm by 3.7 cm, the point of connections were sealed with epoxy adhesive to avoid dripping. Two millimeters (mm) diameter of the hole was drilled into the cover lid of each chamber as wire point inputs. The chambers were filled with water up to the brim for an hour to arrest possible leakages from the joining points. Voltage and current generated from the MFC were measured with the digital multimeter (SUOER SD 9205A) and recorded at 6 hours interval each day (8 am - 8 pm) summarily making up three sessions daily (morning, afternoon, and evening) for a period of forty (40) days.

2.5.1. Proton Exchange Membrane (PEM)

The PEM consisted of NaCl (an electrolyte for proton exchange) according to the work of (Kumar *et al*., 2012; Adegunloye and Faloni, 2020). Twenty gram (20g) of agar-agar powder was dissolved into 1000 ml of distilled water containing 75.5 g of NaCl, the mixture was boiled for about 3 minutes and cooled to room temperature (Akujobi *et al*., 2017). The solution was afterwards dispensed into the polyvinyl chloride pipes with an enclosed end and was left to congeal at normal room temperature.

2.5.2. Filling of the Chambers

The fabricated chambers were disinfected with ethyl alcohol (85%) and radiated for fifteen minutes in an UV inoculating chamber to ensure possible contaminants are ruled out. The anodic chamber was aseptically filled with 50% of the substrate (600 g and 600 ml of pig dung and sterilized water respectively) (Kumar *et al*., 2012). The cathodic chamber was filled with 1200 ml of 0.1 M $KMnO_4$.

2.5.3. Making and Testing the Control MFC:

Six hundred (600) gram of pig dung was sterilized in the autoclave (at 121°C for 15 minutes) to exterminate the organisms embedded while its nutritional and mineral components are preserved. The anodic chamber was aseptically filled with the substrate (600 g and 600 ml of pig dung and sterilized distilled water respectively) and the chamber was air tight. The cathodic chamber was filled with 1200 ml of 0.1 M $KMnO_4$ as electron acceptor.

2.6. Statistical Analysis

Duncan's New Multiple Range Test and Analysis of Variance (ANOVA) using Statistical Packages for the Social Sciences (SPSS) 22.0 version was employed in the test for significance of difference between the samples from the various sites. Data are presented as mean ± standard error (SE). The significance of each test was evaluated at the level of $P \leq 0.05$.

3. Results

3.1. Pig Dung's Proximate Composition

The proximate content of the pig dung from the three sites is presented in Table 1. The outcome specified a significant difference ($p \leq 0.05$) in the nutrient composition of the dung from the three locations. FUTA pig dung had the highest significant ($p \leq 0.05$) values for ash content, crude fat content and protein content with values (12.98 ± 0.00) %, (6.05 ± 0.00) % and (25.36 ± 0.00) % respectively. Apatapiti pig dung had the highest significant ($p \leq 0.05$) values for crude fibre content, carbohydrate content and energy value with values (40.44 ± 0.00) %, (14.77 ± 0.00) % and (703.81± 0.00) Kj/g. There was no significant difference ($p \leq 0.05$) in the moisture content of the dung from the three locations.

Table 1. Pig Dung's Proximate Composition

Parameters	Ash Content (%)	Moisture Content (%)	Crude Fat Content (%)	Crude Fibre Content (%)	Protein Content (%)	Carbohydrate Content (%)	Energy Value (%)
FUTA	12.9780 ± 0.00058^{c}	18.2580 ± 3.00050^{a}	6.0520 ± 0.00058^{c}	33.2260 ± 0.00058^{a}	25.3620 ± 0.00058^{c}	1.3040 ± 0.00058^{a}	677.2460 ± 0.00058^{b}
South Gate	9.0040 ± 0.00058^{b}	16.3140 ± 0.00058^{a}	3.0250 ± 0.00058^{a}	40.4430 ± 0.00058^{c}	21.4280 ± 0.00058^{b}	9.7320 ± 0.00058^{b}	641.6820 ± 0.00058^{a}
Apatapiti	7.0700 ± 0.00577^{a}	22.3500 ± 0.00577^{a}	3.8460 ± 0.00058^{b}	33.7040 ± 0.00058^{b}	18.2630 ± 0.00058^{a}	14.7670 ± 0.00058^{c}	703.8120 ± 0.00058^{c}

3.2. The Total Aerobic Bacterial Load (TABL)

The total aerobic bacterial load is presented in Table 2. The outcome specified a significant ($p \leq 0.05$) difference in the bacterial load of the pig dung from the three locations. The TABL result from the first isolation before the MFC experiment was between 1.72 x 10^5 cfu/g and 2.71 x 10^5 cfu/g; FUTA pig dung had significantly ($p \leq 0.05$) lower TABL compared to South Gate and Apatapiti pig dung which were on the same significant difference ($p \leq 0.05$) level. The TABL result after the MFC experiment was between 5.33 x 10^3 cfu/g and 1.35 x 10^5 cfu/g; the highest significant ($p \leq 0.05$) load was from Apatapiti pig dung. The TABL was higher for the first isolation (before the experiment) across all the sites compared to the final isolation (after the experiment).

Table 2.The Total Aerobic Bacterial Load (TABL)

Pig Dung	Initial (Cfu/g)	Final (Cfu/g)
FUTA	1.72 x 10^5 ± 25.44^{a}	8.90 x 10^4 ± 5.51^{b}
South Gate	2.71 x 10^5 ± 25.10^{b}	5.33 x 10^3 ± 0.88^{a}
Apatapiti	2.67 x 10^5 ± 7.06^{b}	1.35 x 10^5 ± 3.18^{c}

3.3. The Total Aerobic Fungal Load (TAFL)

The total aerobic fungal load is presented in Table 3. The outcome specified a significant ($p \leq 0.05$) difference in the fungal load of the pig dung from the three locations. The TAFL result from the first isolation before the MFC experiment was between 3.99 x 10^3 cfu/g and 2.94 x 10^4 cfu/g; all the dungs from the different locations were on the same significant ($p \leq 0.05$) difference level. The TAFL result after the MFC experiment was between 8.01 x 10^3 cfu/g and 1.60 x 10^4 cfu/g; South Gate pig dung had significantly ($p \leq 0.05$) lower TAFL compared to Apatapiti and FUTA pig dung which were on the same significant difference ($p \leq 0.05$) level. South Gate pig dung had the highest TAFL from the first isolation (before the experiment) while Apatapiti and FUTA pig dung had the highest fungal load from the final isolation (after the experiment).

Table 3. The Total Aerobic Fungal Load (TAFL)

Pig Dung	Initial (Sfu/g)	Final (Sfu/g)
FUTA	1.47 x 10^4 ± 0.88^{a}	1.60 x 10^4 ± 3.06^{b}
South Gate	2.94 x 10^4 ± 13.78^{a}	8.01 x 10^3 ± 0.00^{a}
Apatapiti	3.99 x 10^3 ± 2.00^{a}	1.53 x 10^4 ± 0.67^{b}

3.4. Total Bacterial and Fungal Count (TBC/TFC) under Anaerobic Condition

The TBC and TFC of the pig dung under anaerobic condition after the MFC experiment is presented in Table 4. The outcome specified a significant ($p \leq 0.05$) difference in the fungal load of the pig dung from the three locations. The TBC was in a range of 4.00 x 10^3 cfu/g to 2.20 x 10^4 cfu/g; the highest significant ($p \leq 0.05$) TBC was from FUTA pig dung while the lowest was from South Gate pig dung. The TFC was between 6.33 x 10^3 sfu/g and 1.50 X 10^4 sfu/g; Apatapiti pig dung had significantly ($p \leq 0.05$) the highest TFC while there was no TFC from FUTA pig dung.

Table 4.Total Bacterial and Fungal Count (TBC/TFC) under Anaerobic Condition

Pig Dung	Bacteria (Cfu/g)	Fungi (Sfu/g)
FUTA	2.20 x 10^4 ± 4.04^{c}	9.33 x 10^3 ± 1.33^{a}
South Gate	4.00 x 10^3 ± 0.00^{a}	6.33 x 10^3 ± 0.88^{a}
Apatapiti	1.77 x 10^4 ± .88^{b}	1.50 x 10^4 ± 0.58^{a}

3.5. Total Coliform Count (TCC)

The TCC of the pig dung is presented in Table 5. The outcome specified a significant ($p \leq 0.05$) difference in the fungal load of the pig dung from the three locations. The TCC result from the first isolation before the MFC experiment was between 3.96 x 10^4 cfu/g and 5.98 x 10^4 cfu/g; with all the dungs from the different locations were on the same significant ($p \leq 0.05$) difference level. TCC after the MFC experiment was between 0.00 x 10^3 cfu/g and 3.73 x 10^4 cfu/g; the highest significant ($p \leq 0.05$) load was from FUTA pig dung. The TCC was higher for the first isolation (before the experiment) across all the sites compared to the final isolation (after the experiment).

Table 5. Total Coliform Count (TCC)

Pig Dung	Initial (Cfu/g)	Final (Cfu/g)
FUTA	5.98 x 10^4 ± 3.67^{a}	3.73 x 10^4 ± 3.33^{b}
South Gate	3.96 x 10^4 ± 9.67^{a}	0.00 x 10^3 ± 0.00^{a}
Apatapiti	5.93 x 10^4 ± 3.33^{a}	0.00 x 10^3 ± 0.00^{a}

KEY: Values are calculated as mean ± Standard error of the pig dung. And, values that fall under the same column bearing similar superscript are not apparently different at ($p \leq 0.05$) on applying Duncan's New Multiple Range Test on it.
Cfu/g- Colony-forming unit per gram. Sfu/g- Spore forming unit per gram.

3.6. Biochemical characterization of bacterial isolates

The biochemical characterization of the bacterial isolates is presented in Table 6. The result reveals nine (9) isolates, and the probable identity of the isolates are *Bacillus sp, Escherichia coli, Shigella* sp, *Staphylococcus arlettae, Micrococcus luteus, Klebsiella singaporensis, Paenibacillus sp, Salmonella* sp, *and Yersinia intermedia*.

Table 6. Biochemical Characterization of the Bacterial Isolates

Isolate Code	Gram's Reaction	Cell Morphology	Catalase	Indole	Oxidase	Methyl Red	Voges Proskauer	Citrate Utilization	Urease	Motility	Spore Forming	Glucose	D-Mannitol	Lactose	Fructose	Galactose	Maltose	Sorbitol	Sucrose	H_2S production	Probable Identity
35(o)	+	C	+	-	-	-	-	-	-	-	-	+G	+G	+	+G	+G	+G	+G	(+)	-	*Staphylococcus arlettae*
17(o)	-	R	+	+	-	-	-	-	-	-	-	+	+	+G	+	+G	+G	+G	+G	-	*Yersinia intermedia*
26(o)	+	C	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	*Micrococcus luteus*
C(n)	-	R	+	-	+	-	+	+	+	-	-	+G	+G	+G	+G	+G	+G	+G	+	-	*Klebsiella singaporensis*
30(n)	+	R	+	-	+	-	-	-	+	+	+	+	+	-	+	+	+	+G	+G	-	*Paenibacillus amylolyticus*
17(n)	+	R	+	-	+	+	+	+	-	-	+	+	+	-	-	+	(+)	-	-	+	*Bacillus mycoides*
EC	-	R	+	+	-	-	-	-	-	+	-	+G	+G	+G	+G	+G	+G	+G	+	+	*Escherichia coli*
Shig	-	R	+	-	-	+	+	-	+	-	-	+	+	-	-	-	-	-	-	-	*Shigella* sp
Salm	-	R	+	-	-	-	-	-	-	+	-	+G	+G	+G	+G	+G	+G	+G	+G	+	*Salmonella* sp

KEY: R= Rod, C= Cocci, += Positive reaction, (-) = Negative reaction, (+) = weakly positive reaction, G = gas production

3.7. Microscopic characterization of fungal isolates

The identity of the fungi with the aid of microscopy view are presented in Table 7; this include: *Fusarium* sp, *Aspergillus flavus, Aspergillus funmigatus, Aspergillus niger,* and *Penicillium chrysogenum.*

Table 7.Microscopic Characterization of Fungi Isolates from Pig Dung

Isolate Code	Characteristics	Probable Identity
1	Hyphae are septate and hyaline. Produces both macro- and microconidia from slender phialides. The macroconidia are several celled and the microconidia are one celled in chains, Phialides are cylindrical, conidiophores are medium length.	*Fusarium* sp
2	Conidiophore was thick walled, hyaline and coarsely roughened, often more noticeable with a vesicle at the top. Vesicles are globose with phialides and with short conidial chains.	*Aspergillus flavus*
3	Colonies are spreading, green with pale to bright yellow. Septate hyaline hyphae, conidiophores are branched with yellow exudate, two to three stage branched conidia and mutulae phialides are present.	*Penicillium chrysogenum*
5	Colonies changed from white to black conidia. Septate hyphae, long/tall conidiophores, smooth-walled, branched foot cells, hyaline and contained globose vesicle each covered completely with biseriate phialides. Conidia were globose, dark and rough-walled. Conidia heads were large with radiating heads, dark brown and biseriate.	*Aspergillus niger*
4	Conidial heads are long, globose to prolate. Conidial heads radiate to nearly globose, grayish near the apices with an irregular shape. Conidiophores hyaline slightly coloured, short green, particularly in the upper part, smooth-walled.	*Aspergillus funmigatus*

3.8. Frequency Distribution of Bacterial Isolates from Pig Dung in Percentage

The percentage frequency distribution of bacterial isolates, as represented in Table 8, reveals the frequency distribution of bacterial isolates from Apatapiti, South Gate, and FUTA pig dung. The percentage of occurrence includes *Paenibacillus amylolyticus* (24 %), *Yersinia intermedia* (17.24 %), and *Bacillus mycoides* (16.67 %). These three bacteria occurred before the MFC experiment and after, they had the highest frequency of occurrence in the first three orders, among others.

Table 8. Percentage Frequency Bacterial Isolates

Bacteria	Number	Frequency (%)
Staphylococcus arlettae	1	3.70
Yersinia intermedia	7	25.93
Micrococcus luteus	1	3.70
Klebsiella singaporensis	2	7.40
Paenibacillus amylolyticus	6	22.22
Bacillus mycoides	8	29.63
Escherichia coli	2	7.40
Total Number	27	100

3.9. Frequency Distribution of Fungal Isolates from Pig Dung in Percentage

The percentage frequency distribution of fungal isolates, as represented in Table 9, reveals the percentage frequency distribution of fungal isolates from Apatapiti, South Gate, and FUTA pig dung. The percentage of occurrence include *Aspergillus funmigatus* (55.56 %), *Aspergillus flavus* (52.94 %), and *Fusarium* sp (50 %). These three fungi occurred before the MFC experiment and after, they had the highest frequency of occurrence in the first three orders, among others.

Table 9. Percentage Frequency of Fungal Isolates

Fungi	Number	Frequency (%)
Aspergillus funmigatus	9	34.62
Aspergillus flavus	6	23.08
Fusarium sp	4	15.38
Penicillum chrysogenum	2	7.69
Aspergillus niger	2	7.69
Total	23	100

3.10. Molecular Based Identification of Bacteria Species with the highest percentage of occurrence

The three most occurred organisms across the sample sites were molecularly identified as; *Paenibacillus amylolyticus*, *Bacillus mycoides*, and *Yersinia intermedia*. Observation of the gels after electrophoresis of PCR products of the bacterial and fungal DNA isolated from pig dung are shown on Plates 1 and 2, respectively. The extracted DNA bands for each of the bacteria isolated from pig dung were shown to have a molecular weight of approximately 1500 bp with highly bright, bold, and clean DNA bands. Plate 2 shows the gel electrophoresis image detailed view of the amplified 18S and 26S rRNA from the fungal isolates. The extracted DNA bands for each of the fungi isolated from pig dung were shown to have a molecular weight of approximately 600 bp with highly bright, bold, and clean DNA bands.

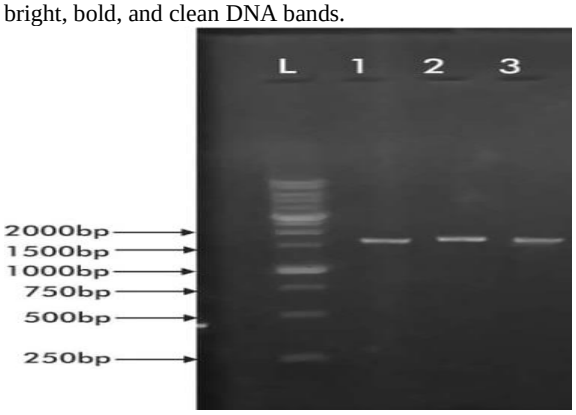

Plate 3.1: PCR amplification of genomic DNA targeted to amplify the 16S rRNA gene of the bacterial isolate on 1.5% agarose gel electrophoresis

L- Molecular marker

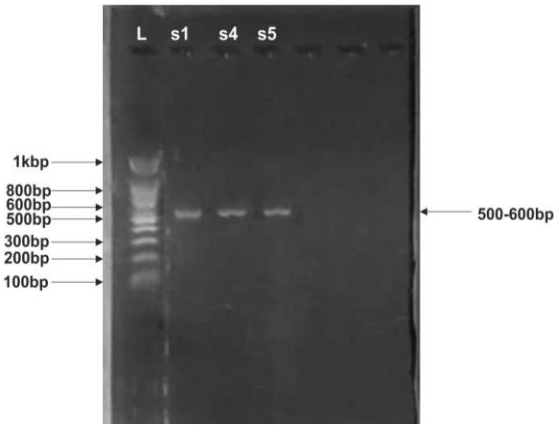

Plate 3.2. PCR amplification of genomic DNA targeted to amplify the 18S and 26S rRNA gene of the fungal isolate on 1.5% agarose gel electrophoresis.

L- Molecular marker

3.10.1. Genetic Relationship between the Bacterial Isolates

The constructed phylogenetic tree showing the relatedness of the bacterial isolates is represented in Plate 3. The phylogenetic tree of the bacterial isolates in Plate 3 showed that the isolates had two clades; from the first clade, *Yersinia enterocolitica* and *Bacillus amyloliquefaciens* (NR_028624 and NR_117946) are closely related but shared the same ancestor with *Bacillus funiculus* which is on the second clade. The evolutionary distance between the bacterial isolates is 0.04.

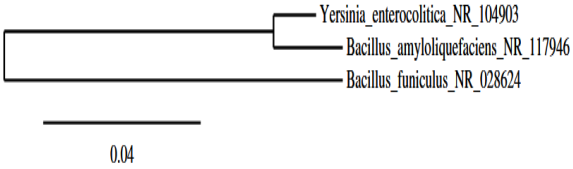

Plate 3.3. Phylogenetic tree of the bacterial isolate from pig dung.

3.10.2. Molecular Identification of isolated microorganisms from pig dung

The molecular identities of the bacterial and fungal isolates are represented in Table 10. The bacterial isolates had more than 80 % similarity with those in the NCBI Gene Bank by BLASTn. The BLASTn results confirmed the bacterial isolates were similar to *Bacillus amyloliquefaciens* NR_117946, *Bacillus funiculus* NR_028624, and *Yersinia enterocolitica* subsp. palearctica NR_104903, which were culturally identified as *Paenibacillus amylolyticus*, *Bacillus mycoides* and *Yersinia intermedia*, respectively. The fungal isolates had more than 70% similarity with those in the NCBI Gene Bank by BLASTn. The BLASTn results confirmed the fungal isolates were similar to *Fusarium verticillioides* XR_001989346, *Aspergillus heteromorphus* XM_02554151 and *Aspergillus tamari* (MK_638758.1) which were culturally identified as *Fusarium* sp, *Aspergillus funmigatus*, *and Aspergillus flavus* respectively.

Table 10. Molecular Identification of isolated microorganisms from pig dung

Cultural and Biochemical Identification	Gene sequence Identification	Max Identity	Accession Number
Paenibacillus amylolyticus	*Bacillus amyloliquefaciens*	98.23%	NR_117946
Bacillus mycoides	*Bacillus funiculus*	88.92%	NR_028624
Enterobacter asburiae	*Yersinia enterocolitica*	93.62%	NR_104903
Fusarium sp	*Fusarium verticillioides*	90.1%	XR_001989346
Aspergillus funmigatus	*Aspergillus heteromorphus*	96.95%	XM_025541519
Aspergillus flavus	*Aspergillus tamarii*	73.17%	MK_638758.1

3.11. Voltage Generated from FUTA Pig Dung

The electricity generation from FUTA pig dung in terms of voltage is presented in Figure 3.1. The outcome specified a significant ($p \leq 0.05$) difference across the three sessions of the day. In the morning session; the least voltage was recorded on day 1 as 673.0000 ± 0.57735 mV while the peak voltage was recorded on day 20 as 1220.6667 ± 0.33333 mV. In the afternoon session; the least voltage ($p \leq 0.05$) were recorded on days 1 and 2 as 873.0000 ± 0.57735 mV and 885.0000 ± 0.57735 mV respectively while the peak voltage was recorded on day 15 as 1214.6667 ± 0.33333 mV. In the evening session; the least voltage was recorded on day 1 as 585.0000 ± 1.15470 mV while the peak voltage was recorded on day 7 as 1301.0000 ± 0.57735 mV.

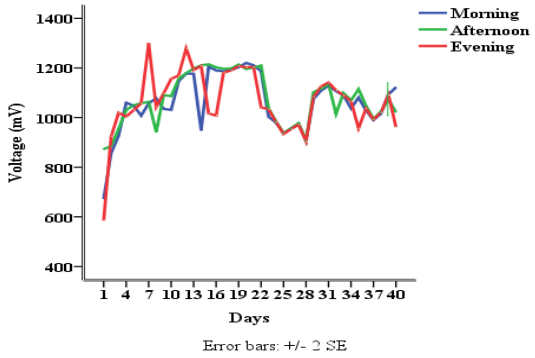

Figure 1. Voltage generated from FUTA pig dung

3.12. Current Generated from FUTA Pig Dung

The electricity generation from FUTA pig dung in terms of current is presented in Figure 3.2. The outcome specified a significant ($p \leq 0.05$) difference across the three sessions of the day. In the morning session; the least current was recorded on day 2 as 0.92167 ± 0.000882 mA while the peak current was recorded on day 5 as 2.30200 ± 0.000577 mA. In the afternoon session; the least current was recorded on day 2 as 0.93500 ± 0.000577 mA while the peak current was recorded on day 5 as 2.60167 ± 0.000333 mA. In the evening session; the least current was recorded on day 2 as 0.93100 ± 0.000577 mA while the peak current was recorded on day 5 as 2.63233 ± 0.000882 mA.

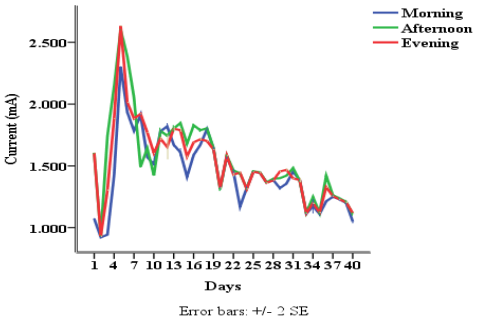

Figure 2. Current generated from FUTA pig dung

3.13. Voltage Generated from Apatapiti Pig Dung

The electricity generation from Apatapiti pig dung in terms of voltage is presented in Figure 3.3. The outcome specified a significant ($p \leq 0.05$) difference across the three sessions of the day. In the morning session; the least voltage was recorded on day 5 as 345.6667 ± 2.84800 mV while the peak voltage were recorded on days 14, 17 and 18 as 1183.3333 ± 1.20185 mV, 1187.0000 ± 0.57735 mV and 1209.0000 ± 0.57735 mV respectively. In the afternoon session; ; the least voltage was recorded on day 4 as 386.0000 ± 0.57735 mV while the peak voltage was recorded on day 18 as 1209.3333 ± 0.88192 mV. In the evening session; the least voltage were recorded on days 3 and 4 as 557.0000 ± 0.57735 mV and 563.0000 ± 0.57735 mV respectively while the peak voltage were recorded on days 17 and 18 as 1183.0000 ± 0.57735 mV and 1204.0000 ± 0.57735 mV respectively.

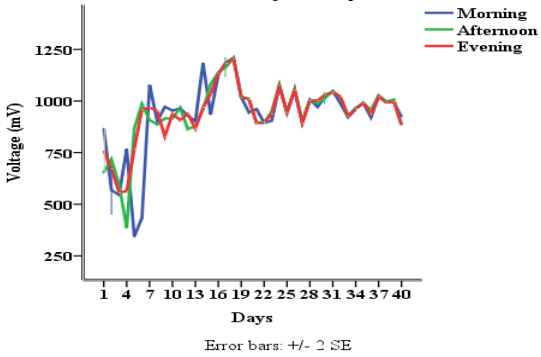

Figure 3. Voltage generated from Apatapiti pig dung

3.14. Current Generated from Apatapiti Pig Dung

The electricity generation from Apatapiti pig dung in terms of current is presented in Figure 3.4. The outcome specified a significant ($p \leq 0.05$) difference across the three sessions of the day. In the morning session; the least current was recorded on day 2 as 0.92167 ± 0.000882 mA while the peak current was recorded on day 5 as 2.30200 ± 0.000577 mA. In the afternoon session; the least current was recorded on day 2 as 0.93500 ± 0.000577 mA while the peak current on day 5 as 2.60167 ± 0.000333 mA. In the evening session; the least current was recorded on day 2 as 0.93100 ± 0.000577 mA while the peak voltage was recorded on day 5 as 2.63233 ± 0.000882 mA.

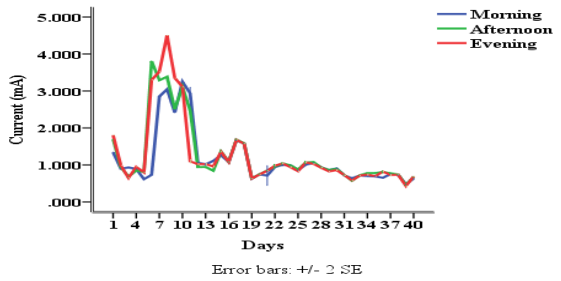

Figure 4. Current generated from Apatapiti pig dung

3.15. Voltage Generated from South Gate Pig Dung

The electricity generation from South Gate pig dung in terms of voltage is presented in Figure 3.5. The outcome specified a significant ($p \leq 0.05$) difference across the three sessions of the day. In the morning session; the least voltage was recorded on day 3 as 391.3333 ± 1.85592 mV while the peak voltage was recorded on day 17 as 1206.0000 ± 0.57735 mV. In the afternoon session; the least voltage was recorded on day 3 as 346.3333 ± 2.18581 mV while the peak voltage was recorded on day 17 as 1214.6667 ± 2.40370 mV. In the evening session; the least voltage was recorded on day 8 as 358.0000 ± 1.15470 mV while the peak voltage was recorded on day 17 as 1203.0000 ± 0.57735 mV.

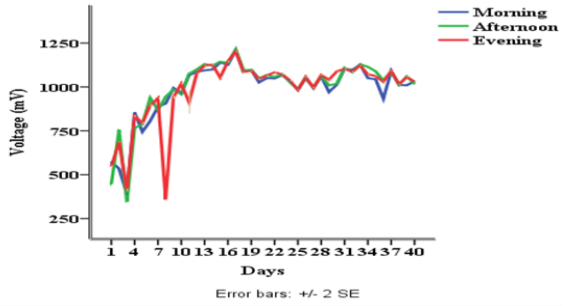

Figure 5. Voltage generated from South Gate pig dung

3.16. Current Generated from South Gate Pig Dung

The electricity generation from South Gate pig dung in terms of current is presented in Figure 3.6. The outcome specified a significant ($p \leq 0.05$) difference across the three sessions of the day. In the morning session; the least current was recorded on day 2 as 0.3050 ± 0.000577 mA while the peak current was recorded on day 18 as 1.96333 ± 0.008819 mA. In the afternoon session; the least current was recorded on day 2 as 0.31233 ± 0.004702 mA while the peak current was recorded on day 18 as 1.98000 ± 0.005774 mA. In the evening session; the peak current was recorded on day 2 as 0.33000 ± 0.006557 mA while the peak voltage was recorded on day 12 as 2.12333 ± 0.061734 mA.

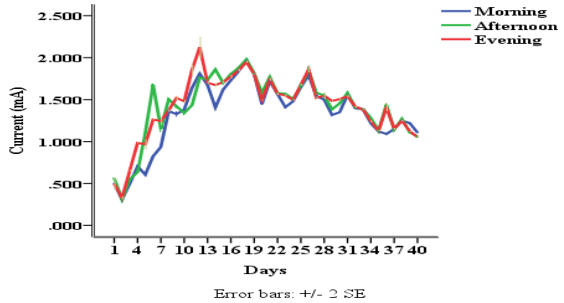

Figure 6. Current generated from South Gate pig dung.

3.17. Voltage Generated from Control MFC

The electricity generation from the control MFC in terms of voltage is presented in Figure 3.7. The outcome specified a significant ($p \leq 0.05$) difference across the three sessions of the day. The peak current was recorded on day 1 as 379.00 ± 0.00 mV. After this, there was an observable steady decrease to the minimum values till day 8.

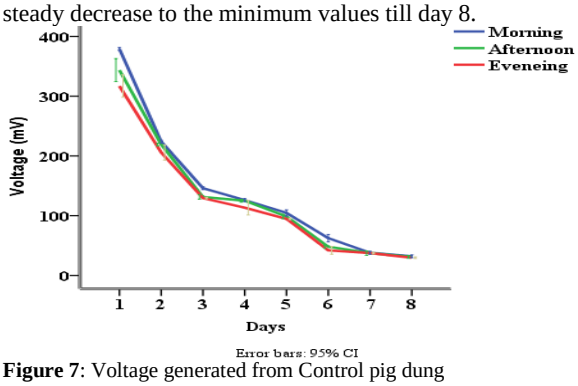

Figure 7: Voltage generated from Control pig dung

3.18. Current Generated from Control MFC

The electricity generation from the control MFC in terms of current is presented in Figure 3.8. The outcome specified a significant ($p \leq 0.05$) difference across the three sessions of the day. The peak current was recorded on day 1 as 0.030 ± mA. After this, there was an observable steady decrease to the minimum values till day 8.

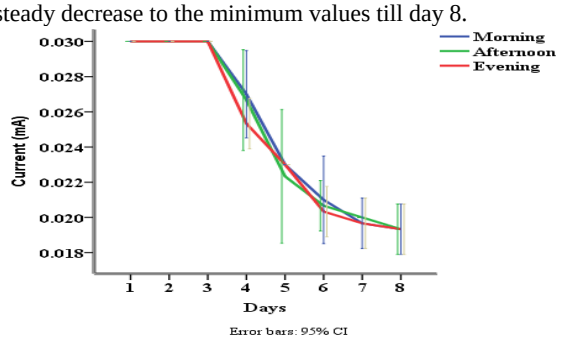

Figure 8. Current generated from Control pig dung

3.19. Testing the MFC on low appliances

The result of the combined voltage generated from the serial connection of the pig dung based MFC with a voltage generation of 3003 mV is represented in figures 9 and 10 (Light-emitting diode (LED) bulbs (red and yellow) and wall clock powered by the combined MFCs respectively). The red bulb, yellow bulb, and wall clock were powered at a voltage of 1640 mV, 1880 mV, and 1760 mV, respectively.

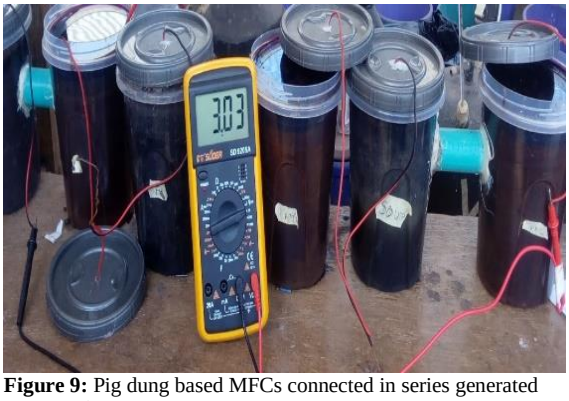

Figure 9: Pig dung based MFCs connected in series generated voltage of 3003 mV

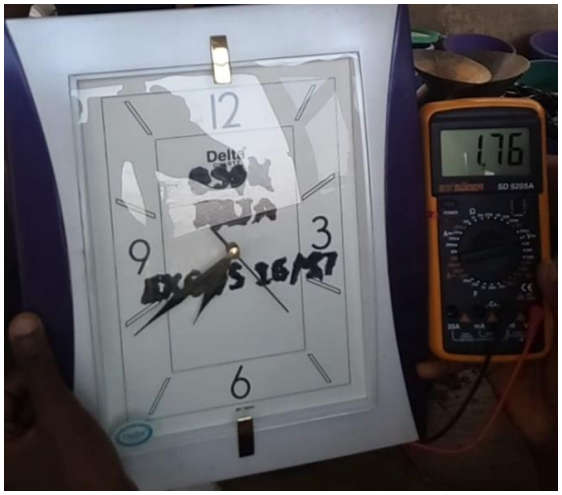

Figure 10: MFC powered wall clock at a voltage of 1760 mV

4. Discussion

Pig dung was evaluated for electricity generation using KMNO4 as an electron acceptor. Based on BLAST in the National Centre for Biotechnology Information (NCBI) database, the dominating bacterial isolates confirmed include *Bacillus amyloliquefaciens, Bacillus funiculus,* and *Yersinia enterocolitica*. The result revealed a slight difference in the cultural identity of these three organisms, which have been identified based on biochemical activities as *Paenibacillus amylolyticus, Bacillus mycoides,* and *Yersinia intermedia*, respectively. Akinyemi and Oyelakin, (2014), also reported differences between the conventional and molecular methods of bacterial identification. Conversely, results from this work demonstrated the importance of introducing 16S rDNA gene sequencing method in bacteria identification and that combining the two methods will help to improve further the identification authentication above the probable identity obtainable from the sole use of the cultural method.

Polymerase chain reaction revealed the molecular weight of the DNA of fungi in this study were between 500 bp and 600 bp. Selected fungi had more than 70 % similarity with those in the NCBI Gene Bank by BLASTn. The BLASTn results confirmed the fungal isolates were *Fusarium verticillioides, Aspergillus heteromorphus* and *Aspergillus tamari*. The result revealed a slight difference in the cultural identity of these three organisms, which have been identified as *Fusarium* sp, *Aspergillus funmigatus*, and *Aspergillus flavus*, respectively. This was also reported by Bechem and Afanga (2017), who reported a slight difference between the conventional and molecular methods of fungal identification.

Bacteria isolation before and after the MFC experiment revealed Bacillus mycoides of the phylum Firmicutes as the organism with the highest occurrence, this is in consonance with the report of Lim *et al*. (2018); they reported phylum Firmicute as the dominant phylum in all the cultured swine manure samples when the bacterial community were identified on phylum basis.

Microbial load before and after electricity generation revealed a significant population of microorganisms before and after the electric energy generation period; their presence connotes and establishes their function in facilitating the release of protons and electrons, which brings about the overall generation of current and voltage. The control MFC generated low voltage and current; it is deduced that other factors, mainly chemical and biological owing to the difference of potential between the two chambers, contributed to the generated voltage and current. The subsequent rapid drop in the current and voltage could be hanged on the exterminated organisms (during sterilization) when compared to other MFCs in which current and voltage were continuously generated throughout the experiment as a result of the presence microbial activities in them. This agrees with the description of (Akujobi *et al*., 2017).

The bacterial and coliform count dropped down relatively after the MFC experiment compared to its initial load in all the pig dung samples after power generation. This is a normality expected when microbial growth curve in an anaerobic environment is considered; available nutrients are competitively used up, waste accumulates, release of secondary metabolites, and the mortality rate of cells is on a rise. This is like the report of Adegunloye and Olotu, (2018); they reported that there was a decrease in the microbial population of the benthic mud used in the microbial fuel cell for electricity generation towards the later day of the experiment. The death phase is also characterized with adaptation, succession, and lysing of dying cells (their contents are spilled into the environment and thereby accessible to other bacteria). Sporulating bacteria are better survivor of the harshness in the death phase. They can go through this survival mode and resume normal growth life when the environment becomes conducive again to supports their growth (Nina *et al*., 2017).

Results revealed that all the energy-yielding macronutrients were present with protein as the most abundant. The crude fat, crude fibre, protein, carbohydrate, and ash content showed a significant difference across the pig dung samples while the moisture content did not show significant difference across the pig dung samples. However, the feeding pattern at the various sites may be responsible for the difference in the energy yield; this statement agrees with the work of Stephen *et al*. (2013). They documented that the significant difference in crude protein, carbohydrate, and fibre between dung may be because of the difference in the feeding pattern of the animals. There was an insignificant difference in the moisture content across the samples (Wnetrzak *et al*., 2015). The percentage of crude protein (18.26 ± 0.00 to 25.36 ± 0.00) % obtained in the present study is higher than the 13.79 % reported by Udebuani (2012) and 14.05 % reported by Okoli *et al*. (2019), the ash value (7.07 ± 0.01 to 7.07 ± 0.01) % was much lower than the 23.24 % obtained in Okoli *et al*. (2019), and the moisture content value (16.31 ± 0.00 to 22.35 ± 0.00) was higher than the 12.02 % reported by Okoli *et al*. (2019).

The energy value was high across the samples with a significant difference across the three samples; this can be attributed to the presence of energy-yielding macronutrients. The peak of the energy value obtained was from Apatapiti pig dung (703.81 Kj/Kg), this can be attributed to its highest carbohydrate and moisture content as these form the basis for energy production, this statement agrees with the findings of (Mukhtar and Capareda, 2017). They stated that nutrients in animal feed that are sources of energy include Carbohydrates which

comprises of (carbon, hydrogen, and oxygen) and other nutrients like protein, this later portions into net energy and energy losses such as in the form of faeces and urine, the inherent energy in the manure can be transformed into usable bio-energy. This statement disagrees with the work of Aneta and Grażyna, (2019), they confirmed that the calorific value of the energy of solid biofuel increases with decreasing moisture.

The present study revealed a sinusoid graphical representation in the bioelectricity generation in terms of voltage and current characterized by irregular falls and rises. This diverges from the steady and continuing increase all through the generation period documented by Meignanalakshmi *et al*. (2013) and Adegunloye and Ojo (2019); where voltage and current were generated from decayed wood. The present study revealed that pig dung generated higher current and voltage in MFC with KMnO4 as an electron acceptor compared to H2O as an electron acceptor in the work of Adegunloye and Faloni, (2020). This shows the effect of higher redox potential in permanganate than oxygen (Arbianti *et al*., 2013).

Samples of pig dung from various location generated their peak voltage and current at varied times during the MFC experiment. The least voltage (345 mV) was generated from Apatapiti pig dung but was higher than the maximum voltage (195.6 mV) generated with sewage water as an electron acceptor and the maximum voltage (179.7 mV) generated with vinegar as electron acceptor as reported from the work of Kumar et al. (2012) where they studied cow dung for electricity generation. The highest voltage (1301 mV) was generated from FUTA pig dung in the evening of day 7; this was higher than the peak voltage (1110 mV) generated with potassium permanganate as electron acceptor reported from the work of (Pandit *et al*., 2011), the maximum voltage (1006 mV) generated from sewage sludge with potassium ferricyanide as an electron acceptor in the work of (Parkash, 2018) and the maximum voltage (572 mV) generated from pig dung based MFCs with water as an electron acceptor in the work of (Adegunloye and Faloni, 2020). The highest value of current (4.5020 mA) was generated from Apatapiti in the evening of day 8. This was also higher than the peak current (0.319 mA) generated from pig dung based MFCs with water as an electron acceptor in the work of (Adegunloye and Faloni, 2020). The maximum voltage (1301 mV) generated by pig dung based MFC in this study has exceeded the theoretical documentation of Madigan *et al*. (2000), they stated that it was already documented that MFC voltages will never exceed a theoretical open-circuit voltage of 1140 mV.

The overall highest voltage was generated from three double chambers of pig dung based MFCs connected in series to yield a voltage output of 3003 mV. Devices including LED bulbs (red, yellow, green, and white colour) and wall clock powered with the combined voltage confirmed the functionality of the MFCs (Agho *et al*., 2018).

5. Conclusion

It can be concluded that MFC has a high potential for use in domestic applications to reduce the odious effect of pig dung disposal into the environment and generate electrical energy to operate appliances of low power consumption as demonstrated with LED bulbs and wall-clock, these were powered during the study. However, judging from the result of the highest voltage (3003 mV) generated from the combined MFCs when connected in series, the possibility of higher power generation for higher appliances than those powered in the study can be conceived, and this will be achievable by scaling up the MFC, thereby some of our home appliances can be made to run on renewable energy from organic sources. Results obtained from the study confirmed $KMnO_4$ as a good electron acceptor for electricity generation in microbial fuel cells due to its higher electrode potential, but its use will be unsustainable on a larger scale owing to cost implication. Therefore, research can be directed to source for renewable oxidizing agents to alleviates this consequence. Finally, findings from the study have revealed that pig dung has the capacity to yield a voltage output as high as 3003 mV, which is higher than the previous literature report indicating the enhancing effect of $KMnO_4$ on electricity generation in a microbial fuel cell.

Competing interest statement

The authors have declared that no competing interest exists in the manuscript.

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Contribution of individual authors

Faloni Taiwo Mercy: Conceived and designed the experiments; Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Adegunloye Deke Victoria: Conceived and designed the experiments; Supervised the experiment and the manuscript.

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