STUDY ON THE USE OF MICROBIAL FUEL CELL AS WASTE MANAGEMENT OPTION TO GENERATE ELECTRICITY FROM PIGGERY WASTEWATER

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Abstract. Microbial fuel cells (MFCs) were constructed to demonstrate the feasibility of generating bioelectricity from piggery wastewater. Exoelectrogens in the wastewater were harnessed and 0.1M potassium permanganate ($KMnO_4$) solution served as electron acceptor. Three units of 2-chambers MFCs with carbon – carbon (CC), carbon – copper (CCu) and copper-copper (CuCu) electrodes were constructed. Using piggery wastewater of BOD, 420mg/L and COD, 1057mg/L, the highest open circuit voltage (OCV) recorded were 969.6mV, 1228.5mV and 1338.5mV for CC, CCu and CuCu respectively. The voltage recorded across the MFCs was observed to decrease with decreasing external resistance. The highest power density (at R_{ext} = 1000Ω) were 79.27mW/m^2 (105.7mA/m^2), 156.32mW/m^2 (148.4mA/m^2) and 92.29mW/m^2 (114.0mA/m^2) for CC, CCu and CuCu respectively. Generally, power density increased with decreasing external resistance across each MFC until 200Ω beyond which it decreased. After 25days operation of the MFCs, the coulombic efficiency of the MFCs were 69%, 84% and 74%, while COD removal were 65%, 51% and 47% for CC, CCu and CuCu respectively. Moreover, carbon – carbon electrodes mix was found to be better in generation of electricity and wastewater treatment than copper – copper electrodes or their combinations. Pre and post bacteria isolation and identification revealed the presence of *Lactobacillus* sp., *Corynebacterium* sp., *Streptococcus* sp., *Proteus mirabilis, Enterobacter* sp., *Escherichia coli, Pseudomonas* sp., *Bacillus* sp., *Aeromonas* sp., *Micrococcus luteus*, *Corynebacterium* sp. *and Salmonella* sp. in the test piggery wastewater. This study therefore paved way for further optimization and scale up for better harvest of energy and wastewater treatment.

Key words: Microbial Fuel Cell, Potassium permanganate, Electrodes, Plasmid, Bioelectricity

INTRODUCTION

Environmental pollution is a dangerous and constantly increasing threat to the world. Our world is exposed to hazardous pollutants and wastes being generated on daily basis from different sources. Industries, agriculture, basic domestic and other human activities are among the anthropogenic factors that spread pollutants around the world. These pollutants affect our environmental resources such as air, water and soil. Slowly, our ecosystem is being brought down by the impending danger of pollutants [6].

Specifically, livestock production sustains the employment and incomes of millions of people in rural areas and generates animal power and organic manure for arable farming mainly in the northern part of Nigeria [11]. Inevitably, they also generate several tonnes of high strength manure and wastewater. Activities in livestock production facilities cause environmental problems such as odour nuisance and land pollution resulting from improperly discharged manure and wastewater [1].

Microbial fuel cells (MFCs) are devices that can use bacterial metabolism of a wide range of organic substrates to produce electrical current [4]. Based on the number of compartments or chambers, microbial fuel cells can be grouped into single chamber [12], double chamber [13], stacked [2] and up-flow [19] microbial fuel cells.

MATERIALS AND METHODS

Sample Collection

Piggery wastewater used for this study was obtained from one of the commercial pig farms at Umualum Nekede, Owerri West Local Government Area, Imo State, Nigeria with coordinates, 5°26'48.5"N 7°01'24.5"E. All plastic containers for collection of sample and chambers used for MFCs construction were surface sterilized according to Yee *et al.* [23]. The procedure for collection of samples for physicochemical, microbial fuel cell and microbiological analysis was as described by Ikotun *et al.* [7], Singh *et al.* [21]. Using the surface sterilized plastic container, which was first rinsed thrice with the piggery wastewater, the sample was collected from the drain pipes. Two sterile sample bottles were rinsed with the collected piggery wastewater and then filled to the brim. The samples were properly labeled and transported within an hour to the laboratory for physicochemical and microbial analyses.

Similarly, at the end of 25 days period of treatment using microbial fuel cells, treated samples from each of the six MFCs, as well as an untreated sample (used as control) were carefully collected using sterile sample bottles and analyzed physicochemically. However, the samples for subsequent microbial analysis was collected by aseptically removing the anode of each MFC and using a sterile swap stick to scrape the biofilm on their surfaces into sterilized peptone water contained in different sterile sample bottles. These were done to determine the physiochemical parameters of the treated wastewater and persistent microbes at the end of the treatment.

Measurement of Physicochemical Parameters of Samples

Before and after treatment, the physicochemical parameters of the wastewater samples were determined. The pH, electrical conductivity (EC) and total dissolved solid (TDS) were measured using Hanna Instrument for pH, EC, TDS and Temperature (Model No.: HI9811-5). Dissolved oxygen (DO) was measured using Dissolved Oxygen meter by LT. Luton (Model No.: DO-5509). Concentrations of ammonia - nitrogen, ammonia and ammonium; phosphorus (P), phosphate (PO_4^{3-}) and orthophosphate (P_2O_5); nitrate – nitrogen, nitrate and calcium were determined using Hanna COD and multiparameter photometer (Model No.: HI83099). The chemical dissolved oxygen (COD) and biochemical oxygen demand were also measured.

Culture – Based and Biochemical Identification of Microbial Flora

The microbial flora of the piggery wastewater sample was determined, before and after treatment by preparing ten-fold serial dilution of 1ml of the samples. McConkey Agar, Nutrient Agar were used to culture the sample. Each medium was prepared according to the manufacturer's specification and allowed to gel. Aliquot from 10^{-3}, 10^{-6} and 10^{-8} dilutions each, was separately spread on freshly prepared plates and incubated at $37^{O}C$. After 48hours incubation, the plates were observed for growth and results recorded in terms of number and morphologies of colonies formed. Pure culture of each different bacterial colony was prepared by sub-culturing on fresh nutrient agar and incubating at $37^{O}C$ for 24 hours. Finally, slant culture of each pure isolate was prepared and stored at room temperature. Biochemical tests were carried out to characterize the microorganisms as described by Cheesbrough [3].

Construction and Loading of Microbial Fuel Cell

Three H – type double chambers microbial fuel cells were constructed using two plastic containers, 1 litre each, as anode and cathode chambers. PVC pipes of length 15 cm and diameter 3.81 cm each, were used to join the two chambers, and also formed the encasement for salt bridge which was prepared by dissolving 20g of agar – agar powder into 1000ml of distilled water containing 1M solution of KCl. The solution was boiled for about 3 minutes, poured into the PVC pipes and then allowed to gel. Carbon and copper rods of length 0.155m, diameter 0.014m, and surface area $0.0071m^2$ each were arranged as copper – copper (CuCu), carbon – carbon (CC) and carbon – copper (CCu) to serve as the anodes and cathodes of each microbial fuel cell respectively. Potassium permanganate ($KMnO_4$) solution of 0.1M concentration was used as the electron acceptor.

Using measuring cylinders, 800ml of pig wastewater sample was introduced into the anode chambers of the MFCs and 900ml of 0.1M solution of potassium permanganate ($KMnO_4$) solution into the cathode chambers. Sealant was used to seal the connections to prevent leakages. The chambers were tightly corked and the circuits completed by means of 1.5mm copper wires of length 0.4m each. They were allowed for 24 hours to stabilize before measurement of voltage generated was read as shown on the digital multimeters (DT-830D Series). Open circuit voltage, (OCV) and voltage across 1000Ω, 500Ω, 200Ω and 100Ω resistors which were in turn connected in parallel to the digital multimeters was recorded when the readings stabilized, Readings were taken at three hours intervals from 6.00 am to 6.00 pm and the MFCs were allowed to run for 25 days.

RESULT

Physicochemical Analysis

The result of the physicochemical analysis of the samples before and after 25 days period of treatment using microbial fuel cell constructed with different combinations of copper and carbon electrodes, using potassium permanganate as catholytes are as shown in table 1. Generally, there was an increase in most parameters of all the wastewater samples analyzed after treatment using MFCs. However, marked decline in calcium concentration, dissolved oxygen, biochemical oxygen demand, and chemical oxygen demand components of the wastewater samples.

Table 1. Results of physicochemical analysis of samples before and after treatment

Parameter	Sample before treatment	CC	CCu	CuCu	Untreated sample (Control)
pH	7.1	6.8	7.1	6.8	5.3
Electrical Conductivity (µS/cm)	3800	7030	7820	7500	5490
Total dissolved solid (mg/L)	189	4500	5100	4870	2710
Nitrate-Nitrogen (mg/L)	24	48	64	83	32
Nitrate (mg/L)	104	114	120	231	128
Phosphate (PO_4^{3-}) (mg/L)	90	332.8	217.6	340.8	48
Phosphorus (P) (mg/L)	129.2	88.8	70.4	96.4	45.6
Orthophosphate (P_2O_5) (mg/L)	67.2	248	163.2	254.4	36
Ammonia-Nitrogen (mg/L)	444.8	256.8	319.2	246.8	352
Ammonia (NH_3) (mg/L)	541.6	380	409.6	401.5	428
Ammonium (NH_4^+) (mg/L)	568	426	440.8	417.6	454.4
Calcium (Ca^{2+}) (mg/L)	3200	800	1600	800	2000
Dissolved oxygen (mg/L)	6.00	2.00	2.00	1.80	4.5
Biochemical Oxygen Demand (mg/L)	420	110	100	100	390
Chemical Oxygen Demand (mg/L)	1057	368	516	559	715

Isolation and Identification of Bacteria

The results of pre- (table 2) and post- (table 3) treatment isolation and identification of bacterial isolates in the samples are as shown.

Generation of Voltage

The average open circuit voltage (OCV) generated daily across each MFC are shown on graphs presented in figure 1. Generally, the highest open circuit voltage observed in this study was 1338.5mV generated by CuCu on day 1, while the lowest was 215.5mV generated by CC on day 1 of treatment. This is close to the findings of Momoh and Neayor [16] who reported a maximum open circuit voltage prior to polarization of 1560mV for single dual chamber MFC.

Subsequently, the various voltages recorded across 1000Ω, 500Ω, 200Ω and 100Ω resistors are shown in figure 2. It was observed that the graphs produced the same pattern of variation as the corresponding parent open circuit voltage plots.

$$\text{Current Density} = \frac{\text{Current produced (mA)}}{\text{Surface area of projected anode (m2)}} \quad (1)$$

where V= voltage (V), R= resistance (load) (Ω) and A= surface area of the projected anode (m^2). Using equation (1), the daily average current densities of each microbial fuel cell across different resistors were calculated and the graphs shown in fig 3. As may be expected, at constant external resistance (R), current (I) proportionally varies with voltage (V).

Table 2. Results of biochemical tests for characterization of bacteria present in the sample before treatment

	Biochemical Test							
Isolates	**Gram stain**	**Catalase Test**	**Oxidase test**	**Methyl Red test**	**Voges Proskauer test**	**Indole test**	**Citrate test**	**Bacterial isolates**
1	+	-	+	+	-	-	+	*Lactobacillus* sp.
2	+	+	+	-	+	-	+	*Corynebacterium* sp.
3	+	-	+	+	-	+	-	*Streptococcus* sp.
4	-	+	-	+	-	-	-	*Proteus mirabilis*
5	-	+	-	-	+	-	+	*Enterobacter* sp.
6	-	+	-	+	-	+	-	*Escherichia coli*
7	-	+	+	-	+	-	+	*Pseudomonas* sp.
8	+	+	+	-	+	-	+	*Bacillus* sp.
9	-	+	+	+	-	+	+	*Aeromonas* sp.
10	+	+	+	-	+	-	-	*Micrococcus luteus*

Legend: + = positive test, - = negative test

Table 3. Results of biochemical tests for characterization of bacteria present in the sample after treatment

	Biochemical test								
Samples	**No of colonies**	**Gram test**	**Catalase test**	**Oxidase test**	**Methyl Red Test**	**Indole test**	**Citrate test**	**Voges Proskauer Test**	**Bacterial Isolates**
CC	3	+	+	+	-	-	+	+	*Corynebacterium* sp.
		+	+	+	-	-	+	+	*Bacillus* sp.
		+	+	+	-	-	+	+	*Corynebacterium* sp.
CCu	4	+	-	+	+	-	+	-	*Lactobacillus* sp.
		-	+	-	-	-	+	+	*Enterobacter* sp.
		+	+	+	-	-	+	+	*Bacillus* sp.
		+	+	+	-	-	-	+	*Micrococcus* sp.
CuCu	2	+	+	+	-	-	-	+	*Micrococcus* sp.
		+	-	-	+	+	-	-	*Lactobacillus* sp.

Legends: + = positive test, - = negative test

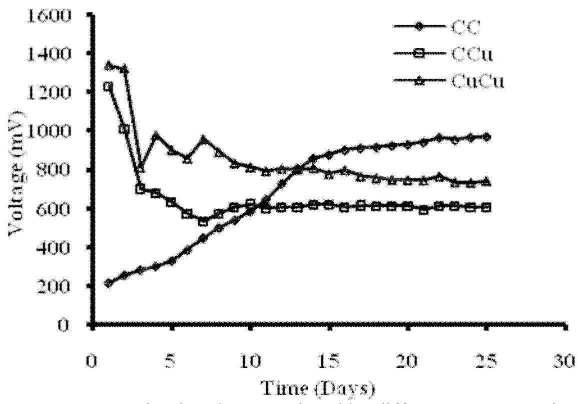

Figure 1. Open circuit voltage produced by different MFCs per time.

Consequently, in this study, the maximum current density recorded across 1000Ω was 148.38mA/m^2 produced by CCuP on day 1 while the least was 26.76mA/m^2 produced by CCP on day 1. However, these increased to 405.63mA/m^2 and 162.68mA/m^2 when 100Ω resistor was connected across the MFCs.

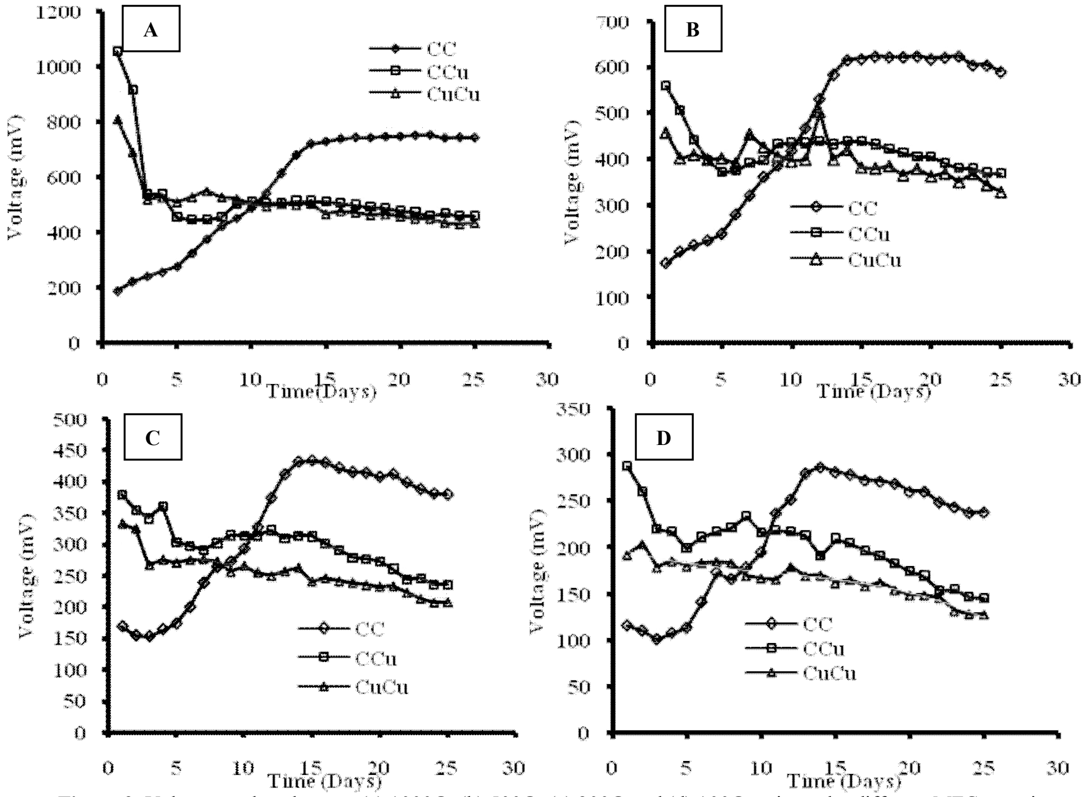

Figure 2. Voltage produced across (a) 1000Ω, (b) 500Ω, (c) 200Ω and (d) 100Ω resistors by different MFCs per time.

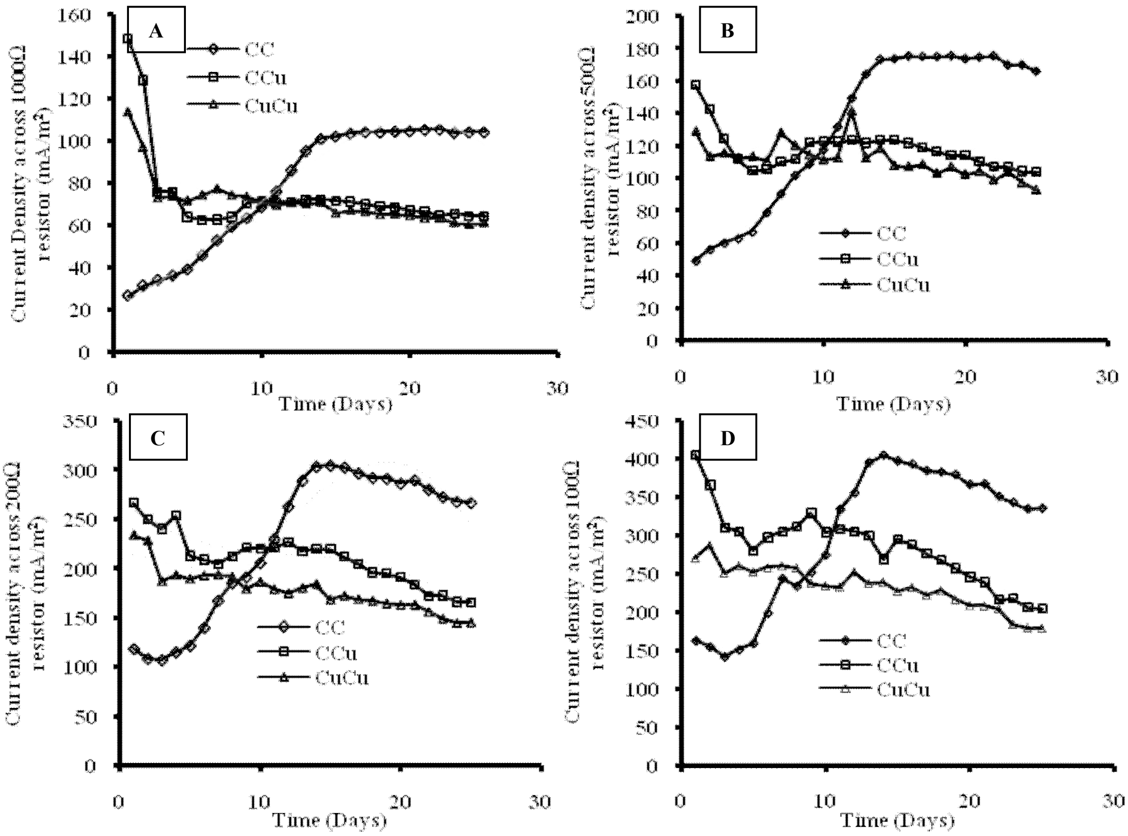

Figure 3. Current density produced of different MFCs across (A) 1000 Ω (B) 500Ω (C) 200Ω and (D) 100Ω resistors.

Effect of Different Electrodes on the Generation of Voltage

As shown on figure 4, the voltage of both MFCs constructed with a mix of copper (i.e., CCu and CuCu) initially showed high voltage before sharply declining until day 3, after which minor increase was observed. They then consistently followed a downward trend until the end of the treatment. However, CCP maintained a sharp increase from day 1 until day 13 when it began to slow down. Although the highest voltage was recorded in the MFC with copper as both electrodes, this trend however didn't last long before significantly declining.

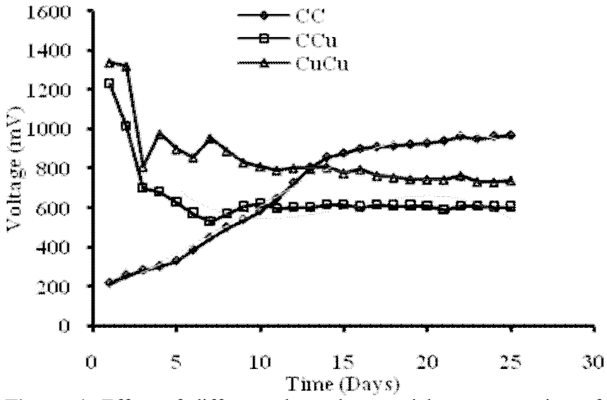

Figure 4. Effect of different electrode materials on generation of voltage.

Power Density

The power density (PD, mW/m^2) was calculated using the equation of Wang *et al.* [22]:

$$P = \frac{V_{cell}^2}{R_{ext.}xA}$$

where A is the projected area (m^2) of the anode, V is the voltage (V) and R_{ext} is the external resistance (Ohm) connected to the cells. The daily average power density for the microbial fuel cells were calculated and graphically presented as shown in figure 5. The MFCs used in the study produced power density in the range of $5.085mW/m^2$ to $156.319mW/m^2$ at $1.21mA/m^2$ and $26.76mA/m^2$ across 1000Ω resistor.

Coulombic Efficiency

Coulombic efficiency is one of the most important indexes that are used to describe MFC performance in terms of power generation [8]. It was calculated from the equation,

$$CE = \frac{M\int_0^t Idt}{FbV_{an}\Delta COD}$$

where V is liquid volume (m^3) at the anode chamber, F is Faraday's constant (96485 C/mol of e-) and b is mol of electrons produced per mol of O_2 (4 mol/mol), M is the molar mass of O_2 (32 g/mol). Using average current (I) derived when R_{ext} = 100Ω, results of the coulombic

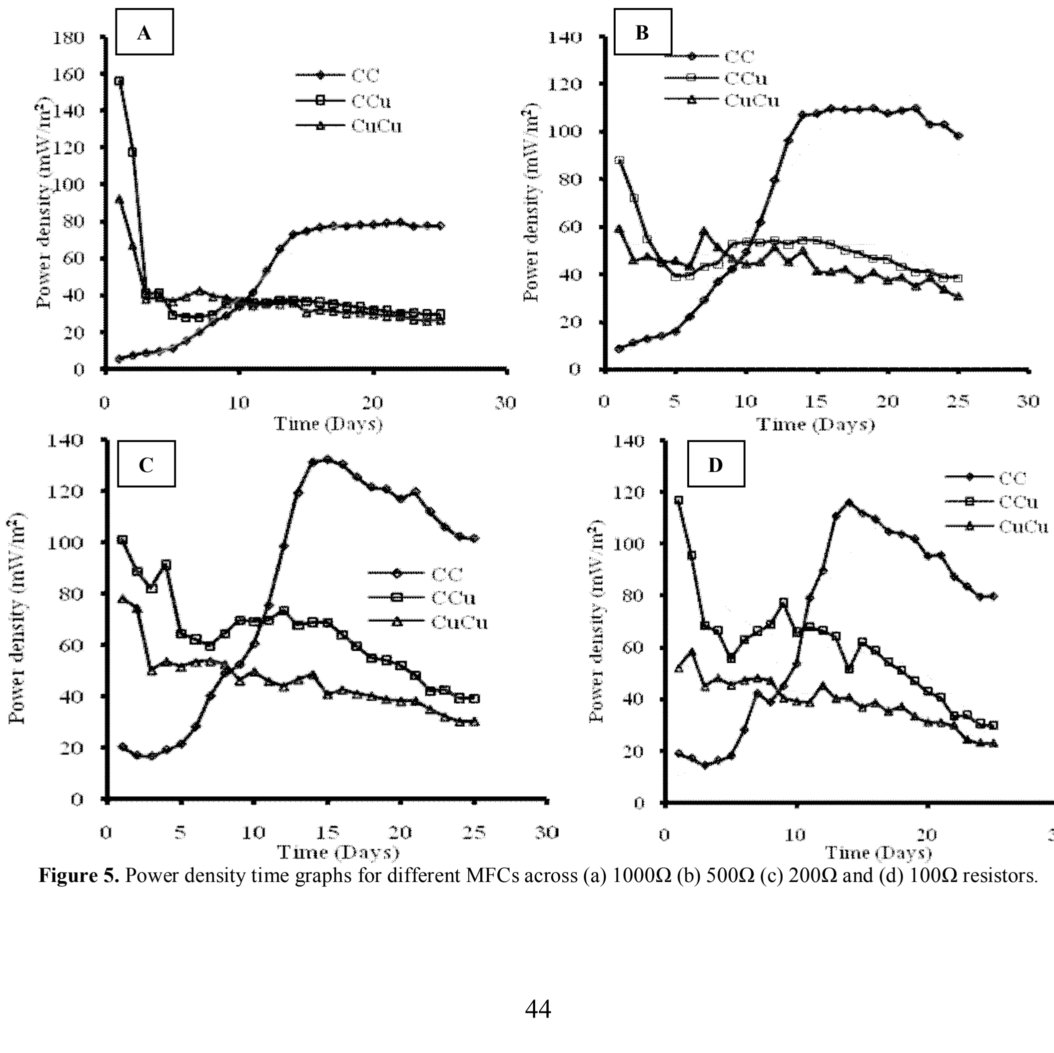

Figure 5. Power density time graphs for different MFCs across (a) 1000Ω (b) 500Ω (c) 200Ω and (d) 100Ω resistors.

efficiency of the microbial fuel cells revealed that the MFCs demonstrated an impressive performance in terms of electricity generation from the quantity of waste consumed. While CCu showed the highest coulombic efficiency of 84% at R_{ext} = 100Ω, the least coulombic efficiency was 69% produced by CC as can be seen in figure 6.

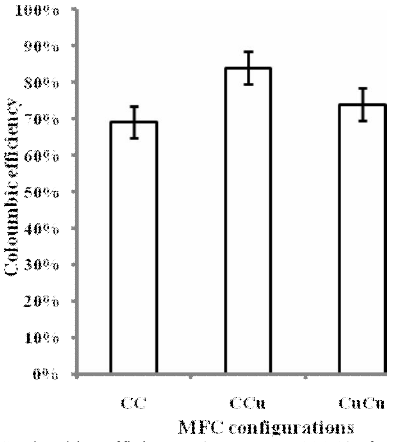

Figure 6. Coulombic efficiency (at R_{ext} = 100Ω) for the MFCs studied.

Relationship between Coloumbic Efficiency And External Resistance

The relationship between Coulombic Efficiency (CE) and external resistance was studied using 100Ω, 200Ω, 500Ω and 100Ω resistors and the results were graphically presented in figure 7. It was observed that in all the MFCs studied, at higher external resistance (R_{ext}), CE gradually reduced.

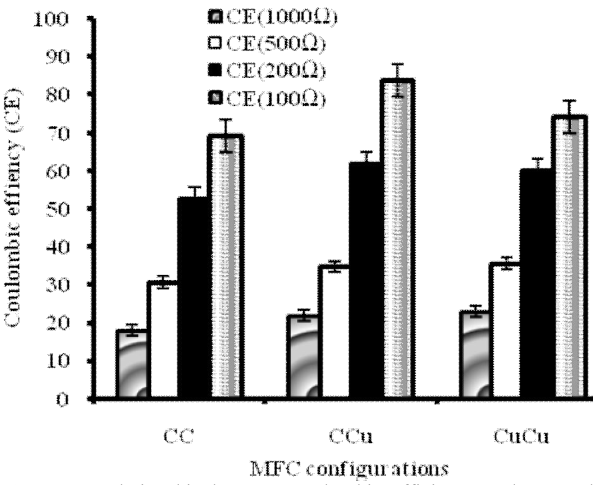

Figure 7. Relationship between coulombic efficiency and external resistance in different MFC configurations.

COD and BOD Removal Efficiency

Using the values of COD and BOD of wastewater in table 1, the efficiency of the MFCs in removing COD and BOD (expressed in percentage) of the pig wastewater was calculated using the formula, and the results are shown in figure 8.

$$\frac{\text{Initial COD (BOD) of wastewater (mg/L) - Final COD (BOD) of wastewater (mg/L)}}{\text{Initial COD (BOD) of wastewater (mg/L)}} \cdot 100$$

The results obtained showed that the MFC configurations studied demonstrated remarkable removal of both COD and BOD of the treated piggery wastewater compared to the original sample. While 73% - 76% removal of BOD in the microbial fuel cells used was observed, 47% - 65% of the COD was removed. On the other hand, control sample produced only 7% BOD reduction and 32% COD reduction. Therefore, it was evident that microbial fuel cell significantly enhanced this reduction.

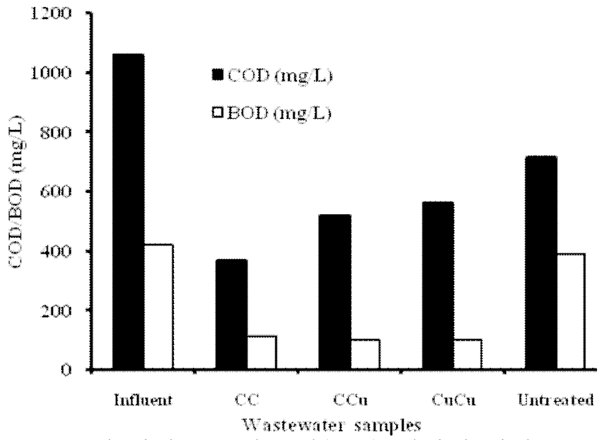

Figure 8. Chemical oxygen demand (COD) and Biochemical oxygen demand (BOD) removal from wastewater samples.

DISCUSSION

Cultural Identification of Microorganisms

Culture and biochemical tests employed in the identification of the isolates of the piggery wastewater showed the presence of *Lactobacillus* sp., *Corynebacterium* sp., *Streptococcus* sp., *Proteus mirabilis, Enterobacter* sp., *Escherichia coli, Pseudomonas* sp., *Bacillus* sp., *Aeromonas* sp., *Micrococcus luteus*, *Corynebacterium* sp. *and Salmonella* sp. This finding corroborates the reports of Zhu [26], which similarly revealed that swine fecal bacteria genera listed in order of quantity from high to low include Gram-positive cocci (ca. 39%), *Eubacterium* (ca. 27%), *Lactobacillus* (ca. 20%), Gram-negative rods (*Escherichia*, ca. 8%), *Clostridium* (ca. 4%), and some other minor groups such as *Propionibacterium acnes* and *Bacteroides* (<2%).

Physicochemical Analysis

The significant reduction in BOD and COD contents of the treated wastewater demonstrate the suitability of MFCs in treatment of wastewaters. The decrease in the organic matter content of the treated wastewater is attributable to the metabolic activities of microorganisms which used them as sources of carbon for energy generation. It was observed that both nitrate – nitrogen and nitrate concentrations in the wastewaters increased. This finding agrees with Min *et al.* [15] who reported that after 100hrs operation using diluted (1:10) wastewater, ammonia was removed by 83 ± 4% (from 198 ± 1 to 34 ± 0mg NH_4-N/L) when the SCOD was reduced from 1240±20 to 120±30mg/L (86±6%

removal). Nitrite and nitrate concentrations increased from 0.4±0.1 to 2.9±0.1mg NO_2-N/L and 3.8±1.2 to 7.5±0.1mg NO_3-N/L. This increase in oxidized nitrogen suggests that nitrification was occurring, which may be due to oxygen diffusion through the cathode. The total amount of ammonia removal (164mg NH_4-N/L) was much larger than the corresponding increases in nitrite (2.5mg NO_2-N/L) and nitrate (3.7mg NO_2-N/L).

Furthermore, Min *et al.* [15] reported that orthophosphate concentrations (PO_4-P mg/L) did not decrease, and may have slightly increased (41±1 – 48±1mg/L) as a result of the low redox potential in the MFC which would stimulate the release of stored phosphates in the bacteria [14], or the conversion of organic phosphorus in the wastewater to orthophosphate.

Generation of Voltage

The generation of voltage in this study indicated the presence of exoelectrogens in the swine wastewater. This is in line with the report that electricity could be generated using swine wastewater, and that the bacteria needed were already present in the wastewater [15]. However, overtime it was observed that voltage recorded in some of the microbial fuel cells maintained gradual increase, especially those MFCs with carbon – carbon electrodes, while others gradually dwindled, especially the MFCs having copper in their electrodes mix. Similar observation was reported by Seop *et al.* [20], who suggested that the implication of voltage generation after inoculation of the MFCs was that electrochemically active bacteria (EAB), or exoelectrogens, propagated in the MFC and that wastewater and/sludge contained EAB at low concentration at the initial stage of enrichment.

Current Density

The ranges of current density across 1000Ω resitor were 26.75mA/m^2 to 105.66mA/m^2 for CCP, 62.93mA/m^2 to 148.38mA/m^2 for CCuP and 60.54mA/m^2 to 114.01mA/m^2 for CuCuP. Similar result was obtained by Gupta *et al.* [5] who also reported that the maximum current density of 108.57mA/m^2 was obtained with graphite rod while 88.01mA/m^2 was obtained with copper rods, both using $KMnO_4$ as the electron acceptor. Moreover, the maximum current densities obtained in this study are close to the result of a study conducted using a two chambered MFC with continuously aerated cathode which demonstrated a maximum current density of 141mA/m^2 at 1000Ω [15].

Power Density

Results obtained indicated that power density measured across 1000Ω resistor, was in the range of 5.085mW/m^2 to 156.319mW/m^2 for the MFCs. It increased with decreasing external resistance upto the 200Ω resistor beyond which it started decreasing. Results obtained showed that the maximum power density obtained from MFCs across 1000Ω resistor was 156.32mW/m^2. This is similar to the report of 116.2mW/m^2 by Pandit *et al.* [18]. You *et al.* [24] have also reported a maximum power density of 115.60mWm^{-2}.

The results further showed that carbon is a better electrode than copper in terms of generation and stabilization of power density in MFC. This conforms to the findings of Gupta *et al.* [5], who reported that the maximum power density was 48.85mW/m^2 with graphite rod and 42.59mW/m^2 with copper electrode both using $KMnO_4$ as the electron acceptor.

COD and BOD Removal Efficiency

This study has proven that MFC significantly enhanced the reduction of COD and BOD of wastewater treated. This is evident when the values of treated and untreated samples are compared. Khan *et al.* [10] recorded COD removal in the range of 70% to 89% using $KMnO_4$ in cathode chamber. MFCs which had carbon – carbon as anode and cathode respectively yielded better COD and BOD removal than other having a combination of copper and carbon or copper – copper.

Consequently, piggery wastewater has been shown to contain both the substrates and appropriate exoelectrogenic consortium needed for its decomposition and generation of electrical energy using microbial fuel cell. Besides, results showed that the MFCs reduced the COD and BOD of the wastewater, making a useful tool in its treatment. Likewise, carbon – carbon electrodes were found to be better than other mix of copper and/or carbon used in the study. The ability of carbon electrode to maintain steady rise in voltage generation over an appreciably long period made it an electrode of choice than copper, which rather gave a transient initial high voltage.

However, further studies should be separately carried out using pure cultures of these microorganisms from piggery wastewater to establish their capabilities and perhaps eliminate species that may be antagonistic in activities. Genetic studies and modifications of the genes (plasmids) of these organisms may be carried out to increase their wastewater degrading and conversion capabilities and enhance electrons transfer potentials through their electrochemically active surface proteins, pili etc.

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Legends for abbreviations used

CC: Carbon-carbon electrode, potassium permanganate as catholyte.
CCu: Carbon-copper electrode, potassium permanganate as catholyte.
CuCu: Copper-copper electrode, potassium permanganate as catholyte.
CE: Coloumbic efficiency

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