International Journal of Bioorganic Chemistry
2016; 1(1): 8-20
http://www.sciencepublishinggroup.com/j/ijbc
doi: 10.11648/j.ijbc.20160101.12

Comparative Study of Effects of Electrode Materials and Catholyte on Simultaneous Generation of Bioelectricity and Waste water Treatment

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To cite this article:

Anuforo Henry Uzoma, Ogbulie Tochukwu Ekwutosi, Akujobi Campbell Onyeka. Comparative Study of Effects of Electrode Materials and Catholyte on Simultaneous Generation of Bioelectricity and Waste water Treatment. *International Journal of Bioorganic Chemistry.* Vol. 1, No. 1, 2016, pp. 8-20. doi: 10.11648/j.ijbc.20160101.12

Received: December 23, 2016; **Accepted**: January 6, 2017; **Published**: January 24, 2017

Abstract: Improvement of the parameters which limit the harvest of energy in microbial fuel cell (MFC) is paramount to increase its output and promote commercial application of the technology. Six dual chamber MFCs with either potassium permanganate or potassium ferricyanide as electron acceptor and various combinations of carbon and copper rods as electrodes produced maximum open circuit voltage (OCV) of 0.97V, 1.23V, 1.34V, 0.75V, 1.03V and 0.63V. The power density (at R_{ext} = 1000Ω), which increased with decreasing external resistance until 200Ω beyond which it decreased, peaked at 79.27mW/m^2 (105.7mA/m^2), 156.32mW/m^2 (148.4mA/m^2), 92.29mW/m^2 (114.0mA/m^2), 60.94mW/m^2 (92.6mA/m^2), 39.94mW/m^2 (75.0mA/m^2) and 14.21mW/m^2 (44.70mA/m^2) for the MFCs. Similarly, coulombic efficiency (CE) were 69%, 84%, 74%, 76%,72% and 5.10%, while COD removal were 65%, 51%, 47%, 83%, 48% and 49%. Above results indicated that potassium permanganate outperformed potassium ferricyanide, while use of carbon as both electrodes was better than other blends copper and/or carbon used in the study. *Lactobacillus spp., Corynebacterium spp., Streptococcus spp., Proteus mirabilis, Enterobacter spp., Escherichia coli, Pseudomonas spp., Bacillus spp., Aeromonas spp., Micrococcus lyteus*, *Corynebacterium spp., Cladosporium, Aspergillus versicolour, Candida albicans, A. flavus, Aspergillus nidulans, Trichoderma spp.* and *Aspergillus fumigatus* were microorganisms isolated from the piggery wastewater. Further studies using cheaper, more sustainable materials with better effects on the setup are necessary.

Keywords: Bioelectricity, Electrodes, Electron Acceptors, Carbon, Copper

1. Introduction

The need for renewable source of energy cannot be overemphasized, in view of the devastating impacts of continued dependence on fossil fuel on man and the environment on one hand, and the ever increasing human population with attendant demands for increased supply of energy for both social and economic development [1, 2, 3, 4, 5, 6]. Fortunately, innumerable reports have affirmed that microbial fuel cell (MFC) is a promising technology in both generation of bioelectricity and waste water treatment [7, 8]. MFCs can generate electrical energy from oxidation of organic matter through the catalytic activity of electrochemically active bacteria [9]. Although diverse substrates, including waste water, have been used to generate bioelectricity in MFC, currently, the output however is still very low to allow economic application of the technology [10, 11, 12].

The output of MFCs is limited by several parameters including the amount of oxidation and electron transfer to the anodes by microorganisms, loading rate, the nature of substrate, the nature of proton exchange membrane (PEM), proton transfer through the membrane to the cathode chamber, oxygen supply in the cathode, the nature and type of electrodes, circuit resistance, the nature of the catholyte

used, operation temperature, pH and sedentary time [13, 14].

The basic properties of MFC electrodes include (i) good electrical conductivity and low resistance; (ii) strong biocompatibility; (iii) chemical stability and anti-corrosion; (iv) large surface area; and (v) appropriate mechanical strength and toughness [15, 16]. Many materials which have found wide application as electrodes in MFCs include carbon paper, cloth, foam, and felt; graphite rod, foil, brush and granules, activated carbon, reticulated vitreous carbon; metals, aluminum, nickel and stainless steel, carbon felt, graphite with Mn^{4+} or Fe^{3+}, platinum, graphite-ceramic composite, cobalt, wood ash cement composite [17, 16, 12].

The electrode material determines the diffusivity of oxygen in single chambered MFCs. If the electrodes are more porous it allows diffusion of oxygen to anode which reduces the efficiency of fuel cells. The electrode material also determines the power loss of fuel cell in terms of internal resistance [18]. The longevity of electrodes is also an important criterion, but the most important criterion is cost. Therefore, by using the most suitable electrode materials with the highest reduction potential, MFC technology can produce high quantities of energy that will foster its wider applications [19].

Moreover, oxygen is widely adopted in several attempts to address the bottleneck arising from consumption of electrons at the cathode chamber due to its availability and high redox potential [20, 21]. However, the poor contact between gaseous oxygen and cathode electrode coupled to the slow rate of oxygen reduction on the surface of carbon electrodes have been major detraction which impede its use in MFCs [21]. This drawback of oxygen is eliminated by increasing dissolved oxygen content of the cathode, which however requires power for agitation and increases oxygen diffusion into the anode, thus causing problems to the MFC. Different types of electron acceptors have been used to circumvent this challenge. Therefore, this study compared the effects of carbon and copper rods as electrodes on MFC using potassium permanganate and potassium ferricyanide as alternative electron acceptors.

2. Materials and Methods

2.1. Collection of Waste Water Samples

Using surface sterilized, nonreactive PVC plastic container, the waste water used was collected after rinsing the container thrice with the waste water from a pig farm in Umualum Nekede, Owerri West Local Government Area, Imo State, Nigeria (5°26'48.5"N 7°01'24.5"E), following the method of [22, 23]. Samples were taken from the container and immediately transported to the laboratory for microbial and physicochemical analyses. Likewise, after the period of treatment in MFC, samples were taken to the laboratory, together with an untreated sample (control experiment), for another round of microbial and physicochemical analyses. However, samples for microbial analysis were collected by aseptically swabbing the surface of the anode with sterile swap to remove the biofilms.

2.2. Physicochemical Parameters

The following physicochemical parameters of the piggery waste water samples were determined before and after treatment; pH, electrical conductivity (EC) and total dissolved solid (TDS) (with Hanna pH, EC, TDS and Temperature instrument, Model No.: HI9811-5) USA. Dissolved oxygen (using Dissolved Oxygen meter by LT. Luton, Model No.: DO-5509), ammonia - nitrogen, ammonia and ammonium; phosphorus (P), phosphate (PO_4^{3-}) and ortho-phosphate (P_2O_5); nitrate – nitrogen, nitrate, calcium, chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) (using Hanna COD and multiparameter photometer, Model No.: HI83099) USA.

2.3. Microbial Identification

All the samples were aseptically serially diluted in ten folds and 0.1ml from 10^{-3}, 10^{-6} and 10^{-8} were inoculated on McConkey Agar, Nutrient Agar, and Saboraud Dextrose Agar (SDA), which were prepared according to the manufacturer's specifications. The plates were incubated at 37°C, except SDA that was left at room temperature. Observation of the plates for growth was done after 24 hours of incubation. Distinct colonies were sub-cultured on fresh nutrient agar to obtained pure cultures. Biochemical analyses were carried out to identify the microorganisms as described by [24]. Lactophenol mounts of fungal isolates were prepared and identification was based on the observed macroscopic and microscopic characteristics.

2.4. Construction of Microbial Fuel Cell

Two groups of three units of MFCs each were made with either 0.1M potassium permanganate ($KMnO_4$) or 0.1M potassium ferricyanide ($K_3[Fe(CN)_6]$) as the electron acceptor. Combinations of carbon and copper rods of surface area 0.0071m^2 each, were made thus; carbon-carbon, copper-copper and carbon-copper to serve as electrodes for each group. Salt bridges made by allowing boiled mixture of 20g agar-agar powder dissolved in 1M solution of KCl to gel in PVC pipes of length 15 cm and diameter 3.81 cm each, was used as the proton exchange membrane which connected the two chambers of the MFCs. While 800ml of pig wastewater sample was aseptically charged into the anode chambers, 900ml each of the electron acceptors was introduced into either group of MFCs respectively. The chambers were tightly closed sealed and external circuits completed using 1.5mm copper wires of length 0.4m each as shown on figure 1. After 24 hours, open circuit voltage (OCV) was recorded, as well as voltage seen on successively connecting 1000Ω, 500Ω, 200Ω and 100Ω resistors in parallel to the digital multimeters (DT-830D Series) connected to the circuit. The observation was done for 25 days at 3 hours interval per day, starting from 6am to 6pm.

2.5. Molecular Base Identification of Bacteria Species

The 16S rRNA molecular base characterization of the

isolates was undertaken to determine the presence of *Clostridium botulinum, Aeromonas hydrophila, Clostridium butyricum* and *Rhodobacter ferrireducens* as possible exoelectrogens in the sample wastewater. Chromosomal DNA of the bacteria isolates from swabbed surface of anode was extracted by boiling method described by [25]. Amplification of the DNA was done using polymerase chain reaction (PCR) technique, with specific primers targeting the 16S rRNA of the four suspected bacteria in 20µl PCR solution containing 13.6µl of nuclease – free water, 0.2µl of forward primer, 0.2µl of backward primer, 4µl of master mix and 2µl appropriate amount of template DNA. The amplification conditions were as follows: an initial step of 95°C for 10 min; 25 cycles consisting of 95°C for 1 min, 50°C for 1 min, and 72°C for 2 min; and a final elongation step at 72°C for 10 min. The PCR (A&E Lab UK, Cy-006-1) was set to run 30 cycles.

Subsequently, gel was prepared by boiling a mixture of 1g agarose powder in 100ml of 1X TBE buffer for about 3 minutes in a water bath and cooling it to about 55°C before adding 10µl of ethidium bromide. It was then poured into the tray of electrophoresis tank (EDVOTEK 220V EVT300) with its comb and stoppers in place, and was allowed to solidify. After which, 1X TBE buffer was poured into the tank to adequately immerse the gel. 20µl of the samples were mixed with 2µl of loading dye and carefully loaded into the wells created by the comb with the standard in lane 1. Electrophoresis was run at 75V for about 45 minutes before the gel was gently placed on a UV – transilluminator and the bands viewed.

Figure 1. *Dual chamber MFC showing voltage generated on multimeter (DT-830D Series).*

3. Results

3.1. Physicochemical Parameters of Waste Water

Piggery waste water samples collected before and after treatment using dual chambers MFC produced the following physicochemical parameters outlined in table 1. There was decline in the DO, BOD, COD, Ammonia-Nitrogen, ammonium and ammonia contents of treated and control samples. However, the values recorded for treated samples were appreciably higher than those of the control sample. This is an indication of the effectiveness of MFC in enhancing reduction of these parameters during waste water treatment.

3.2. Microbial Isolation and Identification

Microorganisms isolated and identified from the wastewater sample before and after treatment are shown on tables 2, 3 and 4. A comparison of the bacteria isolates revealed that *Escherichia coli, Pseudomonas spp.,* and *Aeromonas spp.* were non-persistent after the treatment.

3.3. Molecular Based Identification of Suspected Bacteria Species

Observation of the gels after electrophoresis revealed that none of the extracted bacteria chromosomal DNA products formed bands. This indicates that the DNAs of the bacteria isolates in the wastewater sample were not complementary to the various primers targeting the 16S rRNA genes of the four suspected exoelectrogen, hence their absence. The gels are shown on figure 2.

Table 1. *Physicochemical parameters of wastewater samples collected before and after treatment.*

S/N	Parameter	Sample before treatment	CCP	CCuP	CuCuP	CCF	CCuF	CuCuF	Untreated sample (Control)
1.	pH	7.1	6.8	7.1	6.8	6.7	6.8	6.9	5.3
2.	Electrical Conductivity (µS/cm)	3800	7030	7820	7500	7410	7740	7550	5490
3.	Total dissolved solid (mg/L)	189	4500	5100	4870	4810	5030	4900	2710
4.	Nitrate-Nitrogen (mg/L)	24	48	64	83	128	96	92	32
5.	Nitrate (mg/L)	104	114	120	231	268	146	134	128
6.	Phosphate (PO_4^{3-}) (mg/L)	90	332.8	217.6	340.8	278.4	339.2	165.6	48
7.	Phosphate (P) (mg/L)	129.2	88.8	70.4	96.4	91.2	87.4	53.6	45.6
8.	Phosphate (P_2O_5) (mg/L)	67.2	248	163.2	254.4	208	252.8	123.2	36
9.	Ammonia-Nitrogen (mg/L)	444.8	256.8	319.2	246.8	216.8	219.8	226.8	352
10.	Ammonia (NH_3) (mg/L)	541.6	380	409.6	401.5	371.4	393.2	383.2	428
11.	Ammonium (NH_4^+) (mg/L)	568	426	440.8	417.6	424.2	436.8	442.8	454.4
12.	Calcium (Ca^{2+}) (mg/L)	3200	800	1600	800	800	800	2000	2000
13.	Dissolved oxygen (mg/L)	6.00	2.00	2.00	1.80	1.50	3.00	2.10	4.5
14.	Biochemical Oxygen Demand (mg/L)	420	110	100	100	130	240	180	390
15.	Chemical Oxygen Demand (mg/L)	1057	368	516	559	542	553	542	715

Table 2. *Bacteria isolates of sample before treatment.*

Isolates	Biochemical Test							
	Gram stain	Cat. Test	Ox. test	MR test	VP test	Indo. Test	Cit. test	Microorganisms
1	+	-	+	+	-	-	+	*Lactobacillus spp*
2	+	+	+	-	+	-	+	*Corynebacterium spp*
3	+	-	+	+	-	+	-	*Streptococcus spp*
4	-	+	-	+	-	-	-	*Proteus mirabilis*
5	-	+	-	-	+	-	+	*Enterobacter spp*
6	-	+	-	+	-	+	-	*Escherichia coli*
7	-	+	+	-	+	-	+	*Pseudomonas spp*
8	+	+	+	-	+	-	+	*Bacillus spp*
9	-	+	+	+	-	+	+	*Aeromonas spp*
10	+	+	+	-	+	-	-	*Micrococcus lyteus*

Legend: + = positive test, - = negative test

Table 3. *Bacteria isolates of sample after treatment.*

Biochemical test									
Samples	No of colonies	Gram test	Cat. test	Oxid. test	MR Test	Indole test	Citr. test	VP Test	Microorganisms
CCP	3	+	+	+	-	-	+	+	*Corynebacterium spp*
		+	+	+	-	-	+	+	*Bacillus spp*
		+	+	+	-	-	+	+	*Corynebacterium spp*
CCuP	4	+	-	+	+	-	+	-	*Lactobacillus spp*
		-	+	-	-	-	+	+	*Enterobacter spp*
		+	+	+	-	-	+	+	*Bacillus spp*
		+	+	+	-	-	-	+	*Micrococcus spp*
CuCuP	2	+	+	+	-	-	-	+	*Micrococcus spp*
		+	-	-	+	+	-	-	*Lactobacillus spp*
CCF	3	+	+	-	-	-	+	+	*Bacillus licheniformis*
		+	+	+	-	-	-	+	*Bacillus alvei*
		+	+	+	-	-	+	+	*Bacillus subtilis*
CCuF	3	+	+	+	-	-	-	+	*Micrococcus spp*
		+	-	+	+	+	-	-	*Streptococcus spp*
		+	+	+	-	-	+	+	*Bacillus spp*
CuCuF	3	+	+	+	-	-	+	+	*Bacillus spp*
		-	+	-	+	-	-	-	*Proteus mirabilis*
		+	+	+	-	-	+	+	*Bacillus subtilis*

Table 4. *Fungal isolates of sample before treatment.*

Samples	Number of colonies		Suspected fungi
Before treatment	17	2	*Aspergillus versicolour*
		6	*Candida albicans*
		3	*Aspergillus flavus*
		4	*A. fumigates*
		2	*Aspergillus nidulans*
CCP	8	3	*Cladosporium*
		5	*Aspergillus nidulans*
CCuP	7	2	*Aspergillus versicolour*
		5	*Aspergillus flavus*
CuCuP	11	7	*Candida albicans*
		4	*Aspergillus versicolour*
CCF	4	4	*A. flavus*
CCuF	9	9	*Aspergillus nidulans*
CuCuF	7	3	*Trichoderma spp.*
		4	*Aspergillus fumigates*

3.4. Generation of Bioelectricity

Average of all the open circuit voltage (OCV) recorded daily across each MFC was computed and plotted as shown in fig. 3. The maximum OCV were 0.97V, 1.23V, 1.34V, 0.75V, 1.03V and 0.63V CCP, CCuP, CuCuP, CCF, CCuF, and CuCuF repetively. While the MFCs with blends of copper and carbon as their electrodes, produced high OCV initially which abruptly declined with time, those having only carbon as their electrodes showed continuous steady increase in their output with time.

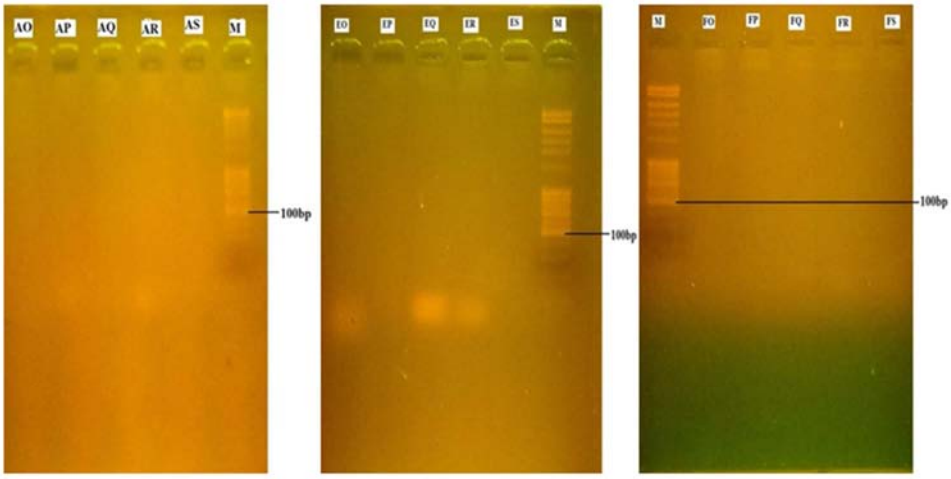

Figure 2. *Agarose gel showing absence (no bands) of DNA of suspected exoelectrogens in the wastewater sample.*

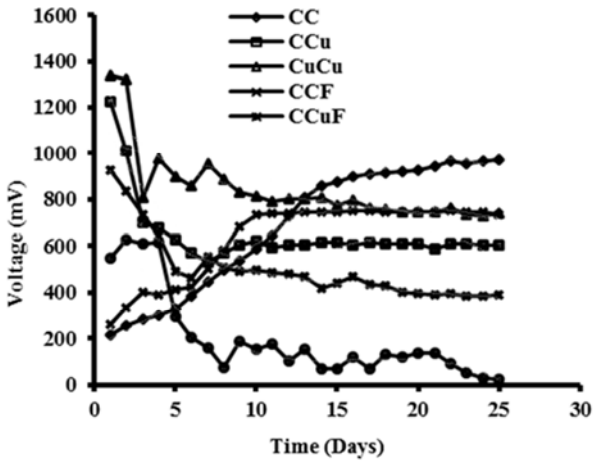

Figure 3. *Graph of open circuit voltage (OCV) generated across different MFCs per time.*

On successive connection of external resistance to the circuit, it was observed that the voltage recorded by multimeters connected in parallel to the resistors increased with increasing external resistance as shown in figure 4.

3.5. Current Density

Using the relationship between voltages recorded across known external resistors and the surface area of the anode, current density produced by the MFCs across different resistors were computed. In line with Ohm's law, at constant voltage (V), current (I) is inversely proportionally to applied external resistance (R). The maximum current density recorded across 1000Ω was 105.7mA/m^2, 148.38mA/m^2, 114mA/m^2, 92.6mA/m^2, 75mA/m^2 and 44.7mA/m^2 for CCP, CCuP, CuCuP, CCF, CCuF and CuCuF respectively. The graphs are shown in fig. 5.

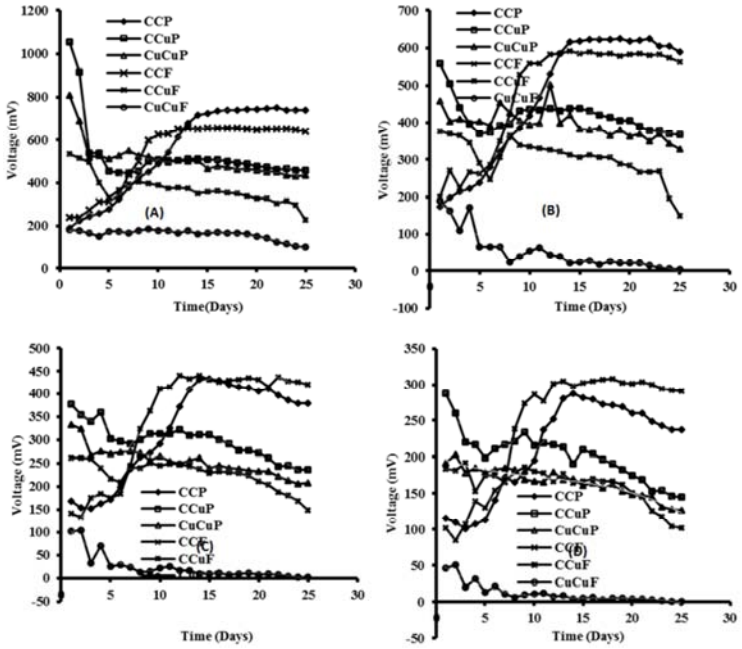

Figure 4. *Graphs of voltage produced across (a) 1000Ω, (b) 500Ω, (c) 200Ω and (d) 100Ω resistors by different MFCs per time.*

3.6. Power Density

The voltage recorded across known external resistors, together with the surface area of the anode was used to compute power derived from the MFCs. The power density obtained ranged from 0.010mW/m^2 to 156.319mW/m^2 across 1000Ω resistor. It was observed that beside in CuCuF and CCuF, power density of other MFCs increased with decreasing external resistance upto 200Ω resistor beyond which it started decreasing with decreasing external resistance. The power density time graph obtained is as shown on fig. 6.

3.7. Effect of Electrode Material on Generation of Bioelectricity

As shown in figure 7, the OCV output of both MFCs constructed with copper only (CuCuP and CuCuF) and combination of copper and carbon (CCuP and CCuP) as the electrodes was initially high. However, it was observed that this sharply declined from day 2 until 9 when relative stability was recorded and maintained till the end of the period of treatment. Conversely, in the MFC made with only carbon (CCP and CCF) as the electrode, though there was initially low OCV output, this abruptly maintained steady increase from day 2 until 16 when it gradually slowed.

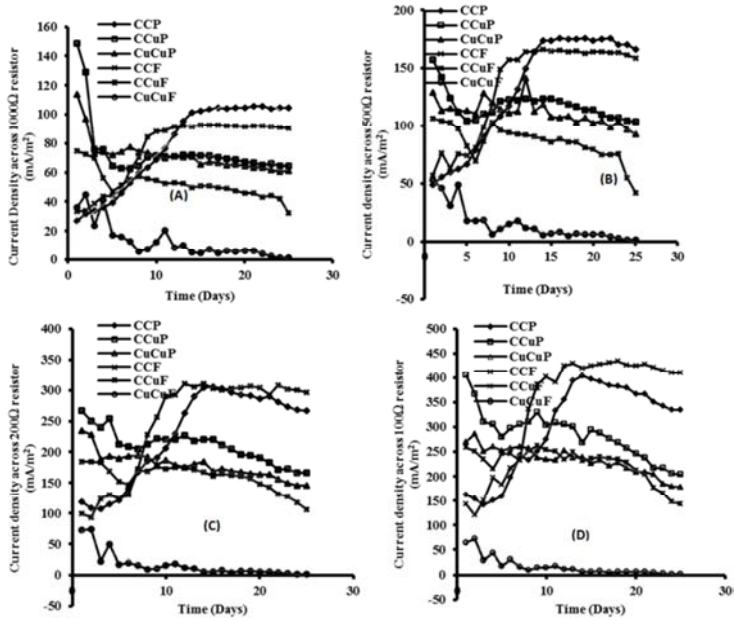

Figure 5. *Graphs of current density for the different MFCs across (A) 1000 Ω (B) 500Ω (C) 200Ω and (D) 100Ω resistors.*

Interestingly, despite the highest OCV recorded in the MFCs which had copper as a constituent of their electrodes, this was not sustained over an appreciable period of time but decreased to low levels that they continuously lagged behind those of MFCs with only carbon as their electrode. In view of this, carbon is considered better electrodes than copper in bioelectricity generation.

3.8. Effect of Catholytes on Bioelectricity Generation

The electrons generated at the anode must efficiently and adequately be transferred and consumed at the cathode chamber together with the protons. Therefore, nature of catholyte used also affects the performance of MFCs. A comparative consideration of the graphs of the OCV output of MFCs constructed using different catholytes and electrodes as shown on figure 8 reveals that potassium permanganate performed better than potassium ferricyanide as electron acceptor in bioelectricity generation.

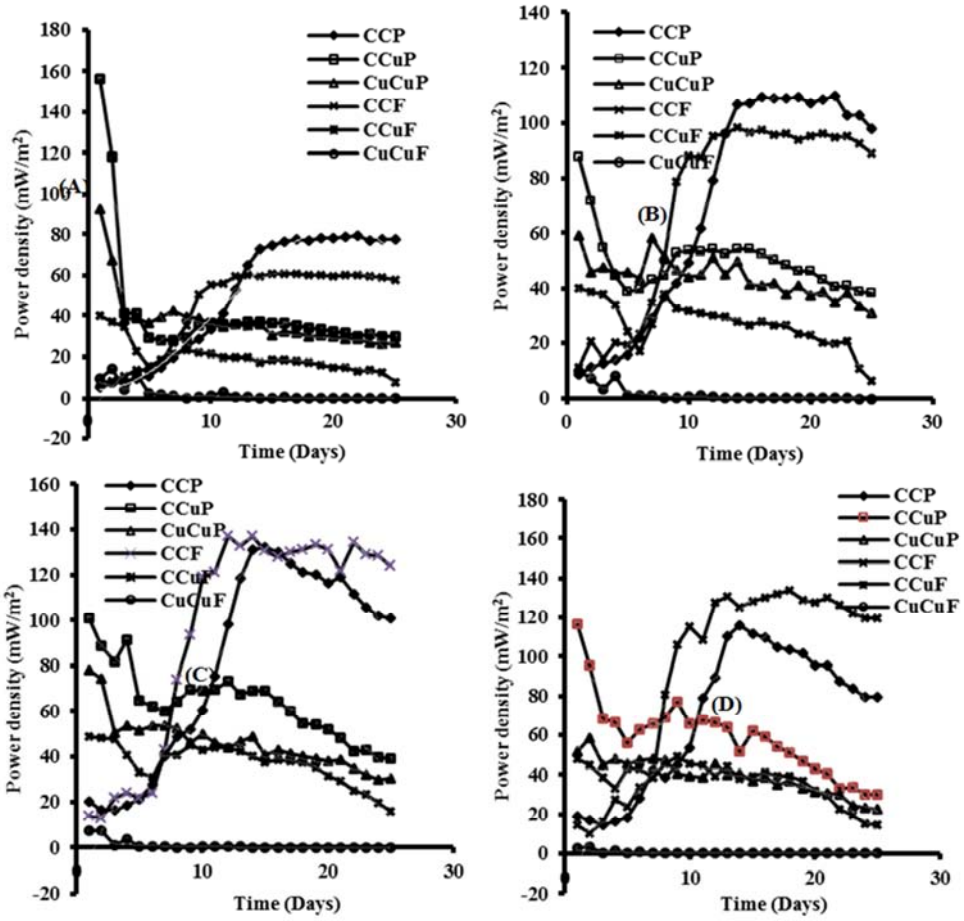

Figure 6. *Power density time graphs for MFCs across (a) 1000Ω (b) 500Ω (c) 200Ω and (d) 100Ω resistors.*

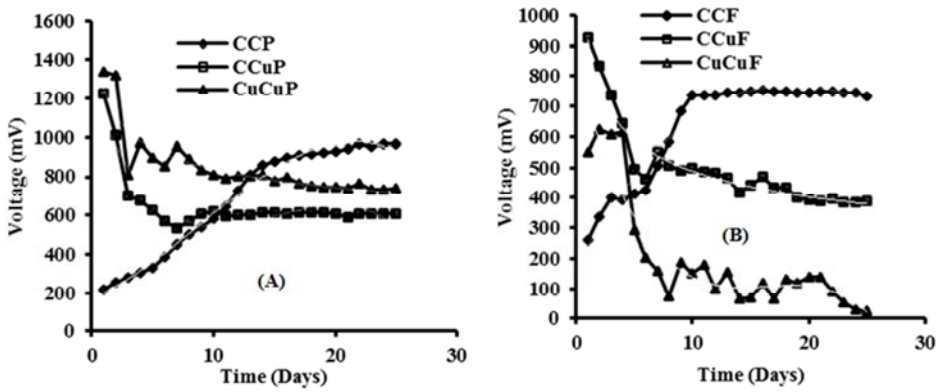

Figure 7. *Effect of different electrodes on generation of voltage using (a) Potassium permanganate (b) Potassium ferricyanide, as catholytes.*

In the MFCs constructed with carbon – carbon rods (graph A) as both electrodes, the highest open circuit voltage of 969.6V and 752.4V; power density (across 1000Ω resistor) of 77.3mW/m^2 and 60.9mW/m^2 were recorded using potassium permanganate and potassium ferricyanide respectively as the electron acceptors. With carbon and copper rods as the anode and cathode (graph B), the highest OCV was 927V and 1228.5V, while power density (across 1000Ω resistor) was 39.9mW/m^2 and 156.3mW/m^2 for MFCs made using potassium ferricyanide and potassium permanganate respectively. Similarly, for copper – copper rods (graph C), the highest OCV and power density values recorded in MFCs using potassium ferricyanide and potassium permanganate were 625.2V and 14.2mW/m^2; 1338.5V and 92.3mW/m^2 respectively.

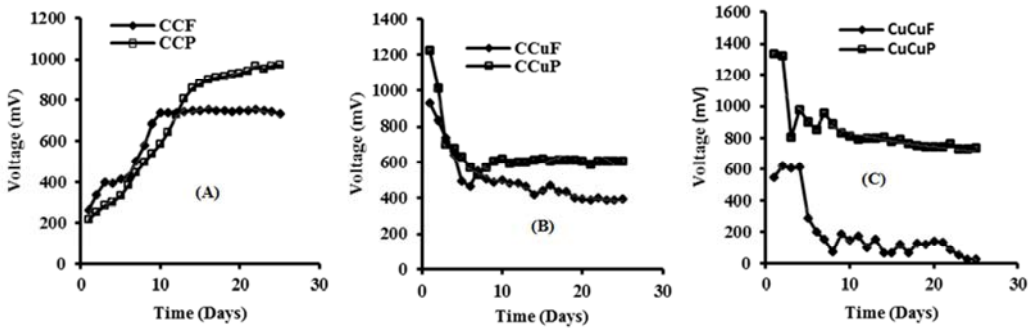

Figure 8. *Effect of different catholytes on generation of bioelectricity (a) carbon – carbon (b) carbon – copper and (c) copper – copper electrodes.*

3.9. Effect of Variable External Resistance on Coulombic Efficiency

The results of coulombic efficiency measured across variable external resistors, shown on figure 9 indicated that coulombic efficiency decreased with increasing external resistance. However, at 100Ω, the coulombic efficiency of CCP, CCuP, CuCuP, CCF, CCuF and CuCuF were 69%, 84%, 74%, 76%, 72% and 5% respectively. Beside 5% recorded from CuCuF, other MFCs performed appreciably well in converting the electrons generated from substrates degenerated to bioelectricity.

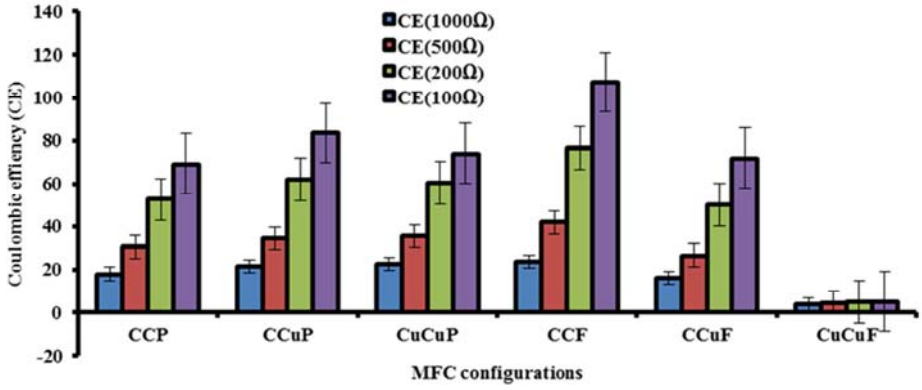

Figure 9. *Effect of variable external resistance on coulombic efficiency of MFCs.*

3.10. Waste Water Treatment Efficiency

The efficiency of the MFCs in treatment of waste water is measured by their ability to remove the COD and BOD parameters of the waste water. This was determined using the relationship below,

$$\frac{C_0 - C_1}{C_0} \times 100 \quad (1)$$

Where C_o is initial COD (or BOD) in mg/L of waste water and C_1 is the final COD (or BOD) in mg/L of waste water. When compared with the result of the control experiment, the MFCs exhibited fairly remarkable capability in removal of both COD and BOD of the piggery wastewater. Result showed that 65%, 51%, 47%, 83%, 48% and 49% of COD were removed by CCP, CCuP, CuCuP, CCF, CCuF and CuCuF respectively, compared to 32% COD removal recorded for the control. On the other hand, BOD removal was 73%, 76%, 76%, 69%, 43% and 57% for CCP, CCuP, CuCuP, CCF, CCuF and CuCuF respectively, while that of the control was 7%. Furthermore, the result showed that MFCs with potassium permanganate as catholyte performed better in removal of both COD and BOD than those with potassium ferricyanide. Similarly, carbon- carbon configuration of the electrodes outperformed other configurations of electrodes studied (figure 10).

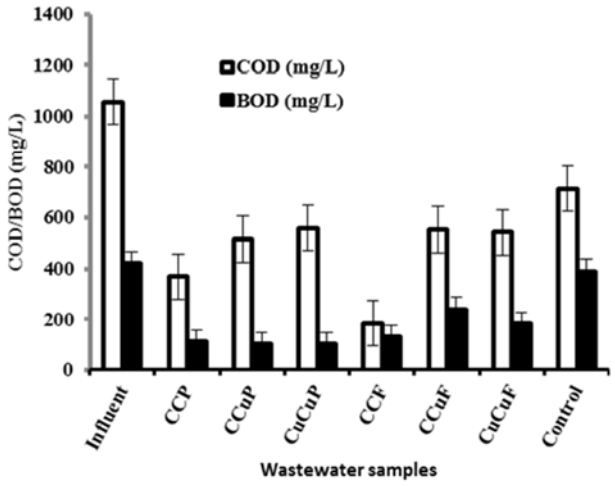

Figure 10. *Graphical comparison of COD and BOD removal efficiency of MFCs.*

4. Discussion

4.1. Microbial Analysis

Microbial analysis of the waste water sample before treatment revealed the presence of Lactobacillus spp., Corynebacterium spp., Streptococcus spp., Proteus mirabilis, Enterobacter spp., Escherichia coli, Pseudomonas spp., Bacillus spp., Aeromonas spp., Micrococcus lyteus, Corynebacterium spp., Cladosporium, Aspergillus versicolour, Candida albicans, A. flavus, Aspergillus nidulans, Trichoderma spp. and Aspergillus fumigates. Similar results have been reported by [26, 27]. Earlier, [28] revealed that the bacterial groups most often represented in terms of phylotype and clone abundance in swine waste were Eubacterium (22% of total sequences), the Clostridium (15% of sequences), the Bacillus–Lactobacillus–Streptococcus subdivision (20% of sequences), the Mycoplasma and relatives (10% of sequences) and the Flexibacter–Cytophaga–Bacteroides (20% of sequences). Furthermore, antibiotic resistant E. coli was isolated from pig as reported by [29]. Likewise, the dominant groups of pig fecal Eubacteria, include Bacteroides-Prevotella, Eubacterium-Clostridiacea, Lactobacillus-Streptococcus [30, 31].

The absence of Escherichia coli, Pseudomonas spp., and Aeromonas spp. in the sample obtained from swabbing the anode after treatment indicates that they did not contribute to the bioelectricity generation. Moreover, the following known exoelectrogens, Clostridium butyricum, Aeromonas hydrophila, Rhodoferax ferrireducens, Clostridium botulinum were not identified by both culture base and molecular base analyses of the sample, hence could not be responsible for generation of bioelectricity recorded in the study. However, [32, 26, 27] have reported that Bacillus, Lactobacillus, Streptococcus and Corynebacterium were isolated from various samples used in generating bioelectricity. Therefore, these are suspected to be the exoelectrogens responsible for the generation of bioelectricity.

4.2. Physicochemical Analysis

Reduction of some parameters of the waste water including BOD, COD, DO, ammonia, ammonium, ammonia-nitrogen indicates the effectiveness of MFCs as a waste water treatment technology. This is further buttressed by the difference in the values for treated and control samples. [27] has reported similar results from their study.

4.3. Generation of Bioelectricity

The initial low OCV recorded immediately after the introduction of piggery waste water into the anode chambers has also been reported by [33, 27]. This was not due to microbial activities, but has been attributed to chemical and biological factors based on difference of potential between the two chambers. However, microorganisms gradually colonized the surface of the anodes, metabolized nutrients in the wastewater and released electrons to the anodes which led to gradual increase in the voltage recorded subsequently. This is corroborated by [34], who reported that it takes time for the bacteria to colonize the electrode and manufacture enzymes or structures needed to transfer electrons outside the cell. The observation of increase in voltage (bioelectricity) generation indicates that piggery wastewater is suitable and contains exoelectogens necessary for bioelectricity generation [33, 26, 27]. OCV recorded in this study were higher than the values reported by [27].

The maximum current density, across 1000Ω resistor, obtained from MFCs with $KMnO_4$ as electron acceptor were 105.66mA/m^2, 148.38mA/m^2 and 114.01mA/m^2. These were higher than 108.57mA/m^2 reported by [35] with graphite rods, 88.01mA/m^2 with copper rods, and $KMnO_4$ as the electron acceptor. Conversely, they were higher than 92.65mA/m^2 for CCF, 75mA/m^2 and 44.73mA/m^2 for MFCs with potassium ferricyanide as electron acceptor in this study. Moreover, [33] have reported 141mA/m^2 as the maximum current density obtained continuously aerated cathode. Results obtained revealed that current density increased with decrease in external resistance. This conforms to Ohm's law that at constant voltage, current increases with decreasing external resistance.

In this study, maximum power density across 1000Ω resistor, was 156.319mW/m^2 recorded from CCuP which had potassium permanganate as electron acceptor, while 136.71mW/m^2 was maximum for MFCs with potassium ferricyanide as electron acceptor. These are close to the maximum power density of 181.48mW/m^3 reported by [6] but higher than 45mW/m^2 reported by [33, 36] have also reported 116.2mW/m^2 for potassium permanganate and 40.6mW/m^2 for potassium ferricyanide. Power density increased with decreasing external resistance.

4.4. Effect of Electrode Material on Generation of Bioelectricity

This study notes that maximum OCV, power density and current density were obtained from MFCs which had copper as a constituent of their electrodes. This has earlier been

observed by [37, 26]. This could be due to better conductivity of electrons by copper than carbon [37]. Conversely, overtime, the consistency and stability of bioelectricity generation recorded across MFCs with copper as electrode was lower than observed from MFCs with carbon electrodes. While copper produced very high voltage within the first few days of the study and copper relatively lower voltage, it was observed that the output across copper electrodes sharply declined and continuously lagged behind those of carbon which gradually increased in output until the end of the study. The sharp decrease in voltage generated with copper as electrodes could be attributed to the gradual degeneration (corrosion) of copper electrodes with time as seen during the study, thus forming thin film layer on the surface of the electrodes. [38] reported that copper cannot be used for a long time in a MFC due to its nature and the ease with which it corrodes. [3] also reported a rather rapid decline in voltage output due to gradual chemical decomposition and electrochemical defects.

Since copper is a heavy metal, when used as the anode, this can inhibit or kill some microorganisms that formed biofilms on its surface, hence reducing voltage generation after an initially high activity. On the other hand, if used as the cathode, such layer may reduce the conductivity of electrons generated (by increasing internal resistance), which results in reduced voltage after initially high output. However, this phenomenon was not particularly envisaged in carbon electrodes and could account for the gradual rise and stability in voltage production in its MFCs as more biofilms were formed and microbial activity increased on the anode.

From the foregoing, it can be inferred that carbon which produced more stable voltage and is non-corrosive is a better electrode for MFCs than copper. Construction of MFCs should be done with materials that can last for prolonged periods to allow maximum treatment of wastewater and harvest of bioenergy. This is in agreement with [40] who reported that in their study of various combinations of anode/cathode materials like copper, zinc, aluminum, carbon, stainless steel, mild steel for MFCs, carbon-carbon was among the combinations that gave highest voltage output. [35] also observed maximum power density of 48.85mW/m^2 and 42.59mW/m^2 when graphite and copper electrodes respectively were used in MFCs.

4.5. Effect of Catholytes on Generation of Bioelectricity

Reports show that different oxidizing agents in the cathode compartment of the MFC effects on its performance [41]. Results of the present study generally indicate that potassium permanganate performed better than potassium ferricyanide as electron acceptor in MFC. This supports the higher maximum OCV and power density output of 1.04V and 7.29mW/m^2 respectively, recorded with potassium permanganate as electron acceptor than 0.71V and 0.92mW/m^2 for potassium ferricyanide and 0.56V and 0.79mW/m^2 for and potassium dichromate [42]. Another study by [36] revealed that the performance of cathodic electron acceptors (CEA) were in the order; potassium permanganate (1.11V; 116.2mW/m^2) > potassium persulfate (1.10V; 101.7mW/m^2) > potassium dichromate, $K_2Cr_2O_7$ (0.76V; 45.9mW/m^2) > potassium ferricyanide (0.78V; 40.6mW/m^2). Similar results have been related by [43, 44]. The increase was due to the high redox potential of the permanganate compared to that of ferricyanide, as seen in equation 2 and 3 [44].

$$MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O \rightarrow E^0 = 1.70V \quad (2)$$

$$[Fe(CN)]^{3-} + e- \rightarrow [Fe(CN)]^{4-} \rightarrow E^0 = 0.36V \quad (3)$$

Moreover, it is worth pointing out that permanganate has no need for catalyst, which makes this process simple and economical. However, like some other liquid-state electron acceptors, permanganate as well as ferricyanide MFCs also require liquid replacements to compensate their depletion. Therefore, their use may be only applied to small-scale power supplies [41, 45].

4.6. Coulombic Efficiency

Coulombic efficiency observed from the MFCs studied were 69%, 84%, 74%, 76%, 72% and 5%, which is quite commendable, implying that more than half of the electrons generated from the metabolizing the substrate was used in bioelectricity generation. The values are relatively higher than 69.1%, 46.1%, 40.6% and 44.0% reported by [46]. [47] reported a CE of the pure culture *E. coli* MFC with sucrose as substrate was found in the range of 69 – 85% while the CE of river water samples was between 71 – 77%.

Besides, MFCs which used potassium permanganate as catholyte relatively gave better coulombic efficiency than those that had potassium ferricyanide as their catholyte. However, there is no significant difference between the coulombic efficiency of MFCs in relation to their electrodes combinations. Coulombic efficiency (CE) increased with decreasing external resistance. This could be due to current, a major factor that affects coulombic efficiency, which is indirectly proportional to external resistance across an MFC. Therefore, any factor that decreases current generation would invariably decrease the coulombic efficiency of the cells. Similar report has been related by [48, 41, 49].

4.7. Waste Water Treatment Removal Efficiency

One of the core objectives of MFC technology is treatment of waste waters (measured by extent of COD and BOD removal). In microbial fuel cell, microorganisms consume carbon content of the substrate and donate the electrons to anode for bioelectricity generation. Owing to the voltage recorded across the MFCs, considerable treatment of the waste water (removal of COD and BOD) was anticipated. [50] recorded 73.34%, 78.71%, 72.54%, 71.38% and 67.31% COD removal at 45^{o}C after 10th day. Interestingly, MFCs with potassium permanganate as catholyte, which produced better electricity, also gave higher percentage COD and BOD removal than those with potassium ferricyanide. [51] stated

that the removal of COD is found to be higher for the cell which showed higher current production. Furthermore, MFCs which had carbon – carbon combination produced higher COD and BOD removal than other studied.

Generally, it was found that values for COD removal efficiency were higher than those of BOD removal. This is in line with the results of [52, 53]. COD removal in an MFC is dependent on microbial growth, current generation, aerobic growth due to oxygen leaking in through the cathode, and anaerobic growth using other terminal electron acceptors in the waste water, including carbon dioxide. Microbial communities can change with different operational conditions, which can affect COD removal rates and current generation [54].

5. Conclusion

Experimental data obtained from this study showed that potassium permanganate is a better electron acceptor than potassium ferricyanide in both generation of bioelectricity and treatment of waste water due to its higher electrode potential. However, as noted earlier, potassium permanganate must be replaced with time to enhance its capability. This however, makes its use unsustainable, possibly environmentally unfriendly due to problems of disposing spent solutions, and increases the cost of running MFCs on it. On the other hand, despite that the highest voltage and power density recorded in this study were from carbon - copper electrodes combination, the output from carbon – carbon electrodes was more stable and consistent. Overtime, copper corroded thus abruptly crashing its initially high output. This may limit its successful application in MFCs. Consequently, now that the role of renewable energy sources in ensuring sustainable power supply has been globally acknowledged, more effort should be made by to optimized all parameters affecting its output using the most sustainable, cost effective and environmentally friendly materials.

Abbreviations/Codes Used

A, E and F represent bacterial isolates from carbon-carbon electrodes, potassium ferricyanide, carbon-copper electrodes, potassium ferricyanide and copper-copper potassium ferricyanide MFCs respective.

O, P, Q R and S are primers for *C. butyricum, A. hydrophila, Rhodoferax ferrireducens, C. botulinum and DNA COM* respectively.

CCP: Sample from carbon-carbon electrodes, potassium permanganate as electron acceptor.

CCuP: Sample from carbon-copper electrodes, potassium permanganate as electron acceptor.

CuCuP: Sample from copper-copper electrodes, potassium permanganate as electron acceptor.

CCF: Sample from carbon-carbon electrodes, potassium ferricyanide as electron acceptor MFC.

CCuF: Sample from carbon-copper electrodes, potassium ferricyanide as electron acceptor MFC.

CuCuF: Sample from copper-copper electrodes, potassium ferricyanide as electron acceptor MFC.

CE: Coloumbic efficiency.

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