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Study on Generation of Bioelectricity Using Potassium Ferricyanide Electron Acceptor in Microbial Fuel Cell

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Abstract: The capability of simultaneously generating bioelectricity and treating piggery wastewater using microbial fuel cell (MFC) with indigenous exoelectrogens was demonstrated. Three units of H – type MFCs were constructed using 0.1M potassium ferricynide ($K_3[Fe(CN)_6]$) as catholyte and carbon – carbon (CC), carbon – copper (CCu) and copper – copper (CuCu) electrodes of surface area 0.0071m^2 each. The BOD and COD of the test piggery wastewater were 420mg/L and 1057mg/L respectively. While coulombic efficiency (CE) of the MFCs after 25 days were 76%, 72% and 5.10%, COD removal were 83%, 48% and 49% for CC, CCu and CuCu respectively. Highest voltage recorded were 752.4mV, 1027mV and 625.2mV across CC, CCu and CuCu respectively. Generation of voltage proportionally decreased with decreasing external resistors. Power density which increased with decreasing external resistance across each MFC until 200Ω beyond which decrease became evident, peaked at 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) across R_{ext} = 1000Ω for CC, CCu and CuCu respectively. This depicts that carbon used as both cathode and anode produced more bioelectricity than other combinations. Bacteria isolated from the surface of anodes include, *Lactobacillus* spp., *Corynebacterium* spp., *Streptococcus* spp., *Proteus mirabilis, Enterobacter* spp., *Escherichia coli, Pseudomonas* spp., *Bacillus* spp., *Aeromonas* spp., *Micrococus luteus*, *Corynebacterium* spp. and *Salmonella* spp. Plasmid profile of the bacteria isolates in the original wastewater sample revealed that *Lactobacillus* spp., *Proteus mirabilis, Escherichia coli, Pseudomonas* spp., *Bacillus* spp., and *Aeromonas* spp had plasmids. These findings show that with better designs and optimization, the performance of the MFCs can be enhanced.

Keywords: Coulombic Efficiency, Bioelectricity, Carbon-Carbon, Microbial Fuel Cell, Potassium Ferricyanide

1. Introduction

Studies have shown that fossil fuel dominates the world's supply of energy, and will stand at about 80% by 2040 [1]. However, this source of energy is not without associated high level of environmental pollution and its attendant health challenges to human, animals, plants and environment, hence the need for renewable, cheap and more environmentally – friendly alternative sources.

A microbial fuel cell (MFC) is a biochemical-catalyzed system which generates electricity by oxidizing biodegradable organic matter in the presence of fermentative bacteria. It is a new form of renewable energy technology that can generate electricity from what would otherwise be considered waste [2].

The outcomes of various experiments show that the nature of electrode materials, together with other parameters have important effect on MFCs [3], as it determines the power loss of fuel cell in terms of internal resistance [4].

2. Materials and Methods

The sample of piggery wastewater used was obtained from a commercial pig farm in Nekede, Owerri West Local Government Area, Imo State, Nigeria with coordinates, 5°26'48.5"N 7°01'24.5"E. The samples for MFC and physicochemical analyses were collected following the method of [5, 6] using a container previously surface sterilized according to [7]. After 25 days, treated samples from each MFC, 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.

Physicochemical parameters including pH, electrical conductivity (EC), total dissolved solid (TDS) (measured using Hanna Instrument for pH, EC, TDS and Temperature, Model No.: HI9811-5), dissolved oxygen (DO) (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 ortho-phosphate (P_2O_5); nitrate – nitrogen, nitrate and calcium (using Hanna COD and multiparameter photometer; Model No.: HI83099) were determined. The chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) were also measured.

2.1. Culture – Based Identification of Microorganisms

Analysis for identification of the microbial flora of the piggery wastewater sample was determined by preparing ten-fold serial dilution of 1ml of the sample and spreading on McConkey Agar and Nutrient Agar. Each medium was prepared according to the manufacturer's specification and incubated at 37°C after inoculation. Observation was done after 24 hours incubation and growths 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°C for 24 hours. Biochemical tests were carried out to characterize the microorganisms and identification was as described by [8].

2.2. Construction of Microbial Fuel Cell

Three H – type double chamber MFCs were constructed as described by [9]. Salt bridge contained in 15 cm length and 3.81 cm diameter PVC pipes used to join the two chambers were prepared by dissolving 20g of agar – agar powder into 1000ml of distilled water containing 75.5g KCl which was boiled for about 3 minutes, poured into the PVC pipes and then allowed to gel. Electrodes used were arranged into carbon – carbon (CC), carbon – copper (CCu) and copper – copper (CuCu) each with surface area 0.0071m^2. Potassium ferricyanide ($K_3[Fe(CN)_6]$) solution of concentration 0.1M served as the electron acceptor.

The anode chambers contained 800ml of pig wastewater while 900ml of Potassium ferricyanide was introduced into the cathodes. The chambers were connected by salt bridges and the circuits completed using 1.5mm copper wires of length 0.4m each. The setups were allowed for 24 hours before records of voltage generated were taken from the digital multimeters (DT-830D Series). Open circuit voltage (OCV) and voltage across 1000Ω, 500Ω, 200Ω and 100Ω resistors were in turn recorded on three hours intervals from 6.00 am to 6.00 pm for 25 days.

2.3. Extraction of Plasmid DNA

The method of [10] was used to extract plasmid DNA of the isolates.

2.4. Preparation of Agarose Gel, Loading and Viewing

1g of agarose powder was dissolved 100ml of 1X TBE buffer and boiled for 5 minutes in a water bath. It was cooled to 50°C and 10µl of ethidium bromide was added and gently shaken before been poured into the tray of electrophoresis tank (EDVOTEK 220V EVT300) with the comb and stoppers in place. It was allowed for about 20minutes to solidify. Two-third of the tank was covered with 1X TBE buffer and 20µl of samples were mixed with 2µl of the loading dye and then carefully loaded into the wells with the marker in lane 1. The electrodes were connected and it was run at 75V till the samples have migrated up to two-third of the gel field before it was transferred to a UV – transilluminator and viewed.

3. Results

3.1. Physicochemical Analysis

Physicochemical analysis of the sample yielded the results shown in table 1. While there was increase in values of other parameters analyzed, dissolved oxygen, calcium concentration, biochemical oxygen demand and chemical oxygen demand significantly decreased. Comparison of the values to the control shows an impressive variation.

3.2. Bacterial Identification

Cultural analysis indicated the presence of organisms in table 2 in original sample. However, after treatment, it was observed that some of the organisms did not persist as shown in table 3.

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	6.7	6.8	6.9	5.3
Electrical Conductivity (µS/cm)	3800	7410	7740	7550	5490
Total dissolved solid (mg/L)	189	4810	5030	4900	2710
Nitrate-Nitrogen (mg/L)	24	128	96	92	32
Nitrate (mg/L)	104	268	146	134	128
Phosphate (PO_4^{3-}) (mg/L)	90	278.4	339.2	165.6	48
Phosphate (P) (mg/L)	129.2	91.2	87.4	53.6	45.6
Phosphate (P_2O_5) (mg/L)	67.2	208	252.8	123.2	36
Ammonia-Nitrogen (mg/L)	444.8	216.8	219.8	226.8	352
Ammonia (NH_3) (mg/L)	541.6	371.4	393.2	383.2	428
Ammonium (NH_4^+) (mg/L)	568	424.2	436.8	442.8	454.4
Calcium (Ca^{2+}) (mg/L)	3200	800	800	2000	2000
Dissolved oxygen (mg/L)	6.00	1.50	3.00	2.10	4.5
Biochemical Oxygen Demand (mg/L)	420	130	240	180	390
Chemical Oxygen Demand (mg/L)	1057	542	553	542	715

3.3. Generation of Voltage

Prior to successively recording the voltage across 1000Ω, 500Ω, 200Ω and 100Ω resistors, shown in figure 3, the average open circuit voltage (OCV) was taken. Highest OCV was found to be 927mV in CCu on day one and 25mV on day 25 in CuCu which was the least, as shown on figure 2.

Table 2. *Results of culture based identification of bacteria in the 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*

Table 3. *Results of identification of bacteria in the samples after treatment.*

Samples	No of colonies	Biochemical tests							
		Gram stain	Catalase test	Oxidase test	MR test	Indole test	Citrate test	VP test	Microorganisms
		+	+	-	-	-	+	+	*Bacillus licheniformis*
CC	3	+	+	+	-	-	-	+	*Bacillus alvei*
		+	+	+	-	-	+	+	*Bacillus subtilis*
		+	+	+	-	-	-	+	*Micrococcus spp*
CCu	3	+	-	+	+	+	-	-	*Streptococcus spp*
		+	+	+	-	-	+	+	*Bacillus spp*
		+	+	+	-	-	+	+	*Bacillus spp*
CuCu	3	-	+	-	+	-	-	-	*Proteus mirabilis*
		+	+	+	-	-	+	+	*Bacillus subtilis*

Legends: Isolates from CC: Carbon-carbon, CCu: Carbon-copper, CuCu: Copper-copper; + = positive test, - = negative test.

3.4. Plasmid Profile

Plasmids were present in Lactobacillus spp., Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Bacillus spp and Aeromonas spp as seen in band on figure 1.

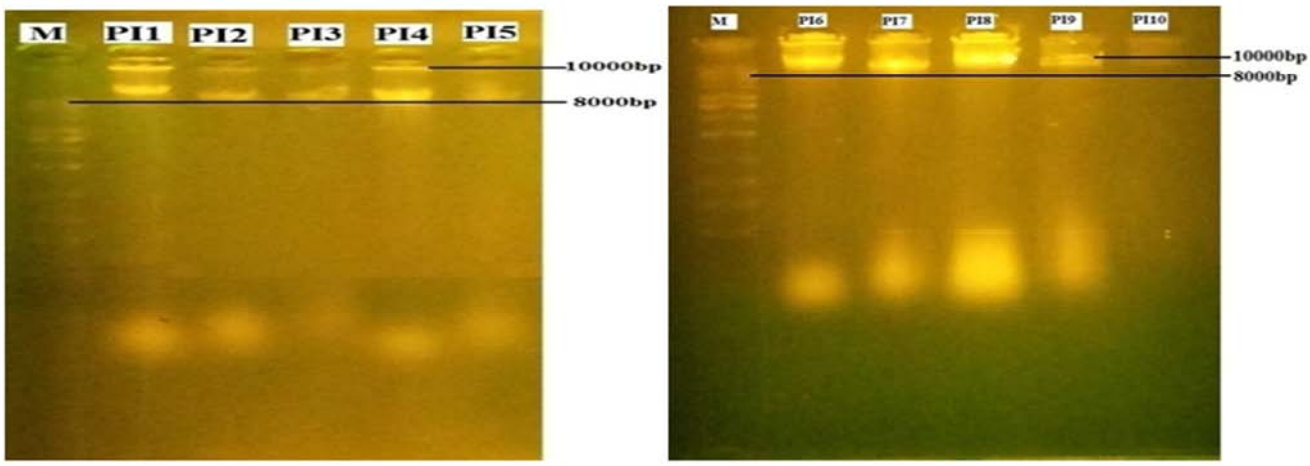

Figure 1. *Bands showing presence of plasmids. Legends: M: Marker; PI1: Lactobacillus spp.; PI2: Corynebacterium spp; PI3: Streptococcus spp; PI4: Proteus mirabilis; PI5: Enterobacter spp.; PI6: Escherichia coli; PI7: Pseudomonas spp.; PI8: Bacillus spp; PI9: Aeromonas spp. and PI10: Micrococcus lyteus.*

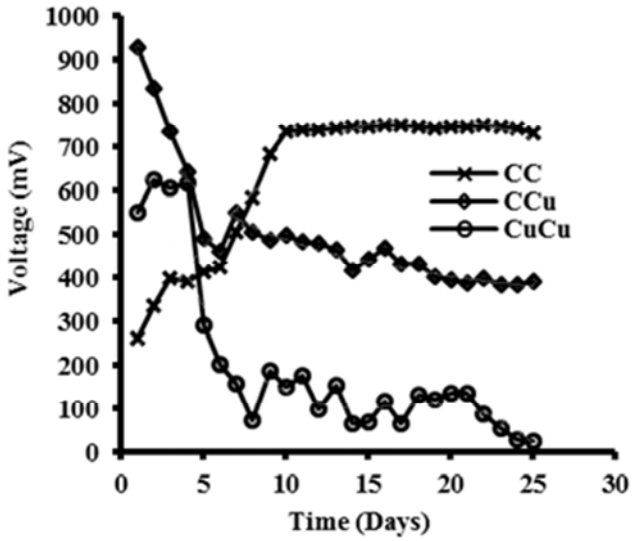

Figure 2. *Open circuit voltage. Legends: CC: Carbon-carbon; CCu: Carbon-copper; CuCu: Copper-copper.*

3.5. Power Density

Using the relationship,

$$PD = \frac{V^2_{cell}}{R_{ext.} \cdot x \cdot A} \quad (1)$$

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, power density (mW/m^2) was computed. The highest power density obtained across 1000Ω resistor was $60.94 mW/m^2$ by CC on day 16, while the lowest was $0.01 mW/m^2$ by CuCu on day 25 as shown on figure 3.

3.6. Effect of Electrodes Materials on Voltage Output

Different combinations of carbon and copper rods were used to determine the effect of electrodes materials on voltage generated in MFCs. As shown on figure 5, the open circuit voltage recorded in both MFCs containing copper as a constituent of their electrodes was initially high before sharply declining afterwards and consistently followed a downward trend until the end of the period of treatment. However, voltage produced by MFC with carbon-carbon electrodes gradually increased until day 13 when it stabilized. While highest voltage of 927V was recorded in CCu, it suddenly declined to 391.2V at day 25. Conversely, CC produced 261.5 on day 1 which gradually increased to 752.4V on day 16.

3.7. Coulombic Efficiency

The measurement of the ratio of amount of actual electrons that is gained from the substrate in the form of electricity against the theoretical amount of electrons which are delivered by the bacteria based on the COD or substrate removal is referred to as coulombic efficiency. The computation is done using,

$$CE = \frac{M \int_0^t Idt}{FbV_{an}\Delta COD} \quad (2)$$

where V is liquid volume (m^3) at the anode chamber, F is Faraday's constant (96485 C/mol of e^-) and b is mole of electrons produced per mol of O_2 (4 mol/mol), M is the molar mass of O_2 (32 g/mol). Using current (I) recorded across 100Ω resistor, results obtained showed that the MFCs performed impressively in converting electrons generated from wastes to electricity, except for CuCu. CC gave the highest coulombic efficiency of 76% at R_{ext} = 100Ω, while least was 5.1% produced by CuCu. By varying external resistance, it was observed that coulombic efficiency decreases with increasing external resistance as can be seen in figure 6.

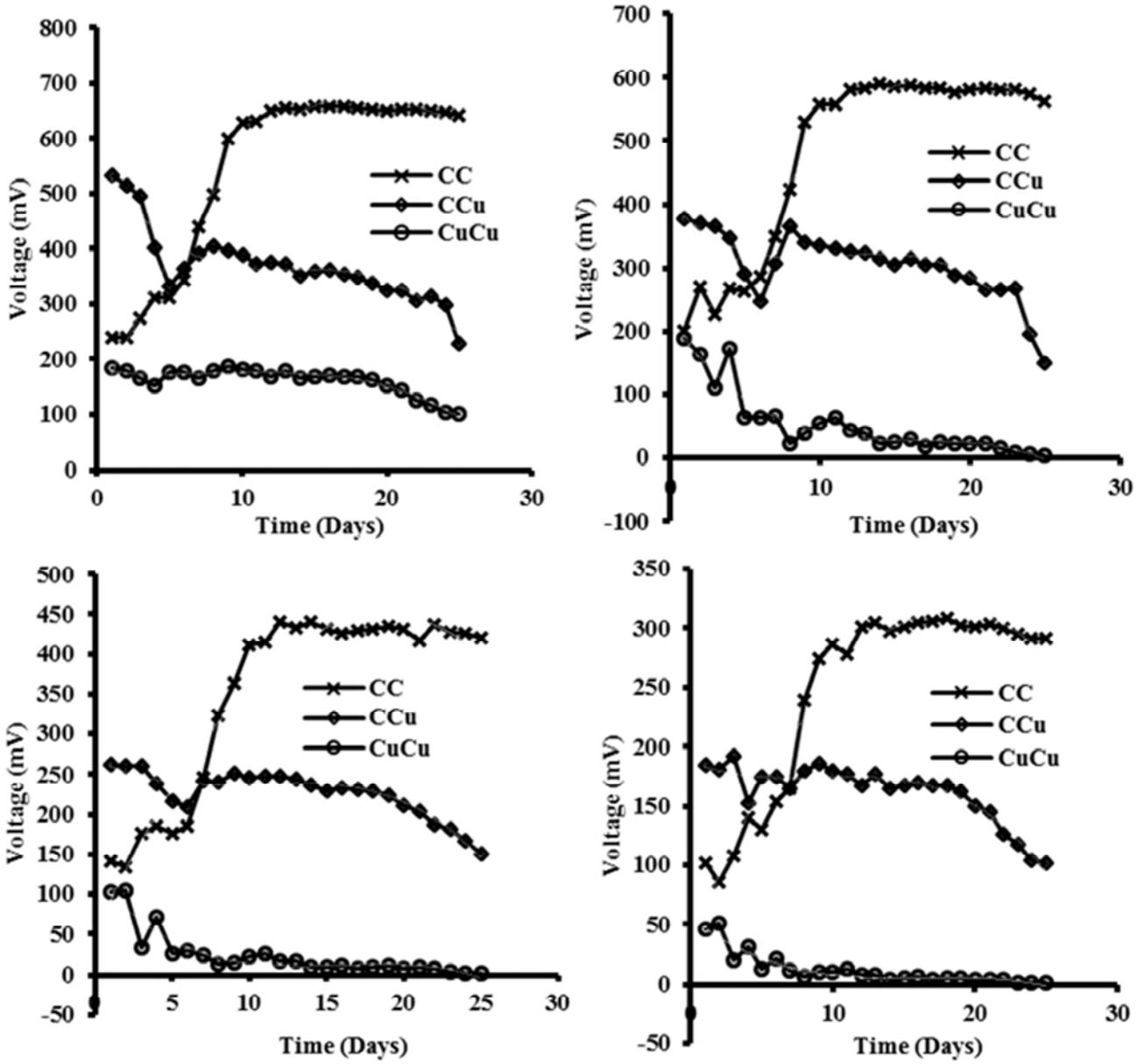

Figure 3. *Voltage produced across (a) 1000Ω, (b) 500Ω, (c) 200Ω and (d) 100Ω resistors by MFCs per time. Legends: CC: Carbon-carbon; CCu: Carbon-copper; CuCu: Copper-copper.*

MFC with only carbon as its constituent electrodes produced higher coulombic efficiency. This corroborates the conclusion that carbon is a better electrode for electricity generation in a MFC.

3.8. Percentage COD and BOD Removal Efficiency

Computation of %COD and %BOD removal efficiencies of the MFCs using the relationship in equation 1 revealed that %BOD removal was in the range of 43% to 69% while %COD removal was 48% to 83%. This is significant compared to 32% COD removal and 7% BOD removal recorded in the control sample. CC showed the highest performance in both parameters. The results are presented in figure 7.

$$\frac{Initial COD\ (BOD)\ of wastewater\ (mg/L)\ ?\ Final\ COD\ (BOD)\ of wastewater\ (mg\ \ L)\times 1}{Initial\ COD\ (BOD)\ of wastewater (mg/L)} \quad (3)$$

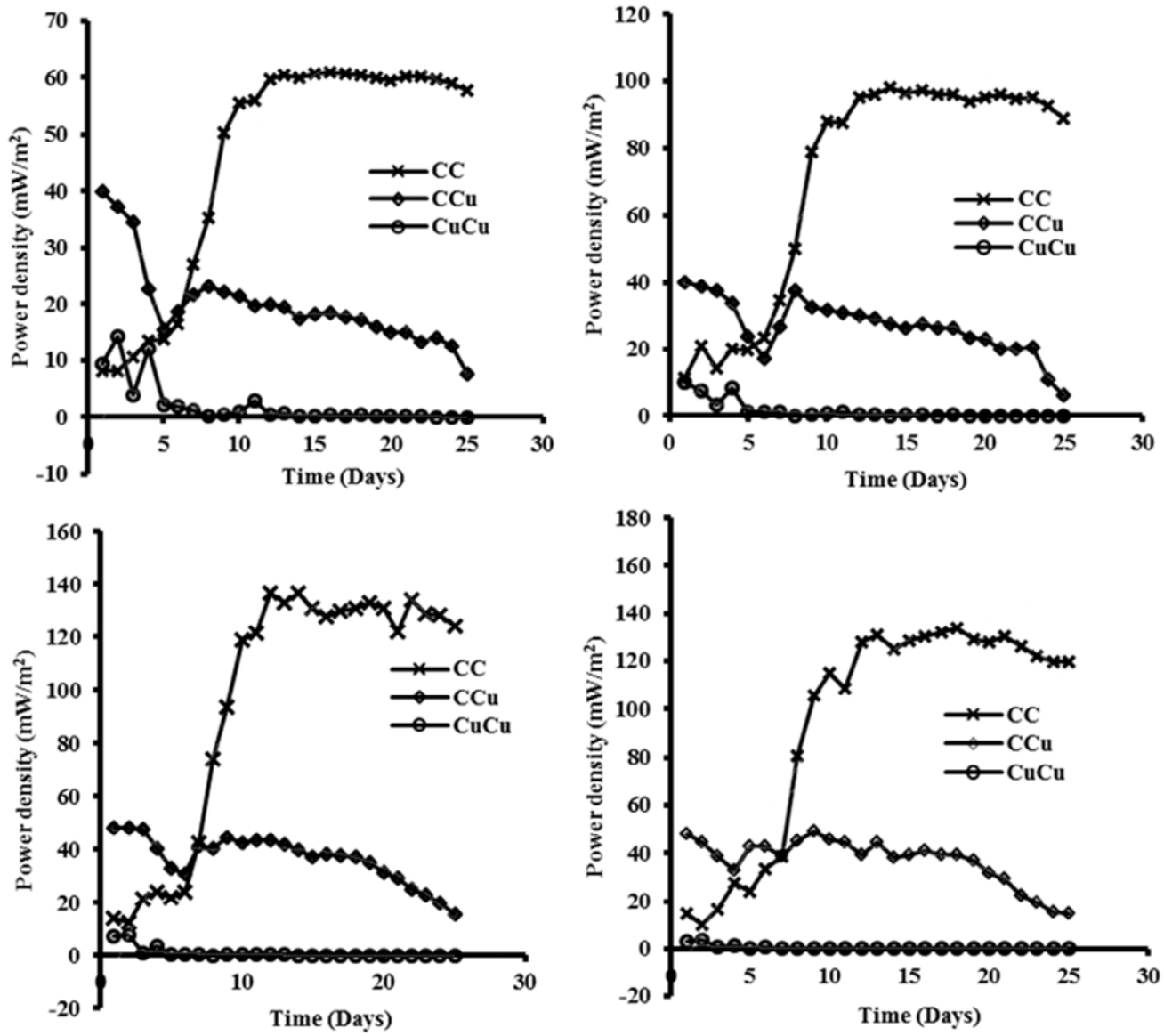

Figure 4. *Comparison of power density time graphs for different MFCs across (a) 1000Ω (b) 500Ω (c) 200Ω and (d) 100Ω resistors. Legends: CC: Carbon-carbon; CCu: Carbon-copper and CuCu: Copper-copper.*

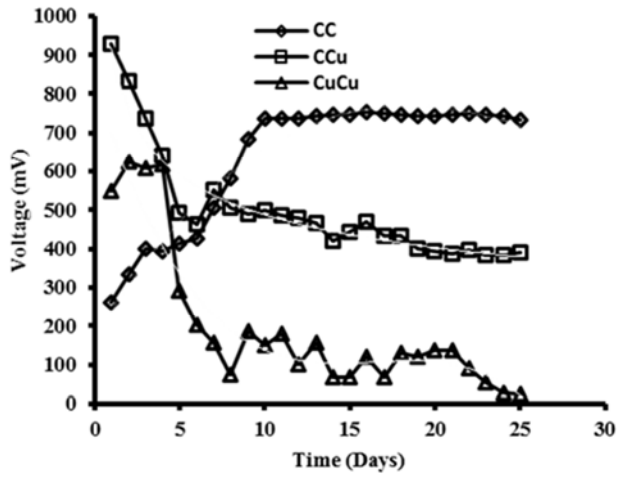

Figure 5. *Effect of different electrodes on generation of voltage. Legends: CC: Carbon-carbon; CCu: Carbon-copper; CuCu: Copper-copper.*

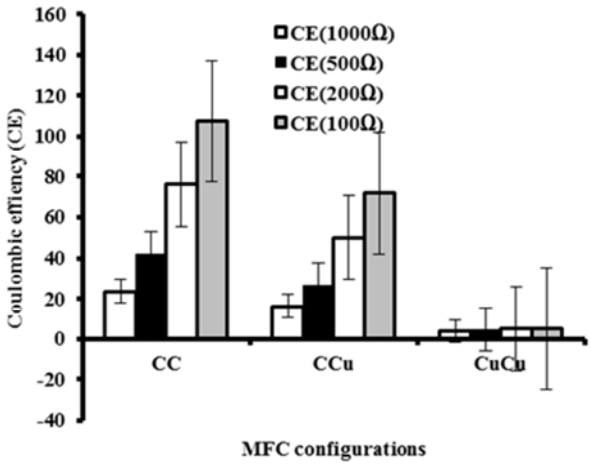

Figure 6. *Charts showing relationship between coulombic efficiency and external resistance. Legends: CC: Carbon-carbon; CCu: Carbon-copper; CuCu: Copper-copper.*

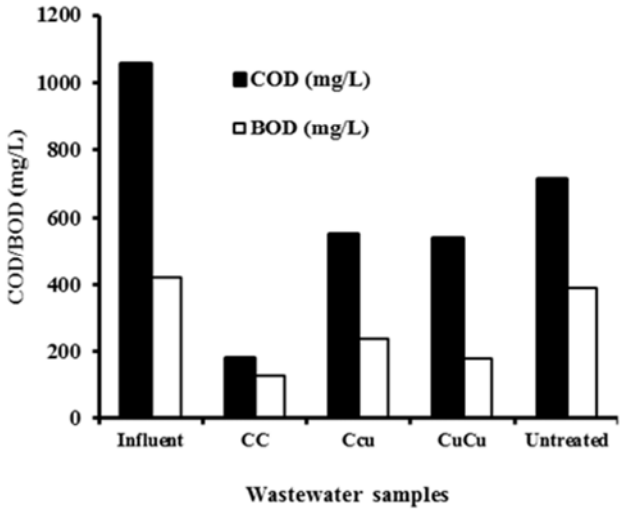

Figure 7. *Chemical oxygen demand (COD) and Biochemical oxygen demand (BOD) removal from wastewater samples. Legends: CC: Carbon-carbon; CCu: Carbon-copper; CuCu: Copper-copper.*

4. Discussion

4.1. Cultural Identification of Microorganisms

Proteus mirabilis, Lactobacillus spp., Escherichia coli, Corynebacterium spp., Aeromonas spp., Streptococcus spp., Enterobacter spp., Pseudomonas spp., Bacillus spp., Micrococcus lyteus and Salmonella spp. were the bacteria isolated from the wastewater sample. This supports [12, 13] who reported that the dominant groups of pig fecal Eubacteria include Bacteroides-Prevotella, Eubacterium-Clostridiacea, Lactobacillus-Streptococcus. [14] also reported isolation of multiple drug resistant E. coli, among other 13 bacteria isolates, from four domestic livestock, including pig. [15] reported that abundance of bacterial isolates from swine feces is in the order, 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%).

4.2. Plasmid Profile of Isolates

Results of plasmid profile showed presence of plasmids in *Escherichia coli, Proteus mirabilis, Aeromonas spp., Pseudomonas spp., Lactobacillus spp.* and *Bacillus spp*. If the plasmids carry resistance genes, then they can impart resistance to drugs, as well as other features to the bacteria. *E. coli* isolated from piggery waste has been reported to exhibit multidrug resistance [14].

4.3. Physicochemical Analysis

Most physicochemical parameters recorded significant variations. The increase recorded in nitrate and nitrate – nitrogen contents of the wastewater after treatment could be attributable to nitrification of nitrogen due to oxygen diffusion through the cathode [16]. This corroborates the observation of [17] that 83 ± 4% ammonia was removed from wastewater after 100hrs operation while 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. Increase in orthophosphate concentration may be attributed to low redox potential in the MFC which probably stimulated the release of stored phosphates in the bacteria [18], or the conversion of organic phosphorus in the wastewater to orthophosphate. The reduction in organic matter content of treated wastewater as depicted by reduced BOD and COD values than untreated sample is an indication of enhanced metabolic activities of microorganisms which used them as sources of carbon for energy generation.

4.4. Power Density

Power density (across 1000Ω resistor) ranged from 0.010mW/m^2 to 60.944mW/m^2. This is close to 40.6mW/m^2 reported by [19], but lower than the maximum resultant MFC output power density of 181.48mW/m^3 produced using 0.1M potassium ferricyanide as the catholyte [20]. Power density inverse proportionally increased with external resistance until 200Ω at which direct proportional relationship was observed. It was observed that carbon-carbon electrode MFC yielded highest and most stable power density. Higher maximum power density was recorded with graphite rod than copper electrode [21].

4.5. Generation of Voltage

Few minutes after setting up the MFCs, low voltage was recorded across the MFCs, which gradually increased with time. This observation has been reported by [17] in a study with two chambers MFC where a circuit voltage of 20 ± 2mV (±SD, n = 90; 8–53h) was immediately generated within only a few hours adding non-diluted swine wastewater. Chemical and biological factors based on difference of potential between the two chambers might be responsible for this initial voltage. Thereafter, the voltage rapidly increased due to biological activity.

4.6. BOD and COD Removal Efficiency

One of the core aims of the MFCs is to serve as sustainable and cost effective alternative technology for wastewater treatment against the conventional treatment plant [22]. Furthermore, this study has proven that the application of MFC significantly enhances the reduction of COD and BOD of wastewater during treatment. This is shown by the difference in the percentage COD and BOD removal for all the treated samples compared to the value for untreated (control) sample, thus confirming the feasibility and suitability of microbial fuel cell in the treatment of pig wastewater. Higher BOD values were recorded than COD and carbon-carbon MFC which yielded better and more stable electricity also recorded highest COD removal. This supports [23] who reported that the removal of COD is found to be higher for the cell which showed higher current production. In order to effectively design MFCs for wastewater treatment, the relationship between current

production and COD removal relative to current generation versus other aerobic and anaerobic processes must be better understood. The amount of substrate lost to processes that do not generate electrical current varies, depending on reactor operation, even for reactors operated over the same period of time [24].

4.7. Coulombic Efficiency

Maximum coulombic efficiency recorded in this study was 76%, which is significantly higher than 69.1%, 46.1%, 40.6% and 44.0% for hydrolysate, rhamnose, xylose and glucose respectively, reported by [25]. Coulombic efficiency (CE) gradually reduced with increasing external resistance. This is attributable to the indirect proportional relationship between external resistance and current generated in a cell. Since CE depends on the quantity of electricity produced per time, any factor that decreases current output of a cell would invariably decrease the coulombic efficiency of the cells. [26] suggested that current flow also affects the CEs. Similar report was given by [27] that when R_{ex} was increased from 1000 to 2000Ω, a general CE decrease was observed. This also agrees with the report of [28].

5. Conclusion

Discharge of untreated piggery wastewater into the environment may pose severe environmental and public health risk especially if the plasmids contain regions that code for antibiotic resistance. Results achieved in the present study attest to capability of MFCs in production of bioelectricity and wastewater treatment. In order to achieve better harvest of bioelectricity from consumed organic matter content of wastewater (coulombic efficiency), external resistance must be minimized. Carbon-carbon electrodes performed better than all other combinations of carbon and copper in the study. The values of bioelectricity recorded in this study though promising, it is still very insignificant to be recommended for useful applications. Therefore, together with further studies of environmental conditions and physicochemical parameters, the performance of more catholytes and electrodes should be undertaken with possibly pure and consortia of exoelectrogens.

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