POTENTIAL EXOELECTROGENIC BACTERIA SPECIES ISOLATED FROM PIGGERY WASTEWATER USED IN GENERATION OF BIOELECTRICITY AND WASTEWATER TREATMENT

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Abstract. The ability of bacteria in anode chambers of microbial fuel cell (MFC) to transfer electrons from their respiratory chains to anode distinguishes it into mediator or mediator-less MFC. Two groups of 3 MFCs each were constructed with either potassium permanganate as electron acceptor, or potassium ferricyanide. Electrodes used were carbon – carbon (CC), carbon – copper (CCu) and copper – copper (CuCu) in each group. The initial BOD and COD of the piggery wastewater were 420mg/L and 1057mg/L respectively. After 25 days, coulombic efficiency recorded were 69%, 84%, 74%, 76%,72% and 5.10%, while COD removal 65%, 51%, 47%, 83%, 48% and 49% for CCP, CCuP, CuCuP, CCF, CCuF and CuCuF respectively. Maximum power density (at R_{ext} = 1000Ω) observed were 79.27mW/m^2, 156.32mW/m^2, 92.29mW/m^2, 60.94mW/m^2, 39.94mW/m^2 and 14.21mW/m^2 for CCP, CCuP, CuCuP, CCF, CCuF and CuCuF respectively. Although *Streptococcus* sp., *Salmonella* sp., *Lactobacillus* sp., *Escherichia coli, Proteus mirabilis, Enterobacter* sp., *Pseudomonas* sp., *Bacillus* sp., *Micrococcus luteus*, *Corynebacterium* sp., *Shigella* sp. *and Aeromonas* sp. were biochemically identified before treatment of wastewater, but *Pseudomonas* sp., *Escherichia coli, Shigella* sp. *and Aeromonas* sp. did not persist after treatment. Molecular analysis confirmed the absence of *Clostridium botulinum, Aeromonas hydrophila, Clostridium butyricum* and *Rhodobacter ferrireducens,* which are known exoelectrogens on the surface of anodes. Plasmid profile revealed that *Lactobacillus* sp., *Proteus mirabilis, Escherichia coli, Pseudomonas* sp., *Bacillus* sp., *and Aeromonas* sp. carried plasmids. Studies should be undertaken using these persistent bacteria in isolation to ascertain their individual capabilities, together with other cheaper, more environmentally friendly catholytes for better outputs.

Keywords: Bacteria, Wastewater, Bioelectricity, Exoelectrogen, Mediator, Piggery, Plasmid.

INTRODUCTION

Gupta, *et al.* [12] and Liu *et al.* [21] defined microbial fuel cells (MFCs) as devices which convert organic waste materials directly into electrical energy by using microorganisms as biocatalysts. The MFC for wastewater treatment is characterized by clean, safe, quiet performance, low emissions, high efficiency, and direct electricity recovery [11]. Typical MFCs comprise of either one or two chambers. Two chamber MFCs have their two chambers connected by a proton exchange membrane (PEM).

In MFCs, electricity is produced by the interplay of aerobic and anaerobic microbes which catalyze organic matter [23]. In the anode chamber of MFCs, fuel (or substrate) is metabolized by bacteria which normally degrade a wide range of substrates and pollutants [12]. When microorganisms consume a substrate such as sugar in aerobic conditions, they produce carbon dioxide and water as shown in equation 1;

$C_6H_{12}O_6 + 6H_2O + 6O_2 \rightarrow 6CO_2 + 12H_2O$ (Equation 1)

However, under anaerobic condition, they produce carbon dioxide, protons and electrons as seen in equation 2 [33].

$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$ (Equation 2)

The electrons generated are deposited on the anode and then transported to cathode by external circuit, while protons are internally transferred to it through the proton exchange membrane. Thus the potential difference is produced between anode and cathode chambers due to dissimilar liquid solutions. Electrons and protons are consumed in the cathode chamber by reducing oxygen, usually from water [12].

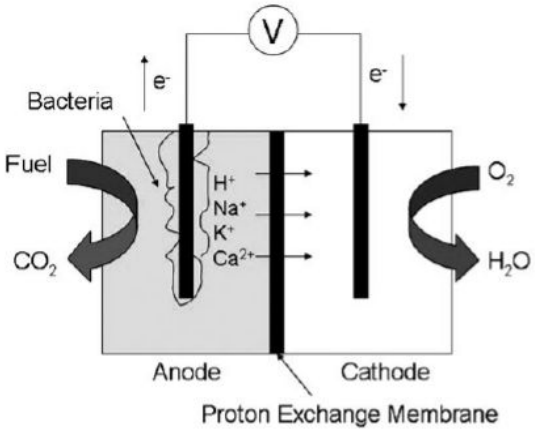

Figure 1. Schematic representation of the principles of a conventional dual chambers MFC [13]

However, in some bacteria, transfer of electrons from their respiratory mechanisms to the anode requires the addition of artificial electron shuttles known as mediators. Mediators are capable of extracting and diverting electrons from the respiratory chain of the bacteria to the anodes. These include *Proteus vulgaris* [40], *Escherichia coli* and *Saccharomyces* sp. [32]. Conversely, others have no need of mediators as the bacteria which transfer the electrons to anodes via c-type cytochromes, biofilms and highly conductive pili (nanowires) [7, 12]. These are termed exoelectrogens and examples include *Shewanella putrefaciens, Aeromonas hydrophila,*

Rhodoferax ferrireducens, Clostridium botulinum, Clostridium butyricum, Desulfotomaculum reducens, Rhodobacter capsulatus and Thiobacillus ferroxidans [5, 25, 36]. *Geobacter metallireducens* [26] *Geobacter sulfurreducens* [2]. According to Logan *et al.* [24], based on existing and new data from individual laboratories, many new types of exoelectrogens or even bacteria capable of interspecies electron transfer (electrons transfer between bacteria in any form) will be discovered.

MATERIALS AND METHODS

Collection of Sample

Using a plastic container surface sterilized as described by Yee *et al.* [43], sample of piggery wastewater was collected following the method of Ikotun *et al.* [14], Singh *et al.* [39]. The samples were collected from some of the drain pipes in a pig farm located at Umualum Nekede, Owerri West Local Government Area, Imo State, Nigeria on coordinates, 5°26'48.5"N 7°01'24.5"E. The collected piggery wastewater sample was immediately transported to the laboratory within 1hour for physicochemical and microbial analyses. As the treatment period of 25 days elapsed, control sample and treated samples were taken from each MFC and analyzed physicochemically. Treated samples for microbiological analysis were aseptically collected by swabbing the surfaces of anodes of each MFC.

Physicochemical Analysis of Samples

The piggery wastewater samples were physicochemically analyzed for pH, electrical conductivity (EC), total dissolved solid (TDS) (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, ammonium, phosphorus (P), phosphate (PO_4^{3-}), ortho-phosphate (P_2O_5), nitrate – nitrogen, nitrate, calcium (using Hanna COD and multiparameter photometer, Model No.: HI83099), chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5).

Microbial Analysis of Piggery Wastewaters

Aliquot from 10^{-6} and 10^{-8} dilutions per sample was inoculated on McConkey Agar, Nutrient Agar, Salmonella-Shigella Agar (SSA) and Saboraud Dextrose Agar (SDA) following tenfold serial dilution. All the media used were prepared as prescribed by their respective manufacturers. The plates were incubated at 37°C, except SDA and SSA plates which were left at room temperatures for 24 – 72 hours after which observation for growth was made. Pure cultures were obtained by sub-culturing each distinct colony on fresh nutrient media. Biochemical tests were used to characterize bacteria isolates and identification was done as described by [4]. Fungal isolates observed on SDA plates were identified macroscopically using lactophenol cotton blue mounts and morphological characteristics.

Construction of Microbial Fuel Cell

The H – type dual chambers MFC described by Jambeck and Damiano [16] was adopted. Six MFC units divided into two groups were constructed using 1000ml capacity plastic containers as the chambers. Salt bridge used as the proton exchange membrane was prepared by dissolving 20g of agar – agar powder into 1000ml 1M solution of KCl. The mixture was boiled for about 3 minutes, poured into 15cm x 3.81cm PVC pipes and then allowed to gel. Carbon and copper rods of surface area $0.0071m^2$ each used as the electrodes were paired thus; copper – copper (CuCu), carbon - copper (CCu) and carbon – carbon (CC) giving the anodes and cathodes of each MFC in each group. Each of the two groups had either 0.1M solution of potassium ferricyanide ($K_3[Fe(CN)_6]$) or 0.1M solution of potassium permanganate ($KMnO_4$) as the catholyte.

Surface sterilized measuring cylinders were used to introduce 800ml of pig wastewater sample into the anode chambers of the MFCs, while 900ml each of the electron acceptors was introduced into either group of MFCs respectively. The chambers were joined by means of salt bridges, tightly corked and external circuits closed by connecting a digital multimeter (DT-830D Series) using 1.5mm copper wires of length 0.4m each. All openings were carefully sealed to prevent leakages. The setups were allowed for 24 hours to stabilize before measurement of voltage generated was read from the multimeters. On each occasion, open circuit voltage (OCV) together voltage across 1000Ω, 500Ω, 200Ω and 100Ω resistors in turn connected in parallel to the digital multimeters were recorded as the readings stabilized. This was repeated on 3 hours intervals from 6.00 am to 6.00 pm and the MFCs were operated for 25 days.

Molecular Characterization of Bacteria Isolates

An attempt to possibly detect the presence of *Clostridium botulinum, Aeromonas hydrophila, Clostridium butyricum and Rhodobacter ferrireducens* in the wastewater and implicate them as been responsible for any bioelectricity generation was carried out. This is due to the fact that they have been known exoelectrogens. This was undertaken using specific primers targeting their 16S rRNA genes in a PCR based assay. Plasmid profile of the isolated microorganisms in the original sample was also undertaken.

a. Extraction of Chromosomal DNA

Total chromosomal DNA of the microorganisms in the samples was extracted using modified boiling method of [34]. This was carried out by dispensing using 1ml each of the broth culture of isolates from treated piggery wastewater samples into pre-sterilized eppendorf tubes and centrifuging at 6000rpm for 4minutes. The supernatant was discarded by decanting and blotting the eppendorf tubes on a paper towel.

Sterilized distilled water (1ml) was added to the eppendorf tubes, vortexed and centrifuged at 6000rpm for 10minutes. The supernatant was again discarded and the tubes blotted. Another 1ml of sterilized distilled water was added, vortexed and centrifuged at 6000rpm for 10minutes. The supernatant was discarded and the tube blotted. Again, 20µl of sterilized distilled water was added and vortexed to homogenize the pellets. The samples were then boiled at 100^{o}C for 10minutes. The tubes were vortexed and then centrifuged at 6000rpm for 10minutes. The supernatants were transferred into another pre-labeled eppendorf tubes by gentle aspiration using a micropipette.

b. Amplification of Target DNA Using PCR

Polymerase chain reaction (PCR) was used to amplify the V3 variable region of the 16S rDNA gene of the bacteria isolates with specific primers for *Clostridium botulinum, Aeromonas hydrophila, Clostridium butyricum and Rhodobacter ferrireducens*. The PCR solution (20µl) contained 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 an initial denaturation step of 95°C for 1min, annealing of primers at 50°C for 30 seconds, and holding temperature of 72°C for 1 min for elongation by Taq polymerase, final holding temperature of 72°C for 4 min. the amplification however was for a period of 30 cycles.

c. Extraction of Plasmid DNA

TENS mini - prep method was adopted as described by Kado and Liu [17], Ojo and Oso [30], Zhou *et al.* [46] to extract plasmid DNA of the isolates in the original wastewater sample.

d. Analysis of PCR Products and Plasmid DNA Using Gel Electrophoresis

Agar gel used was prepared by dissolving 1.5g and 0.08g of agarose powder in 100ml of 1X TBE buffer for PCR product and plasmid DNA respectively. The mixture was dissolved by boiling in a microwave oven. After cooling to about 45^{o}C, 10µl of ethidium bromide was added and gently swirled. It was then poured into the tray in which the comb and stoppers are in place. It was allowed to solidify, combs were carefully removed, and then placed inside electrophoresis tank (EDVOTEK 220V EVT300) filled with 1X TBE buffer. The extracted DNA samples (20µl) were mixed with 2µl of the loading dye and then carefully loaded into the wells with the marker in lane 1. It was run at 75V for 55 minutes and the gels were viewed under UV – transilluminator for presence of bands [28].

RESULTS

Physicochemical Analysis

Following 25 days period of treatment in microbial fuel cells, results of physicochemical parameters of piggery wastewater are as shown in table 1.

Biochemical Identification of Bacteria Isolates

From the results obtained, 10 distinct species of bacteria were identified in the wastewater before treatment as shown in table 2. However, after treatment, anodic surface swap samples from all the MFCs recorded decrease both in the number of colonies formed on various media and diversities of species of bacteria present as shown in table 4. Notably, *Escherichia coli, Pseudomonas* sp.*,* and *Aeromonas* sp. failed to persist after treatment as indicated by their absence from all the samples collected after treatment.

Both Salmonella and Shigella were isolated from the sample before treatment. Nevertheless, Shigella failed to persist in the wastewater after treatment as it

Table 1. Results of physicochemical analysis of samples 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.	Phosphorus (P) (mg/L)	129.2	88.8	70.4	96.4	91.2	87.4	53.6	45.6
8.	Orthophosphate (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.	Biochem. 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

was absent from all the plates cultured with samples from the MFCs studied. Salmonella on the other hand persisted only in some samples though there was decline in the number of colonies recorded as shown on table 3. Fungal analysis using SDA revealed the presence of diverse species of fungi in the wastewater as depicted in table 5. *Aspergillus* sp., *Candida albicans, Trichoderma* sp. and *Cladosporium* were isolated from the sample but *Aspergillus* sp. was most prevalent.

Molecular Based Identification of Microorganisms

Results obtained from the molecular identification of microorganisms in the treated piggery wastewater showed that none of the microorganisms targeted by the specific primers used in this study was present in the sample as shown by the absence of bands on the gels in figure 2.

Plasmid DNA Profile

As shown in figure 3, the formation of bands on agarose gel indicated the presence of plasmids in some

Table 2. Results of biochemical tests used in identification of bacteria present in the sample before treatment

Isolates	Biochemical Test							
	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 Salmonella - Shigella Agar (SSA) culture using piggery wastwater obtained before and after treatment

Sample	Salmonella	Shigella
Before treatment	Growth	Growth
CCP	Growth	No Growth
CCuP	No Growth	No Growth
CuCuP	Growth	No Growth
CCF	No Growth	No Growth
CCuF	No Growth	No Growth
CuCuF	Growth	No Growth

Table 4. Results of biochemical tests used in identification of bacteria present in the piggery wastewater sample after treatment

		Biochemical test							
Samples	No of colonies	Gram stain	Catalase Test	Oxidase test	Methyl Red test	Indole test	Citrate Test	Voges Proskauer Test	Bacterial isolates
CCP	3	+	+	+	-	-	+	+	*Corynebacterium* sp.
		+	+	+	-	-	+	+	*Bacillus* sp.
		+	+	+	-	-	+	+	*Corynebacterium* sp.
CCuP	4	+	-	+	+	-	+	-	*Lactobacillus* sp.
		-	+	-	-	-	+	+	*Enterobacter* sp.
		+	+	+	-	-	+	+	*Bacillus* sp.
		+	+	+	-	-	-	+	*Micrococcus* sp.
CuCuP	2	+	+	+	-	-	-	+	*Micrococcus* sp.
		+	-	-	+	+	-	-	*Lactobacillus* sp.
CCF	3	+	+	-	-	-	+	+	*Bacillus licheniformis*
		+	+	+	-	-	-	+	*Bacillus alvei*
		+	+	+	-	-	+	+	*Bacillus subtilis*
CCuF	3	+	+	+	-	-	-	+	*Micrococcus* sp.
		+	-	+	+	+	-	-	*Streptococcus* sp.
		+	+	+	-	-	+	+	*Bacillus* sp.
CuCuF	3	+	+	+	-	-	+	+	*Bacillus* sp.
		-	+	-	+	-	-	-	*Proteus mirabilis*
		+	+	+	-	-	+	+	*Bacillus subtilis*

Table 5. Result of fungal isolates from piggery wastewater sample obtained before and after treatment

Samples	Number of colonies		Fungal isolates
Before treatment	17	2	*Aspergillus versicolor*
		6	*Candida albicans*
		3	*Aspergillus flavus*
		4	*A. fumigatus*
CCP	8	2	*Aspergillus nidulans*
		3	*Cladosporium*
		5	*Aspergillus nidulans*
CCuP	7	2	*Aspergillus versicolor*
		5	*Aspergillus flavus*
CuCuP	11	7	*Candida albicans*
		4	*Aspergillus versicolor*
CCF	4	4	*A. flavus*
CCuF	9	9	*Aspergillus nidulans*
CuCuF	7	3	*Trichoderma* sp.
		4	*Aspergillus fumigatus*

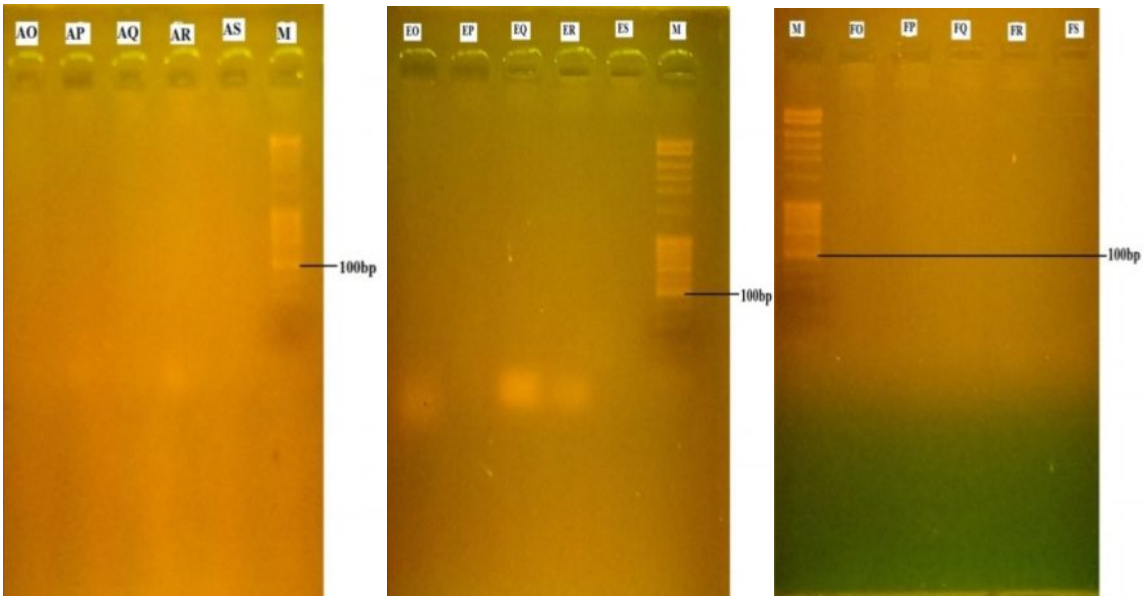

Figure 2. Some agarose gel showing absence of clear bands, which indicates the absence of target microorganisms in the piggery wastewater samples

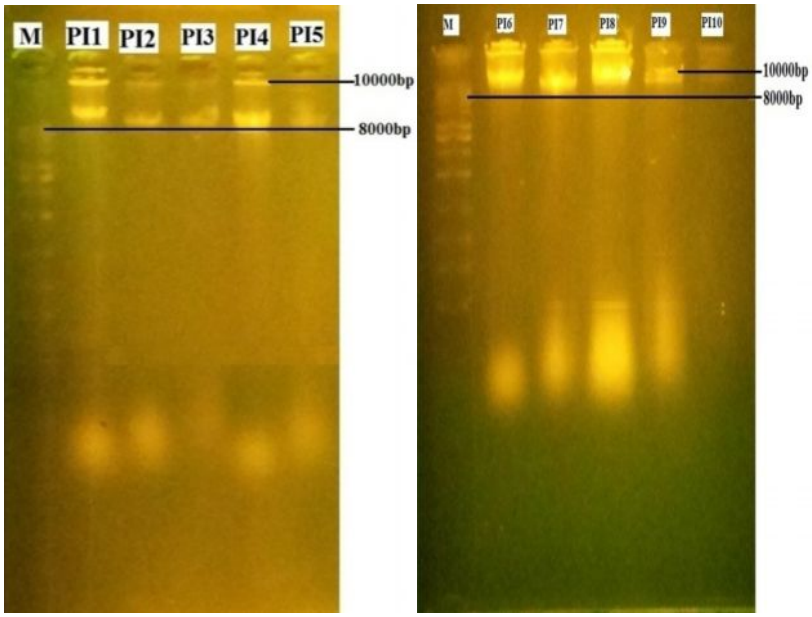

Figure 3. Bands shows plasmids on corresponding isolates

cells including *Lactobacillus* sp., *Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Bacillus* sp. and *Aeromonas* sp.

Wastewater Treatment and Coulombic Efficiency of MFCs

Wastewater treatment capability of the MFCs can be expressed in terms of their COD, and perhaps BOD removal efficiency. The computation was done using the formula from [45],

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

Results showed appreciable removal of both COD and BOD. While 43% - 76% BOD removal was achieved, 47% - 83% COD was removed. Control sample gave only 7% BOD reduction and 32% COD reduction as shown in figure 4.

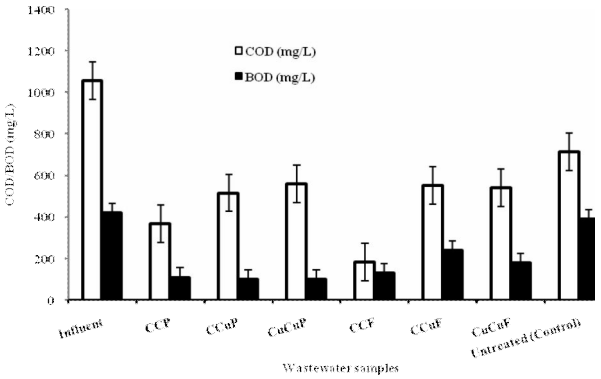

Figure 4. Chemical oxygen demand (COD) and Biochemical oxygen demand (BOD) removal from wastewater samples

Coulombic efficiency is an expression of the number of electrons that are actually recovered from the substrate in the form of electric current in relation to the total amount of electrons theoretically available based on the COD or substrate removed. It was calculated from the equation,

$$CE = \frac{8\int_{0}^{tb} Idt}{Fv_{An}\Delta COD} \quad [27]$$

where 8 is a constant used for COD, based on MO_2 = 32 for the molecular weight of O_2, F = Faraday's constant (96485 C/mol of e-), and V_{An} is the liquid volume in the anode compartment. Using average current (I) obtained when R_{ext} = 100Ω, high coulombic efficiency were calculated for the MFCs as shown in figure 7, except for CuCuF. While CCuP expressed the highest coulombic efficiency of 84%, the least was 5.1% recorded in CuCuF.

Generation of Bioelectricity

CCuP, CuCuP and CCuF yielded their maximum open circuit voltage of 1.2V, 1.34V and 0.93V respectively on day 1 while CuCuF produced 0.63V on day 2 as shown on figure 4. On the other hand, it was 0.97V and 0.75V for CCP and CCF on days 25 and 16 respectively. Unlike others, CCP and CCF recorded more stable and gradual increase in voltage over time. With external resistors in turn connected in parallel to the digital multimeters, there was clear decrease in voltage output which was maintained with reducing external resistance as shown on figure 5.

Power Density

Power density produced by the cells was computed with the equation,

$$P(mW/m^2) = \frac{V^2_{cell}}{R_{ext.} \cdot A} \quad [19]$$

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. Results showed that power density of the MFCs ranged from 0.010mW/m^2 to 156.319mW/m^2 across 1000Ω resistor as shown on figure 6. Generally, it was observed that power density of the microbial fuel cells increased with decreasing external resistance upto the 200Ω resistor beyond which it started decreasing.

DISCUSSION

Microbial Identification

The characteristics of swine waste vary with a number of factors, including the age and diet of the pigs, type of housing or confinement, and waste removal and pre-processing [6]. Ogugbue *et al.* [29] have reported the isolation of *Bacillus, Citrobacter, Pseudomonas, Lactobacillus, Escherichia coli, Aspergillus* and *Rhizopus* from swine wastewater and concluded that these microbes acted as primary and secondary utilizers, utilizing carbon and other organics of the wastewater. Egbadon *et al.* [9] in another study stated that *Corynebacterium* sp., *Bacillus* sp., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus faecalis* were isolated from swine wastewater. These reports lend credence to isolation of *Streptococcus* sp., *Salmonella* sp., *Lactobacillus* sp., *Escherichia coli, Corynebacterium* sp., *Proteus mirabilis, Enterobacter* sp., *Pseudomonas* sp., *Bacillus* sp., *Micrococcus luteus*, *Corynebacterium* sp., *Shigella* sp. *and Aeromonas* sp. in this study. Fungal isolates were *Aspergillus versicolor, Candida albicans, Aspergillus flavus, A. fumigatus, A. nidulans,* and *Trichoderma* sp.

Moreover, *Bacillus* sp., *Corynebacterium* sp., *Staphylococcus* sp., *Enterococcus* sp. and *Micrococcus* sp. have earlier been reported as possible exoelectrogenic bacteria having been isolated from hostel wastewater used in bioelectricity generation [1]. Likewise, *Corynebacterium* sp. [20], *Enterobacter cloacae* [35], *Lactococcus lactis* [10] and *Bacillus megaterium* [3] are all exoelectrogens. *Escherichia coli* [32], *Shigella* sp. *and* some *Pseudomonas* sp. [16, 37], are not exoelectrogens. This may explain why they were not isolated from the sample obtained from the surface of the anode since they could not utilize it as an insoluble electron acceptor. Furthermore, result of the molecular base analysis of the sample obtained from surface of anode indicated the absence of *Aeromonas hydrophila, Clostridium butyricum, Clostridium botulinum and Rhodobacter ferrireducens* which

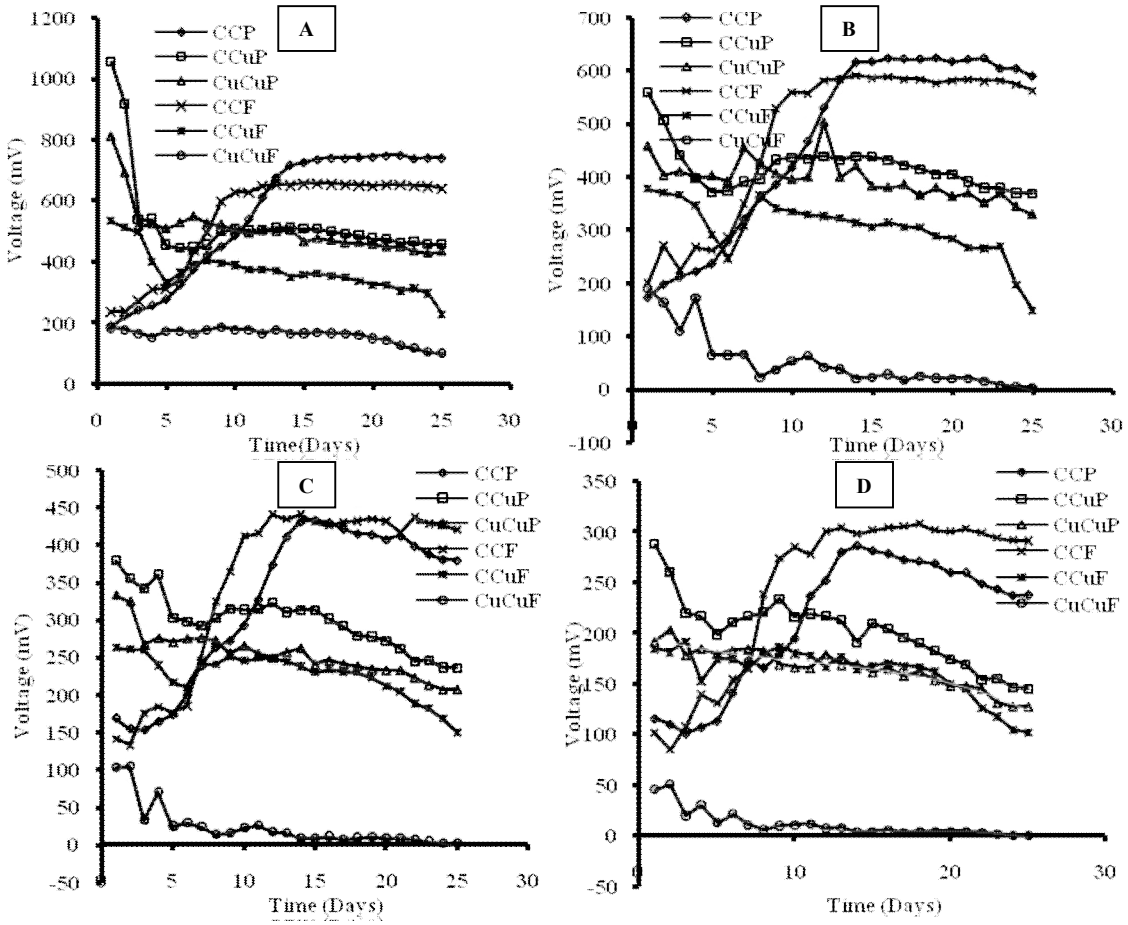

Figure 5. Voltage produced across (a) 1000Ω, (b) 500Ω, (c) 200Ω and (d) 100Ω resistors by different MFCs per time

further gives credence that they were not responsible for the generation of bioelectricity recorded.

Wastewater Treatment

One of the objectives of various wastewater treatment techniques is to reduce the concentration of organic matter before been discharged to the environment. Activated sludge process (ASP) has been the mainstay of wastewater treatment. However, its application is becoming limited due to its high energy intensive process. The emphasis of today's waste management is on reuse and recovery of energy, which has led to new views on how these streams can be dealt with [31]. MFC is considered to be a promising sustainable technology to meet increasing energy needs, especially using wastewaters as substrates, which can generate electricity and accomplish wastewater treatment simultaneously, thus may offset the operational costs of wastewater treatment plant [22]. The significant reduction in BOD and COD contents of the treated wastewater, with respect to the control, further demonstrates and supports the capability 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.

Generally, it was found that the MFCs produced higher COD removal efficiency than BOD removal. The findings above are in line with the results obtained by Ismail and Jaeel [15] who observed COD removal efficiency of 84% and 90%, and BOD removal efficiency of 70% and 82% for MFCs inoculated with activated sludge and *Bacillus subtilis*, respectively. A COD removal in the range of 70–79% has also been reported [41]; 85.92% and 51.74% COD and BOD removal [9]. The rate of COD removal in MFCs is affected by microbial growth, current generation, aerobic growth due to oxygen leaking in through the cathode, and anaerobic growth using other terminal electron acceptors in the wastewater, including carbon dioxide [44]. Furthermore, Ogugbue *et al.* [29] recorded 75% decrease in biochemical oxygen demand and 3.3% reduction of chemical oxygen demand.

The outcome of the study confirms that piggery wastewater does not require biostimulation or addition of mediator to be used in generation of bioelectricity in MFC. This is advantageous because mediated microbial fuel cells tend to be inefficient, expensive, and produce low levels of power [38]. Maximum open circuit voltage of 1.2V, 1.34V, 0.93V, 0.63V, 0.97V and 0.75V as recorded for CCuP, CuCuP, CCuF, CuCuF, CCP and CCF were comparable to 836mV reported by Egbadon *et al.* [9]. However, the voltage is directly proportional to external resistance connected in parallel to the multimeter.

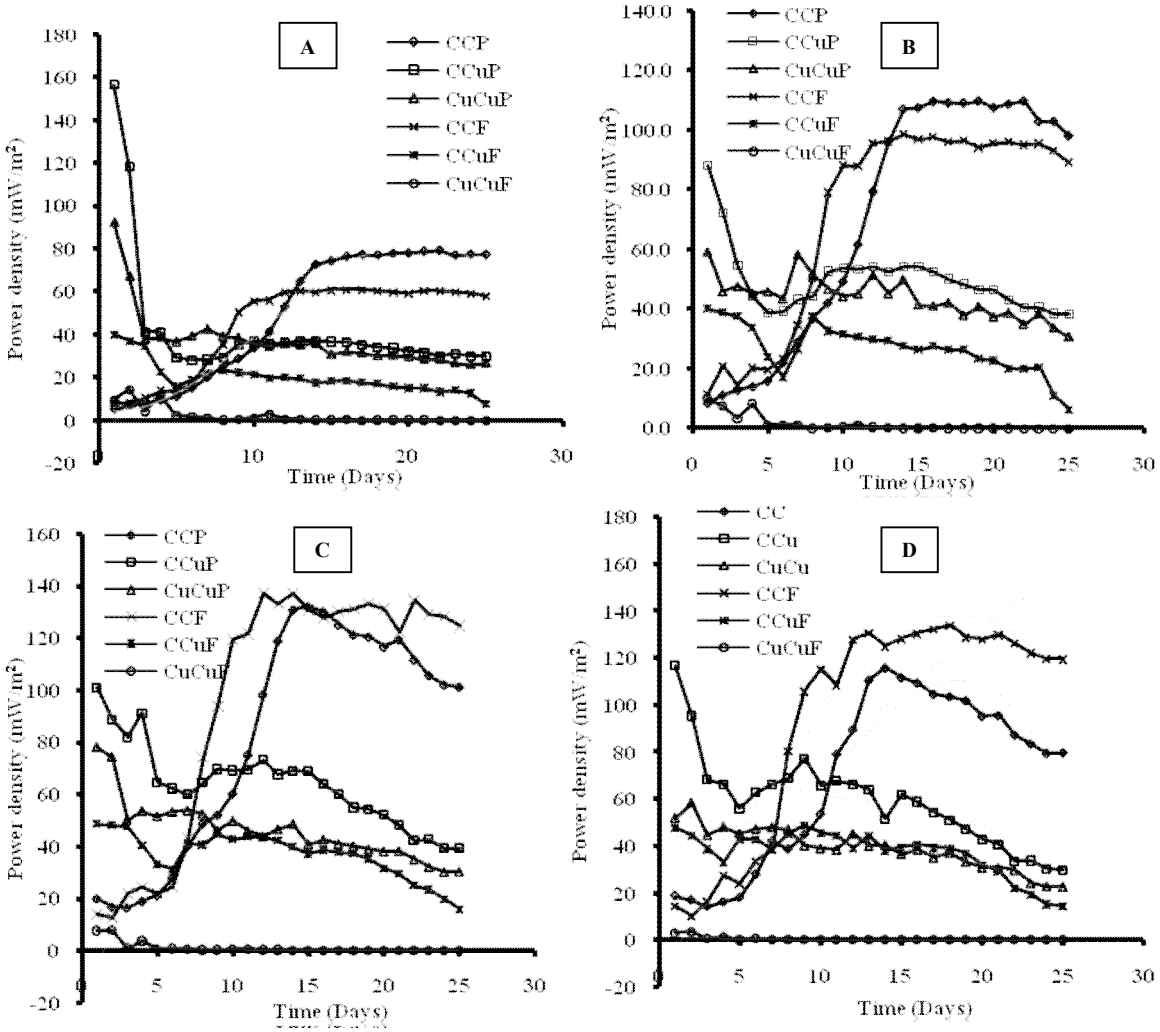

Figure 6. Comparison of power density time graphs for different MFCs across (a) 1000Ω (b) 500Ω (c) 200Ω and (d) 100Ω resistors

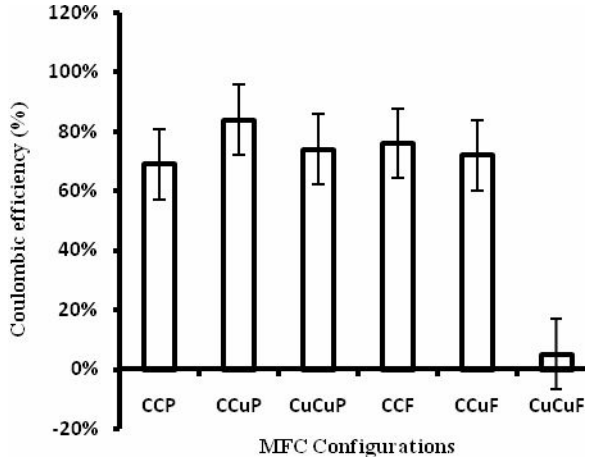

Figure 7. Coulombic efficiency (at R_{ext} = 100Ω) recorded for various MFCs

Devasahayam and Masih [8] reported similar range of 69 – 85% coulombic efficiency in pure culture *E. coli* MFC with sucrose as substrate, while that of river water samples was between 71 – 77%. In line with the findings obtained, Kim *et al.* [18] also observed that the coulombic efficiency obtained from the MFCs increased as the current density increased from higher resistance (1000Ω) to lower resistance (50Ω), indicating that current flow also affects the CEs.

Generation of Bioelectricity

Results of power density measured across 1000Ω resistor, was in the range of 0.010mW/m^2 to 156.319mW/m^2 for the MFCs. This is close to the maximum resultant MFC output power density (181.48mW/m^3) produced using potassium ferricyanide with concentration of 0.1M as the catholyte [42]. Min *et al.*[26] reported that a study conducted using two chambered MFC with continuously aerated cathode demonstrated a maximum power density of 45mW/m^2

($141mA/m^2$) at 1000Ω. The maximum power output obtained using copper and carbon electrodes were 250.54 and 52.33µW, respectively [29].

The outcome of this study confirms that piggery wastewater which has constituted nuisance to the environment around piggeries may become a useful resource, if appropriately utilized using technologies like microbial fuel cell. This is because it contains both the substrates and appropriate exoelectrogenic consortium needed for its decomposition and generation of electrical energy using microbial fuel cell. This is impressive, especially now that many crude oil-producing, developing nations including Nigeria may be diversifying their economic base into other sectors like agriculture, of which pig farming is expected to have its fare share of the revolution. The implication of this would be increased generation of agricultural wastes and wastewaters like piggery wastewater. Moreover, the cost of treating wastewaters is usually very high and may not be affordable to most farmers in developing nations. However, microbial fuel cell provides a very cheaper alternative method of treating wastewaters as well as generating electricity, which has remained a major challenge to the economic and social development of many developing nations, like Nigeria.

Further studies should be carried out using pure cultures of these bacteria reported to establish their capabilities and perhaps eliminate possible antagonistic species, if present. Moreover, studies to ascertain the mode of electron transfer in these identified bacteria should be embarked upon with the aim of enhancing it for better generation of electricity. Genetic studies and modifications of the genes (plasmids) of these organisms may be carried out to increase their wastewater degrading, and electrons transfer potentials through their electrochemically active surface proteins, pili, etc.

REFERENCES

[1] Adeleye, S.A., Okorondu, S.I., (2015): Bioelectricity from students' hostel waste water using microbial fuel cell. International Journal of Biological and Chemical Science, 9(2): 1038-1049.

[2] Bond, D.R., Lovley, D.R., (2003): Electricity production by *Geobacter sulfurreducens* attached to electrodes. Applied Environmental Microbiology, 69: 1548-1555.

[3] Borah, D., Sejal, M., Yadav, R.N.S., (2013): Construction of double chambered microbial fuel cell (MFC) using household materials and *Bacillus megaterium* isolate from tea garden soil. Advancement in Biological Resources, 7(5): 136-140.

[4] Cheesbrough, M., (2006): Biochemical tests to identify bacteria. pp. 62-70. In Cheesbrough M. (ed.): District laboratory practice in tropical countries, Part 2, 2nd Edition. Cambridge University Press, UK.

[5] Clauwert, P., Aelterman, P., Pharm, H.T., DeSchamphelaire, L., Carballa, M., Rabaey, K., Verstraete, W., (2008): Minimizing losses in bio-electrochemical systems: the road to application. Application of Microbial biotechnology, 79: 901.

[6] Day, D.L., Funk, T.L., (1998): Processing manure: physical, chemical and biological treatment. pp. 243-282. In Hatfield, J.L., Stewart, B.A. (eds): Animal waste utilization: effective use of as a soil resource, Ann Arbor Press, Chelsea.

[7] Derek, R.L., (2008): The microbe electric: conversion of organic matter to electricity. Current Opinion in Biotechnology, 19: 564-571.

[8] Devasahayam, M., Masih, S., (2012): Microbial fuel cell demonstrates high coulombic efficiency applicable for water remediation. Indian Journal of Experimental Biology, 50: 430-438.

[9] Egbadon, E.O., Akujobi, C.O., Nweke, C.O., Braide, W., Akaluka, C.K., Adeleye, S., (2016): Simultaneous generation of bioelectricity and treatment of swine wastewater in a microbial fuel cell. International Letters of Natural Sciences, 54: 100-107.

[10] Freguia, S., Rabaey, K., Yuan, Z., Keller, J., (2007): Electron and carbon balances in microbial fuel cells reveal temporary bacterial storage behavior during electricity generation. Environmental Science Technolology, 4(18): 2915-2921.

[11] Guo, Q., Zhao, S., Wang, X., Yue, X., Hou, L., (2010): Electricity generation characteristics of an anaerobic fluidized bed microbial fuel cell. The 13th International Conference on Fluidization - New Paradigm in Fluidization Engineering, Art. 43: 1-8. Retrieved from http://dc.engconfintl.org/fluidization_xiii/43.

[12] Gupta, P., Parkhey, P., Joshi, K., Mahilkar, A., Bhatia, J. K., Meena, L. N., (2012): Comparative study of microbial fuel cell for electricity generation by enriched exoelectron generating bacteria from environmental samples. Asian Journal of Biotechnology, 4(3): 137-142.

[13] He, Z., Angenent, L.T., (2006): Review: Application of bacterial biocathodes in microbial fuel cells. Electroanalysis, 18: 19-20.

[14] Ikotun, O. O., Olafusi, O. S., Quadri, H. A., Bolarinwa, O. A., (2012): Influence of human activities on the water quality of Ogun river in Nigeria. Civil and Environmental Research, 2(9): 36-48.

[15] Ismail, Z.Z., Jaeel, A.J., (2013): Sustainable power generation in continuous flow microbial fuel cell treating actual wastewater: influence of biocatalyst type on electricity production. The Scientific World Journal, 1-7.

[16] Jambeck, J.R., Damiano, L., (2010): Microbial fuel cells in landfill applications. Final report prepared for the Environmental Research and Education Foundation, Alexandria, VA., 1-112.

[17] Kado, C.I., Liu, S.T., (1981): Rapid procedure for detection and isolation of large and small plasmids. Journal of Bacteriology, 145: 1365-1373.

[18] Kim, G.T., Webster, G., Wimpenny, J.W.T., Kim, B.H., Kim, H.J., Weightman, A.J., (2006): Bacterial community structure, compartmentalization and activity in a microbial fuel cell. Journal of Applied Microbiology, 101: 698-710.

[19] Li, Z., Zhang, X., Zeng, Y., Lei., L., (2009): Production by an overflow-type wetted-wall microbial fuel cell. Bioresource Technology, 100: 2551-2555.

[20] Liu, M., Yuan, Y., Zhang, L. X., Zhuang, L., Zhou, S. G., Ni, J. R., (2010): Bioelectricity generation by a Gram-positive *Corynebacterium* sp. strain MFC03 under alkaline condition in microbial fuel cells. Bioresource Technology, 101(6): 1807-1811.

[21] Liu, T., Rao, L., Yuan, Y., Zhuang, L., (2014): Bioelectricity generation in a microbial fuel cell with a self-sustainable photocathode. The Scientific World Journal, pp. 1-8.

[22] Liu, Z., Liu, J., Zhang, S., Su, Z., (2009): Study of operational performance and electrical response on mediator-less microbial fuel cells fed with carbon - and protein-rich substrates. Biochemical Engineering Journal, 45: 185-191.

[23] Logan, B.E., (2009): Exoelectrogenic bacteria that power microbial fuel cells. Nature Reviews Microbiology, 7: 375-381.

[24] Logan, B.E., Hamelers, P., Rozendal, R., Schroder, U., Keller, J., Freuguia, S., Alterman, P., Verstraete, W., Rabaey, K., (2006): Microbial fuel cells: Methodology and technology. Environmental Science and Technology, 40: 5181-5192.

[25] Logan, B.E., Logan, J.M., (2006): Electricity-producing bacterial communities in microbial fuel cells. Trends in Microbiology, 14: 512-518.

[26] Min, B., Cheng, S., Logan, B.E., (2005): Electricity generation using membrane and salt bridge microbial fuel cells. Water Resources, 39: 1675-1686.

[27] Min, B., Logan, B., (2004): Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environmental Science and Technology, 38: 5809-5814.

[28] Ogbulie, T.E., Ezeji, E.U., Nsofor, C.A., (2014): A concise guide to in-vitro recombinant DNA technology: Laboratory manual Unique books, Webs Media Communications LTD Owerri Nigeria, ISBN978-978-52588-5-6, 56 p.

[29] Ogugbue, C.J., Ebode, E.E., Leera, S., (2015): Electricity generation from swine wastewater using microbial fuel cell. Journal of Ecological Engineering, 16(5): 26-33.

[30] Ojo, O.A., Oso, B.A., (2008): Isolation and characterization of synthetic detergent degraders from wastewater. African Journal of Biotechnology, 7(20): 3753-3760.

[31] Pant, D., Bogaert, G.V., Diels, L., Vanbroekhoven, K., (2010): A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresource Technology, 101: 1533-1543.

[32] Potter, M.C., (1911): Electrical effects accompanying the decomposition of organic compounds. Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character, pp. 260-276.

[33] Pranab, K.B., Deka, D., (2010): Electricity generation from biowaste based microbial fuel cells. International Journal of Energy, Information and Communications, 1(1): 77-92.

[34] Sambrook, J., Russell, D.W., (2001): Molecular cloning: A laboratory manual. 3rd Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 483 pp.

[35] Samrot, A.V., Senthikumar, P., Pavankumar, K., Akilandeswari, G.C., Rajalakshmi, N., Dhathathreyan, K.S., (2010): Electricity generation by *Enterobacter cloacae* SU-1 in mediator less microbial fuel cell. International Journal of Hydrogen Energy, 35(15): 7723-7729.

[36] Schamphelaire, L.D., Bossche, L.D., Dang, H.S., Hoftte, M., Boon, N., Rabaey, K., Verstraette, W., (2008): Microbial fuel cells generating electricity from rhizodeposits of rice plants. Environmental Science Technology, 8: 3053-3058.

[37] Sengodon, P., Hays, D.B., (2012): Future fuel technologies. National Petroleum Council (NPC) Study, Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas – 77843. *Microbial fuel cells*, pp. 1 -19. Retrieved from http://www.npc.org/FTF_Topic_papers/13-Microbial_Fuel_Cells.pdf

[38] Seop, C.I., Moon, H., Bretschger, O., Jang, J. K., Park, H.I., Nealson, K.H., Kim, B.H., (2006): Electrochemically active bacteria and mediator-less microbial fuel cells. Journal of Microbiology and Biotechnology, 16(2): 163-177.

[39] Singh, S.N., Srivastav, G., Bhatt, A., (2012): Physicochemical determination of pollutants in wastewater in Dheradun. Current World Environment, 7(1): 133-138.

[40] Thurston, C.F., Bennetto, H.P., Delaney, G.M., Mason, J.R., Roller, S.D., Stirling, J.L., (1985): Glucose metabolism in a microbial fuel cell. Stoichiometry of product formation in a thionine-mediated *Proteus vulgaris* fuel cell and its relation to coulombic yields. Journal of' General Microbiology, 131: 1393-1401.

[41] Wang, X., Feng, Y.J., Lee, H., (2008): Electricity production from beer brewery wastewater using single chamber microbial fuel cell. Water Science and Technology – WST, pp. 1117-1121.

[42] Wei, L., Han, H., Shen, J., (2012): Effects of cathodic electron acceptors and potassium ferricyanide concentrations on the performance of microbial fuel cell. International Journal of Hydrogen Energy, 30: 1-7.

[43] Yee, B.C., Maynard, J.A., Wood, T.K., (1998): Rhizoremediation of trichloroethylene by a recombinant root-colonizing *Pseudomonas fluorecens* strain expressing toluene ortho-monoxigenase constitutively. Applied and Environmental Microbiology, 64(1): 112-118.

[44] Zhang, Y., Min, B., Huang, L., Angelidaki, I., (2011): Electricity generation and microbial community response to substrate changes in microbial fuel cell. Bioresources Technology, 102(2): 1166-1173.

[45] Zhen, H., Shelley, D.M., Angenent, L.T., (2005): Electricity generation from artificial wastewater using an upflow microbial fuel cell. Environmental Science and Technology, 39: 5262-5267.

[46] Zhou, C., Yang, Y., Yong, A.Y., (1990): Mini-prep in ten minutes. Biotechniques, 8(2): 172-173.

List of Abbreviations used

CCP: Carbon-carbon as electrodes and potassium permanganate as electron acceptor.

CCuP: Carbon-copper as electrodes, and potassium permanganate as electron acceptor.

CuCuP: Copper-copper as electrodes, and potassium permanganate as electron acceptor.

CCF: Carbon-carbon as electrodes, and potassium ferricyanide as electron acceptor.

CCuF: Carbon-copper as electrodes, and potassium ferricyanide as electron acceptor.

CuCuF: Copper-copper as electrodes, and potassium ferricyanide as electron acceptor.

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

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 luteus.*

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