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Bioelectricity Generation Using Marine Sediment and Cow Dung

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Abstract

In this study, the aim is to determine the application of marine waste and sediment mixed with cow dung as the substrate for generation of electricity by means of microbial fuel cell technology. The waste water sample and sediments were collected from Abonema water site at Port Harcourt Rivers State. The method employed for isolation, characterization and identification of electrogenic microbes involves the isolation on Nutrient agar, microscopy, biochemical tests (Analytical Profile Index (API) kit) and comparison with Bergey' Manual of Determinative Bacteriology. The electrogenic organisms isolated include: *Pseudomonas aeruginosa, Corynebacterium* sp., and *Lactobacillus casei.* Three (3) MFCs were fabricated with 1L transparent plastic for the cathode and anode chambers containing graphite electrode and PVC pipe salt bridge. The experiment was performed at room temperature with continuous monitoring and readings were taken after 24h of inoculation. The method employed for the multimetric measurement and physicochemical parameters involves voltage, current, power density, temperature, pH, conductivity; total dissolved solid (TDS) and total organic carbon (TOC). The method involves for biofilm formation and development involved coverslip overlay microscopic techniques. The result showed that MFC setup 1 generated the highest voltage and current of 391 mV and 126 mA. The pH and temperature of the MFC setups revealed slight acidicity to neutrality as well as moderate temperatures. There was biofilm development from 24h to 120h. Statistically, there is significant difference ($P < 0.05$) between the test sample and the control in all the parameters measured. This study revealed that the microbial fuel cell fabricated with the substrates above has a higher voltage and current productivity than the positive and negative control.

Keywords: *Bacteria; Bioelectricity; Marine Ecosystem; Microbial Fuel Cell*

Introduction

A microbial fuel cell (MFC) is a bioelectrochemical device that can generate electricity by the use of electrons obtained from the anaerobic oxidation of substrates [1]. It is a system that uses bacteria as biocatalyst to convert biodegradable substrates into electricity. A Technology using microbial fuel cell (MFC) that convert the energy stored in chemical bonds in organic compounds to electrical energy achieved through the catalytic reactions by microorganisms has generated considerable interests among academic researchers in recent years. Microbial fuel cells (MFCs) are a promising technology for electricity production from a variety of materials [2].

Every year the global energy demand increases especially in Nigeria with high population explosion and great problems of her electricity system. Approximately 86% of the world energy production comes from fossil fuels especially petroleum. Coals are being exhausted, leading to an energy crisis in the near future [3]. Furthermore, the combustion of the fossil fuels adds CO_2 to the atmosphere and causes

global warming. Consequently, there is a need to develop a new type of energy source as alternative to fossil fuels [4]. To overcome this energy requirement mankind has been exploring the possibility of alternative sources of energy and has been trying tapping the energy resources of all origin; solar power, nuclear power, water power, wind power, geothermal power, tidal power, wave and ocean currents etc. One particular method of generating power is with the help of fuel cell, which can minimize the usage of fossil fuels [5].

Unlike chemical fuel cell, such as methanol and hydrogen fuel cells, biofuel cells operate under mild reactive conditions, mainly ambient operational temperature and pressure. They also employ neutral electrolyte and use inexpensive catalyst such as copper rods. In Microbial Fuel Cell (MFC) the catalyst is either a microorganism or an enzyme. Biological fuel cell converts the chemical energy of carbohydrates such as sugar and alcohol, indirectly into electrical energy [6]. MFC may be best described as a bioreactor, where microbes act as biocatalyst in metabolizing the organic substances containing the organic carbon to generate electricity [7]. Electrons are produced by the oxidation of organic materials in which microbes act as a catalyst [8]. These types of microbes are called exogenic microbes and can be utilized to generate electricity within an MFC. The advantages of MFC are easily available exogenic materials which are used as substrate and microbes which act as biocatalyst [9].

This MFC mainly consists of two chambers, one of the chambers, where, oxidation takes place is called anodic chamber (anode) and the other chamber where reduction takes place is called cathodic chamber (cathode) [10]. In the presence of oxygen, microbes oxidize organic compounds to produce CO_2 and H_2O, but if the reaction takes place in anaerobic environment then microbes decomposes organic materials to produce CO_2, while proton and electrons are produced simultaneously [11]. Electrons thus produced are transfer to the cathodic chamber via an external circuit while protons are transferred through salt bridge [12]. These flows of electrons generate voltages. Unique design adjustments utilized these years have given huge yields and opened wide in the multidisciplinary MFC research [13].

Cow dung which is usually a dark brown colour (usually combined with soiled bedding and urine) is often used as manure (agricultural fertilizer). In many parts of the developing world, and in the past in mountain regions of Europe, caked and dried cow dung is used as fuel. Dung may also be collected and used to produce biogas to generate electricity and heat. The gas is rich in methane and is used in rural areas of India and Pakistan and elsewhere to provide a renewable and stable source of electricity [14]. To our knowledge, there are dearth of literatures regarding the bioelectricity generation of marine sediment or waste water as well as cow dung in Nigeria and therefore, justifies this study.

Aim of the Study

The aim of this research is to determine the application of waste materials like marine water and sediment mixed with cow dung as a double chambered MFC for electricity generation.

Materials and Methods

Study area

The study sites are Abonema Wharf with latitude 08°56”99” N and longitude 50°47”59” E at an elevation of 15m, Onne Port with latitude 07°7”047” N and longitude 52°40”79” E at an elevation of 18m and Nembe water with latitude 08°37”38” N and longitude 50°68”87” E at an elevation of 11m, all situated in Rivers State, Nigeria.

Sample collection

Ten waste water and sediment samples were collected at different sampling points using a 2 L sterile clean plastic containers and round mouthed plastic containers from Abonema Wharf, Onne Port and Nembe water site, Rivers State, Nigeria. Samples were transported to the laboratory and stored at 4°C until further studies.

Sources of substrates

Cow dungs were aseptically collected using both sterile plastic containers and polyethene bags from cow slaughter house located at Odumodu Market, Umunya in Anambra State. The glucose (JHD, China) were purchased from chemical dealers at Head Bridge Market, Onitsha Anambra State, Nigeria.

Preparation of substrates

Cow dungs of 200g was sun-dried for 3 days until moisture was driven off completely, grinded properly until those large particles where no longer visible and was later stored for usage. Fifty grams of cow dung was measured using a digital weighing balance. Five hundred millilitres of waste water and then 200g of the bottom sediments was also measured. The substrates were all poured into the sterile anode chamber and properly covered. Phosphate buffer with composition: MnCl- 0.005g, K_2HPO_4- 3.4g, KH_2PO_4- 4.4g, KCl- 0.1g, NH_4Cl- 1.5g, NaCl- 2.0g, $MgSO_4$- 0.1g and $MgCl_2$- 0.1g, 1000 mL of distilled water were prepared and then transferred into the sterile cathode chamber.

Materials for MFC construction/fabrication

Electrodes

This was carried out using the modified methods of Adeleye and Okorondu [15] and Singh., *et al* [16]. A total of 6 electrodes graphite rods were purchased from Onitsha main market including a brass mash net. The electrodes were coated with brass to increase their surface area thereby encouraging microbial biofilm formation as well as to increase conductivity. The electrodes were pretreated in 100% ethanol for 30 minutes. After this, the electrodes were washed in 1M hydrochloric acid for 10 minutes to neutralize and to remove possible inorganic contaminants. They were stored in distilled water before use.

Salt bridge preparation

The method of Adeleye and Okorondu [15] was used in the preparation of the salt bridge. A PVC pipe of about $^1/_2$ inch was cut into 8 cm each to make a salt bridge. One side of the PVC pipe was covered properly with masking tape after which 2.2g of Agar-Agar and 3.75g of NaCl was dissolved in 50 mL of distilled water which makes up the content in the salt bridge. The mixture was heated until a homogenous solution was formed. The solution was poured into the PVC pipe and properly covered with their caps after which it was allowed to gel. After some min, the masking tape acting as the cap was removed.

Coupling of double-chambered MFC

Two 1L plastic containers served as the anode and cathode chambers. A hole was made on those plastic containers with the aid of an electric drill on the lid of both chambers to allow the passage of the copper wire and an extra hole on the cathode chamber lid for the aeration of the buffer with the aid of an air pump. Another hole, equal in diameter to the $^1/_2$-inch adopter was made by the sides of the chambers and adopter was attached 6 cm from the base of each chamber. The adopter serves as a point of attachment for the salt bridge which interconnects each chamber, respectively. The edges of the contact between the adopter and the chambers were sealed with araldite to avoid leakages after which the coupled chambers were allowed to air dry as shown below [15].

Figure 1: *Fabricated microbial fuel cell (doubled chambered).*

Composition of MFC

MFC	Anode	Cathode
Set up 1 (test sample)	500 mL of waste water + 200g marine sediments + 50g of cow dung	700 mL of phosphate buffer
Set up 2 (Positive control)	500 mL of waste water + 200g marine sediments + 50g of glucose	700 mL of phosphate buffer
Set up 3 (Negative control)	500 mL of waste water + 200g marine sediments	700 mL of phosphate buffer

Table 1: *Composition of anode and cathode chambers in MFC set up.*

Measurement of physicochemical and electrical properties of the MFC

The physicochemical parameters which includes pH, temperature, total dissolved solid (TDS) and conductivity were measured using a multimeter (DSS - 11A, China) by adopting the standard method of AOAC [17]. The total organic carbon (TOC) was determined using colorimetric method of Nelson and Sommers [18], by titrating blank containing oxidant (potassium chromate) and sulphuric acid as against the sample and the titre value was recorded. The basic electrical quantities such as voltage, current and resistance were also measured using a multimeter.

Isolation of bacteria from the experiment setup

One millilitre of the samples were mixed with saline water (0.85% w/v NaCl) and serially diluted into five test tubes labelled dilution 10^{-1} to 10^{-5}, respectively. From the 10^{-5} dilution, 0.1 mL was aseptically pipetted and spread on the surface of sterile Nutrient agar contained in Petri dishes using a sterile spreader and incubated by inversion at 37°C for 24h at room temperature. Morphologically distinct bacterial colonies were counted, expressed in CFU/mL, purified and stored for future use for identification and also to serve as biocatalyst for the microbial fuel cell [19].

Characterization and identification of the bacterial electrogens

Colonial morphology

The colour, shape, elevation, and other typical features of the colonies were observed as described by Willey., *et al* [20].

Microscopic characterization

The method described by Cheesbrough [21] was employed for the Gram staining and spore staining of all the bacterial electrogens.

Biochemical characterization

The method described by Cheesbrough [21] and as described in the manual instructions of Analytical Profile Index (API) kit was employed for the biochemical characterization of all the bacterial electrogens.

Biofilm formation and development

A clean grease free sterile cover slip of about 18 mm was provided together with well sterilized forceps. The forceps were used to hold the tip of the cover slip firmly and then aseptically dipped into the anode chamber to check for the biofilm formation for 5 days. The cover slips were recovered from the chambers, washed thoroughly in 1% saline solution aseptically, air-dried and Gram-stained. Formation of

biofilm was viewed under 100X oil immersion objective using microscope (Life Assurance, 153H UK). The formation of biofilm on thin glass cover slips was also studied for hourly development of film by the bacterial electrogens and stained at different time intervals of growth at 24th, 48th, 72nd, 96th and 120th h [22].

Statistical analysis

The statistical analysis was carried out on the experimental study using Graph Pad Prism statistical software USA, version 7.00. Two-way ordinary analysis of variance (ANOVA) was used to check the significance of the control and substrates used. Probability values less than 0.05 were considered significant.

Results and Discussion

Microbial fuel cells (MFC) have recently received increasing attention due to their promising potential in sustainable wastewater treatment and contaminant removal. In Nigeria three major issues our country faces include the fuel crisis, waste disposal crisis and power (electricity) crisis. Waste generated was obtained from both waste water and sediments which consist of different populations of microorganism in microbial fuel cell to support the generation of electricity. In this study, the result of the physico-chemical parameters of the MFCs setups are presented in table 2-4. From the results, MFC set up 2 (positive control) had highest conductivity value of 2.28 µS/cm, MFC set up 3 (Negative control) had the highest TOC, SDE and microbial count values of 6.21%, 79.07% and 8.38 CFU/mL while MFC set up 1 had the highest TDS value of 1243.33 mg/L. There were acidic to slight neutral pH (3.90 - 7.79) as well as moderate temperatures range (27.56 - 32.23°C) after 120h of monitoring. The extraordinarily low or high pH values could likely limit MFCs' performance in aspects of power generation [23,24]. The addition of phosphate buffer solution to the MFC set ups in this study shielded the negative impacts that could have arisen due to variations in pH and similar observation was reported by Wang., *et al* [25]. The fact that set up 2 had the highest conductivity value showed that it contained higher ionic and salty contents. Set up 3 had the highest chemical oxidation of the chemical compound in it leading to higher substrate degradation efficiency and more microbial count. Wang., *et al*. [26] reported that a substrate degradation efficiency (SDE) of 75.90% was achieved with 48.70% attributed to the anaerobic process and 27.20% to the aerobic process when generating electricity with waste water and almost comparable to the finding in this study. Previous microbial cultures in minimum and enriched mediums with glucose, starch and protein suggest the role of microorganisms cultured under aerobic condition into the bioelectrogenic process [19]. Inside MFCs positive correlations were not always observed between the abundance of microbes and electricity generation. The predominance of electrogenic microorganisms is not a guarantee of high performance of MFCs because minor members in the community could contribute to the current production [27] and similar feature was observed in this study.

Time (h)	CD (µS/cm)	pH	Temp (°C)	SDE (%)	TOC (%)	MC (logCFU/mL)	TDS (mg/L)
24	16.51	7.05	31.00	47.20	8.79	7.23	17066.66
48	14.93	6.67	30.03	79.25	6.12	8.31	8713.33
72	16.02	6.88	28.60	80.29	6.66	8.12	15336.33
96	4.54	7.26	29.30	79.75	7.26	8.43	3083.33
120	1.35	7.97	30.93	74.13	4.44	8.02	1243.33

Table 2: *Physico-chemical parameters of the MFCs setup 1.*
Legend: CD: Conductivity; Temp.: Temperature; SDE: Substrate Degradation Rate; TOC: Total Organic Carbon; MC: Microbial Count; TDS: Total Dissolved Solid.

Time (h)	CD (µS/cm)	pH	Temp (°C)	SDE (%)	TOC (%)	MC (logCFU/mL)	TDS (mg/L)
24	17.36	5.29	29.20	16.47	4.92	7.87	13403.33
48	19.10	5.37	29.96	66.58	8.49	7.69	19900.00
72	17.61	4.19	30.30	67.77	7.80	8.00	13405.33
96	5.70	3.90	32.23	67.68	5.34	8.03	3083.33
120	2.28	4.89	31.00	63.91	2.67	8.36	1140.00

Table 3: *Physico-chemical parameters of the MFCs setup 2.*
Legend: CD: Conductivity; Temp.: Temperature; SDE: Substrate Degradation Rate; TOC: Total Organic Carbon; MC: Microbial Count; TDS: Total Dissolved Solid.

Time (h)	CD (µS/cm)	pH	Temp (°C)	SDE (%)	TOC (%)	MC (logCFU/mL)	TDS (mg/L)
24	14.56	6.84	30.70	52.53	3.63	7.43	13460.00
48	13.88	6.47	30.30	80.82	8.46	7.86	12413.33
72	7.29	6.76	27.56	81.83	8.46	7.91	6563.33
96	3.98	7.15	29.96	81.18	7.68	8.14	1946.66
120	1.26	7.52	31.30	79.07	6.21	8.38	1176.66

Table 4: *Physico-chemical parameters of the MFCs setup 3.*
Legend: CD: Conductivity; Temp.: Temperature; SDE: Substrate Degradation Rate; TOC: Total Organic Carbon; MC: Microbial Count; TDS: Total Dissolved Solid.

The results of the electrical parameters in MFC set ups is presented in table 5-7 while the results of the voltage and current against time intervals for the three aerated chambers are shown in figure 2 and 3. From the results, MFC setup 1 generated the highest voltage, current and power density of 391 mV, 126 mA and 2413.92 mW/cm^2 with the lowest resistance of 3.10 Ω. Wang., *et al.* [26] reported that the MFC fed with a continuous flow of 2 g/day acetate produced a power density of 30 mW/m^2 and current density of 245 mA/m. In line with findings of Logroño., *et al.* [19], the highest voltage output was found in samples with organic wastes. However, there were an inverse correlation between the voltage generated and CFU/g soil into the SMFCs and the report of these authors is similar to our finding because set up 1 with the highest voltage output had the lowest microbial count out the three MFC set ups. The result in figure 2 and 3 showed that there is a positive correlation between voltage output and current in MFC setups and is in line with previous research of Wang., *et al* [26].

Time (h)	V (mV)	C (mA)	R (Ω)	P.D. (mW/cm^2)
24	161	65	2.48	511.60
48	215	84	2.56	883.83
72	252	92	2.74	1134.44
96	269	122	2.20	1609.95
120	391	126	3.10	2413.92

Table 5: *Electrical parameters in MFC set up 1.*
Legend: V: Voltage; C: Current; R: Resistance; PD: Power Density.

Time (h)	V (mV)	C (mA)	R (Ω)	P.D. (mW/cm²)
24	216	70	3.09	739.06
48	235	75	3.13	863.62
72	267	76	3.51	994.14
96	330	82	4.02	1325.97
120	368	99	3.72	1781.90

Table 6: *Electrical parameters in MFC set up 2.*

Legend: V: Voltage; C: Current; R: Resistance; PD: Power Density.

Time (h)	V (mV)	C (mA)	R (Ω)	P.D. (mW/cm²)
24	248	34	7.29	412.96
48	297	35	8.49	508.55
72	325	36	9.03	572.55
96	334	43	7.76	703.66
120	365	44	8.30	785.67

Table 7: *Electrical parameters in MFC set up 3.*

Legend: V: Voltage; C: Current; R: Resistance; PD: Power Density.

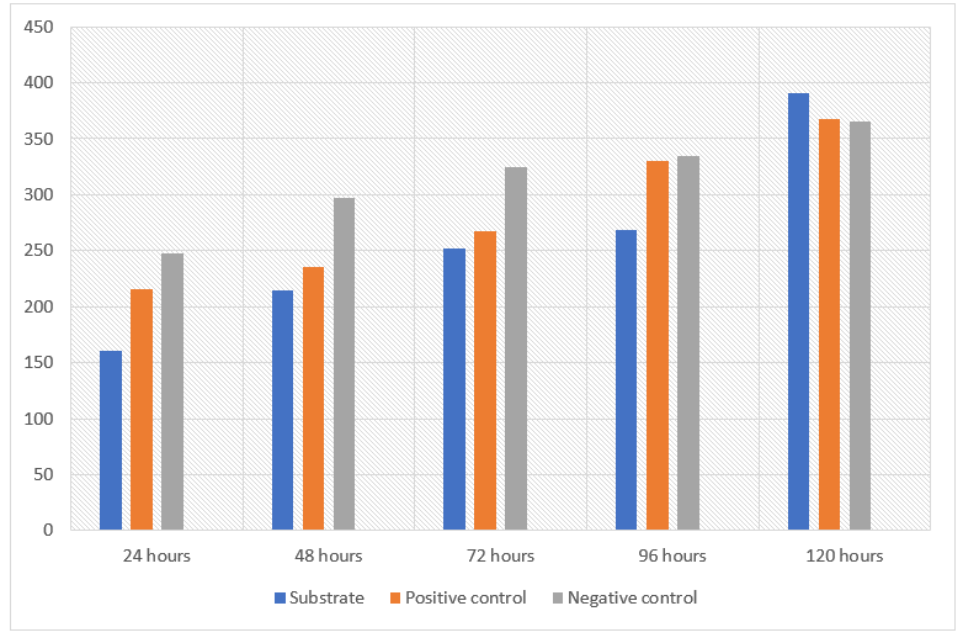

Figure 2: *Voltage against time interval for the three aerated chambers.*

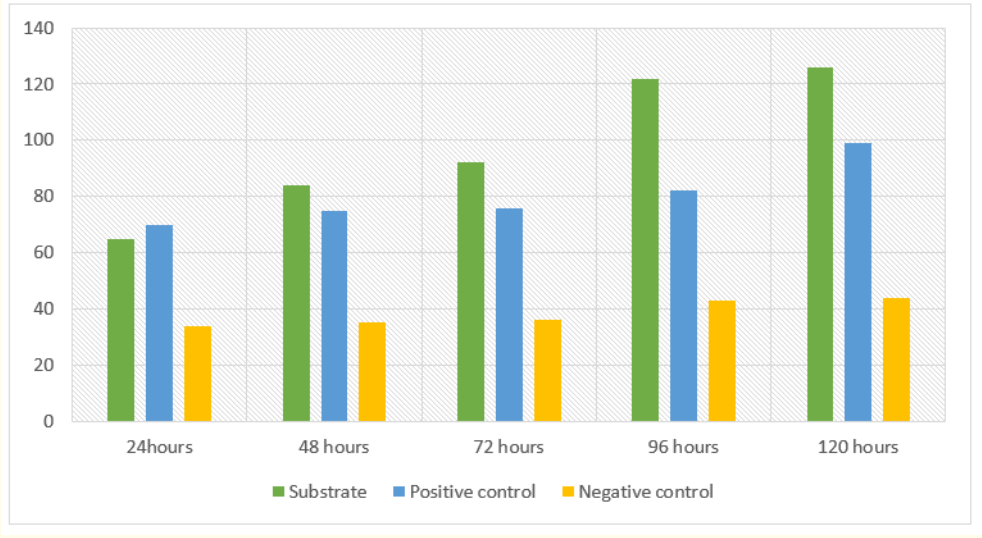

Figure 3: *Current against time interval for the three aerated chambers.*

The results of the morphological characteristics of the bacterial electrogens is presented in table 8. From the result, isolate A had a bluish - greenish colouration; short rods, motile and non-spore former. Isolate B had a white/milky coloration, long rods, non-motile and non-spore former and isolate C had a white coloration, long rods (single), non-motile and non-spore spore-former. The results of the biochemical properties of the bacterial electrogens is presented in table 9. From the results, most of the isolates were positive to catalase test, oxidase test, o-nitrophenyl-B-D-galactopyranoside (ONPG), arginine dehydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), citrate, urease, tryptophan deaminase (TDA), indole, gelatin (GEL), glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), saccharose (SAC), nitrogen gas (N_2), amigdalina (AMY), melbiose (MEL) but negative to hydrogen sulphide (H_2S) test, Voges-Proskauer (VP), nitrite (NO_2) and arabinose (ARA) tests. The bacteria species B and C were Gram-positive bacilli and non-motile except for isolate A which was Gram-negative bacilli and motile. They were identified using API identification key as *Pseudomonas* sp., *Lactobacillus casei* and *Corynebacterium* sp. The myriad of electrogens isolated and identified with different morphological features in this study revealed the biodiversity of these electrogens in the marine ecosystem. Similar observation was reported by Logroño., *et al.* [19] that the morphological differences between colonies such as different color, form and edge were suggestive of the diversity of microbial populations.

Morphology	**Bacterial isolate**		
	A	**B**	**C**
Gram staining	-Ve	+Ve	+Ve
Colony color	Greenish	White/milky	White
Colony morphology	Smooth	Smooth	Slightly curved
Structure	Short rods	Long rods	Long rods (single)
Motility	Motile	Non-Motile	Non-Motile
Spore	Non - sporing	Non - sporing	Non - sporing
Identity	*Pseudomonas aeruginosa*	*Lactobacillus casei*	*Corynebacterium* sp.

Table 8: *Morphological characteristics of the bacterial electrogens.*

Biochemical reaction	Bacterial isolate		
	A	B	C
Catalase	+VE	+VE	+VE
Oxidase	+VE	-VE	+VE
ONPG	+VE	+VE	+VE
ADH	+VE	+VE	+VE
LDC	+VE	+VE	+VE
ODC	+VE	+VE	+VE
CIT	+VE	+VE	+VE
H_2S	-VE	-VE	-VE
Urease	+VE	+VE	+VE
TDA	+VE	+VE	+VE
Indole	+VE	+VE	+VE
Voges Proskauer	-VE	-VE	-VE
GEL	-VE	+VE	+VE
GLU	+VE	+VE	+VE
MAN	+/- VE	+VE	+VE
INO	+VE	+VE	+VE
SOR	+/- VE	+VE	+VE
RHA	+VE	+VE	+VE
SAC	+VE	+VE	+VE
N_2	+VE	+VE	+VE
NO_2	-VE	-VE	-VE
ARA	-VE	-VE	-VE
AMY	+VE	+VE	+VE
MEL	+VE	+VE	+VE

Table 9: *Biochemical properties of the bacterial electrogens.*
Legend: +VE: Positive; - VE: Negative; +/- VE: Positive or Negative.

The results of the biofilm development from 24h to 144h by the bacterial electrogens are shown in figure 4A-4F. From the figures, the initial event of bacterial electrogens adhesion was found in the first twenth four hour of growth (Figure 4A). In the next 48 - 72h, the multicellular aggregation emerged on the glass cover slip and release from the substratum (Figure 4B and 4C). At around 96 - 144h of growth, multicellular aggregation become more mature and form cell matrix (Figure 4D-4F). Moreso, biofilm formation trails a dynamic cycle of adhesion and release from its substratum during its nourishment on substrates as observed by Gram staining at different time intervals. Moreover, there is a wealth of proof supportive of the fact that biofilm development and maturation follow a cycle of attachment and release. Establishment of biofilm on gravel particles and glass slides was reported by Dasgupta., *et al.* [22] who stated that the enhancement of degradation is profound in all oil degrading naturally exiting strains with various degrees of biofilm forming capacity. Similar phenomenal was observed in our study although different substrates were used.

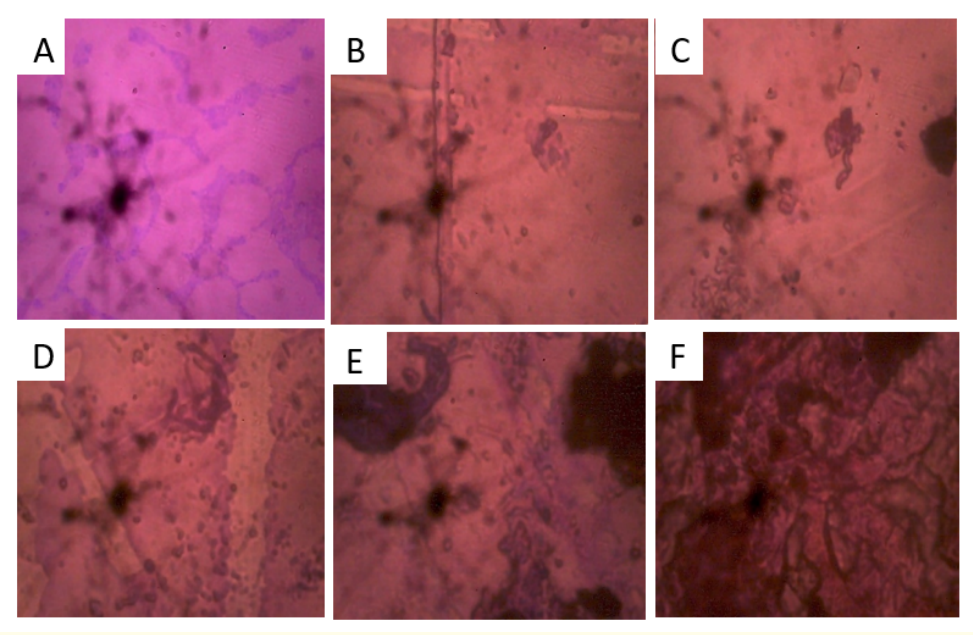

Figure 4A-4F: *Biofilm development from 24 h to 144 h by the bacterial electrogens.*
Legend: Figure 4A: 24h biofilm; Figure 4B: 48h biofilm; Figure 4C: 72h biofilm; Figure 4D: 96h biofilm; Figure 4E: 120h biofilm; Figure 4F: 144h biofilm.

Conclusion

This study revealed that the microbial fuel cell set up 1 had a higher output of voltage, current and power density than the MFC set up 2 and MFC set up 3. The bacterial electrogens present in the anodic chambers as evidenced with biofilm development influenced the bioelectricity production. Hence, in order to obtain a higher productivity of voltage, current and power density, further research on higher concentration of substrates and media is therefore recommended.

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Conflict of Interests

The authors hereby declare no conflict of interests exist.

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