ISSN No:-2456-2165

Electricity Generation From Petroleum Contaminated Wetland Sediment Obtained from Ekerekana-Ama Creek in Rivers State.

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Abstract:- This research study was designed to generate electrical power through a biolelectrochemical system: microbial fuel cell (MFC) from petroleum contaminated wetland sediment obtained from Ekerekana-Ama Creek, Okrika, in Rivers State. Sediment Microbial Fuel Cells (SMFCs) consisting of an anode embedded in the anaerobic sediment containing petroleum hydrocarbon contaminants and a cathode suspended in the overlying aerobic water were used. Seven 300 ml sterile transparent bottles labelled T_1 to T_7 subjected to different treatments were used for this electrical power generation study. Digital multimeter was used to measure the voltage and current of electricity generated. It was observed that voltage of electricity generated increased for some weeks and finally fall which is as an indication that organic substrates depletion was caused by electrochemically active bacteria (EAB). Maximum power output generated from T_1, T_2, T_4, T_5 and T_6 were 0.95 mW, 306.50 mW, 0.18 mW, 126.60 mW and 0.49 mW respectively. Methylene blue (100 µM) aided electron transfer by microbes than neutral red (100 µM) while NPK 15-15-15 did not significantly improve electricity generation as observed in this study Distinct bacteria isolated were characterized biochemically and molecularly. The identity of the bacteria nucleotide sequences (genus to species) as shown by Basic Local Alignment Search Tool (BLAST) identified *Clostridium sporogenes* (MF623797), *Desulfobulbus propionicus* (MF623798), *Ewingella Americana* (MF623799), *Bacillus amyloliquefaciens* (MF623800), *Helicobacter sp.* (MF623801), *Alcaligenes faecalis* (MF623802), *Clostridium botulinum*, *Bacillus subtilis* (MF623803), *Klebsiella oxytoca* (MF623804), and *Burkholderia cepacia* (MF623805) associated with T_1 to T_7. These isolated Bioelectrochemically Active Bacteria (BEA) interplayed metabolically to bring about electrical power generation in this study. Installation of biolectrochemical devices in water associated with Niger Delta, Nigeria where oily activities are predominant will go a long way in remediating such environment and concurrent generation of electricity for sustainable development is attainable.

I. INTRODUCTION

A more ecofriendly alternate sustainable electrical power becomes necessary as the world demand for energy increases continually. Microbial fuel system is a less energy input technology that brings about anaerobic oxidation of reduced petroleum hydrocarbons in the waterlogged soils or sediments leading to electricity generation.

Microbial Fuel cell (MFC), a bioeletrochemical system consists of anodic electrode which pulls out liberated electron during anaerobic oxidation of organic pollutants in sediment into air-cathode electrode where reduction reaction occurs leading to bioremediation and simultaneous generation of electrical power.

The cost of aeration in order to supply electron acceptors for bioremediation is not sustainable. Alternative electron acceptors such as Iron III oxides, sulfates, nitrates into contaminated sediments can stimulate anaerobic oxidation of hydrocarbons but the use of these electron acceptors can be reduced under anoxic and it is not sustainable. Thus, a need for a system with sustainable electron acceptors becomes necessary.

In sediment microbial fuel cells (SMFCs) otherwise known as benthic microbial fuel cell (BMFC); microorganisms are used as biocatalysts to oxidize biodegradable organic substrates such as petroleum hydrocarbon in the sediment and transfer electrons to the anode embedded in or rested on top of the sediment, and then the electrons are transferred to the cathode suspended in the overlying seawater, where electron and proton chemically combine with dissolved oxygen in a reductive reaction leading to production of water (Donovan *et al*., 2011).

In comparing sediment microbial fuel SMFC with other types of microbial fuel cells, Sediment Microbial Fuel Cell system is one of the membranes-less bioelectrochemical systems designed to eliminate pH imbalance.

ISSN No:-2456-2165

A Proton Exchange Membrane (PEM) has slow proton transfer capacity and could result in a rapid accumulation of acidity in the anode, which can decrease the activity of exoelectrogens (Harnisch *et al*., 2008). Thus, omitting membrane from MFC is an effective way to balance pH in the anode and cathode (Liu and Logan, 2004).

Sediment microbial fuel cell can be employed for sediment as power sources for fresh water or marine studies (Donovan *et al.*, 2008).

II. MATERIALS AND METHODS

➢ *Sample collection:*

Petroleum hydrocarbon contaminated Sediment and sea water used for laboratory investigations were collected from Ekerekana-Ama creek in Rivers State of the Niger Delta where oily activities are predominant. Samples were collected randomly with a mini-shipek grab sampler at a depth of 3 cm from subsurface sediment and sea water into sterile bottle from 0-3 cm surface. Random Samples were homogenized for even distribution of contaminants and to ensure representativeness of the sample area and thereafter transported to the laboratory at 4 °C in ice pack.

Fig. 1.0: A pictorial view of Ekerekana-Ama creek where oily activities are predominant

Parameters	Values
pH	6.8
Temperature	25 °C
Conductivity	3742
Sulphate	104
Phosphate	0.18
Nitrate	0.7
Moisture content	26 %
TOC	0.97 %
TOM	2.88 %
Cd	2.127 mg/kg
Pb	3.848 mg/kg
TPH	272.62 ppm

Table 1.0: **Baseline features of Sediment for bioeletrochemical study**

TOC=Total Organic Carbon, TOM=Total Organic Matter, TPH= Total Petroleum Hydrocarbon

➢ *Sediment Microbial Fuel Construction*

Sediment microbial fuel cells as shown in Fig. 2 were constructed. Seven (7) sterile transparent empty bottles (350 ml, 265 g) were used and labeled T1 to T7 for set up which were subjected to different treatment.

Each bottle was filled with petroleum hydrocarbon contaminated sediment weighing 1000 g from the sample area occupying about 6 cm height of the bottle.

Ten (10) graphite electrodes obtained from batteries were used as anodes and cathodes and copper wires were also used as conductors in the construction of sediment microbial fuel cell according to Hayat *et al.* (2014).

The copper wires and the electrodes were sanitized with 99 % alcohol so as to minimize or eliminate contamination. Each bottle was filled with 150 ml of sea water (salty) from the sample area which served as the aerobic layer of the microbial fuel cell (MFC) while the sediment serves as the anaerobic layer.

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The anode and cathode electrodes to which sanitized copper wire had been connected were introduced into the bottles. The anode was buried into the sediment containing the petroleum hydrocarbon while the cathode electrode was made to stay afloat of the sea water in the aerobic layer.

The copper wires from the anode and cathode electrode were connected to Digital Multimeter which helped to measure the voltage and current produced by the sediment microbial fuel cell (SMFC) continuously.

➢ *Experimental Setup*

The experiment was conducted and observed for 80 days so as to know the effects of contaminated petroleum hydrocarbon sample, mediators, and NPK-15-15-15 on microbial fuel cells performance. Seven (7) sterile transparent empty bottles (350 ml, 265 g) were used labeled T1 to T7 for set up. T1 was sterilized before installation of MFC and 100 µM of mediators (methylene blue and neutral red) were used according to Taskan *et al.* (2014) for T4 and T5 respectively and 5 g of sterile N-P-K 15-15-15 fertilizer as biostimulant was used for 1000 g sediment in the bottle.

The Treatment for the samples could be summarized as follow:

T1 = sterile (sediment + seawater) + MFC
T2 = Sediment + Seawater + MFC
T3 = Sediment + Sea water
T4 = Sediment + Seawater + Mediator (Neutral Red) + MFC
T5 = Sediment + Seawater + Mediator (Methylene Blue) + MFC
T6 = Sediment + Seawater + NPK + MFC
T7 = Sediment + Seawater + NPK

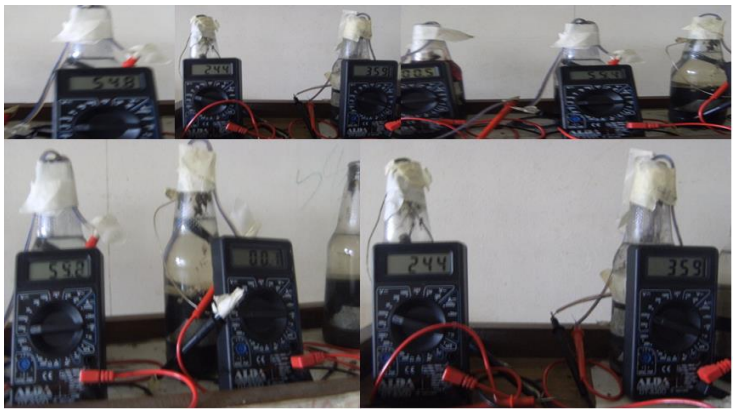

Fig. 2:- Functioning Sediment Microbial Fuel Cells in a Bioelectrochemical system showing voltage of electricity being generated from electron donors in the sediment for investigation.

III. MICROBIOLOGICAL ANALYSES

➢ *Enumeration of Total Culturable Heterotrophic Bacteria (TCHB) (aerobes and anaerobes)*

Plate count agar (PCA) was prepared according to the specification of manufacturer and sterilized by steam under pressure (i.e. autoclaved). Spread plate technique was used on plate count agar (PCA) to culture the bacteria as previously described by Pelzar *et al,* (2004). 0.1 ml aliquots of appropriate dilutions were spread on duplicates of sterile PCA plates the inoculated plates were incubated for period of 18-24 hours in the incubator at $28^0C \pm 2^0C$ to observe colonial formation. In the same way but with little modification and extra carefulness; isolation of total heterotrophic culturable anaerobic bacteria was done by using Gaspak to create anaerobic condition for the bacteria during incubation.

Colonies formed during the incubation period were counted using colony counter. Colonies on plates within the range of 30-300 were recognized. The colony forming unit per gram (cfu/g) was calculated with the relation:

$$\text{cfu/g} = \frac{\text{No of colonies}}{\text{Volume of inoculum used}} \times \text{dilution factor}$$

➢ *Enumeration of Total Culturable Hydrocarbon Utilizing Bacteria (aerobes and anaerobes)*

Bushnell-Haas Agar (Sigma-Aldrich, USA) was prepared according to the specification of the manufacturer, sterilized and used in vapour-phase technique as previously reported by Hamamura *et al.* (2006). Hydrocarbons were supplied through the vapour phase to putative hydrocarbon utilizers by placing sterile Whatman No.1 filter papers impregnated with 5 ml sterile crude oil on the lids of the inverted plates and incubated for 7 days at 30 °C.

In the same way, anaerobic bacteria that are capable of utilizing petroleum hydrocarbon were isolated with sterile BHA with inclusion of Gaspak during incubation so as to create anaerobic condition. Colonies of the organisms were counted as usual.

Moreover, Post gate agar was formulated as previously reported by Wargin *et al.* (2007) with exclusion of sodium lactate and used in vapour phase as in BHA to screen for sulphur reducing bacteria that are bioeletrochemically active and possessing the potentiality of petroleum hydrocarbon utilization leading to electrical power production.

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IV. RESULTS AND DISCUSSION

DAY		T1	POWER		T2	POWER		T4	POWER		T5	POWER		T6	POWER
	V(Mv)	I(Ma)	(Mw)	V(Mv)	I(Ma)	(Mw)	V(Mv)	I(Ma)	(Mw)	V(Mv)	I(Ma)	(Mw)	V(Mv)	I(Ma)	(Mw)
1	0.00	0.00	0.00	46.85	0.03	1.41	0.00	0.00	0.00	11.89	0.01	0.12	1.25	0.01	0.01
2	0.00	0.00	0.00	58.38	0.03	1.75	0.10	0.00	0.00	12.81	0.01	0.13	1.34	0.01	0.01
3	0.00	0.00	0.00	64.86	0.03	2.16	0.21	0.01	0.00	13.89	0.01	0.14	1.70	0.01	0.02
4	0.85	0.01	0.01	72.52	0.03	2.42	0.30	0.01	0.00	14.74	0.01	0.15	1.92	0.01	0.02
5	2.13	0.01	0.02	86.63	0.04	3.18	0.43	0.01	0.00	15.86	0.01	0.16	1.96	0.01	0.02
6	2.45	0.01	0.02	113.71	0.04	4.55	0.62	0.01	0.01	17.04	0.01	0.17	2.67	0.01	0.03
7	2.95	0.01	0.03	128.56	0.04	5.14	0.80	0.01	0.01	30.00	0.02	0.60	2.96	0.01	0.03
8	3.52	0.01	0.04	143.60	0.09	12.92	2.83	0.01	0.03	38.37	0.02	0.77	2.90	0.01	0.03
9	4.52	0.01	0.05	161.26	0.13	20.43	5.88	0.01	0.06	35.86	0.02	0.72	2.77	0.01	0.03
10	6.44	0.01	0.06	192.00	0.18	34.56	6.03	0.01	0.06	35.90	0.02	0.72	3.97	0.01	0.04
11	9.70	0.01	0.10	200.94	0.22	44.21	8.15	0.01	0.08	39.20	0.02	0.78	3.81	0.01	0.04
12	11.55	0.01	0.12	211.26	0.18	38.73	9.56	0.01	0.10	39.60	0.02	0.79	3.50	0.01	0.04
13	12.23	0.01	0.12	220.74	0.18	40.47	10.74	0.01	0.11	40.23	0.02	0.80	4.23	0.01	0.04
14	8.56	0.01	0.09	230.71	0.19	43.07	13.67	0.01	0.14	40.88	0.02	0.82	4.37	0.01	0.04
15	14.82	0.01	0.15	250.75	0.30	76.06	12.15	0.01	0.12	40.63	0.02	0.81	4.42	0.01	0.04
16	16.88	0.01	0.17	278.85	0.33	92.95	15.68	0.01	0.16	43.33	0.02	0.87	4.79	0.01	0.05
17	17.78	0.01	0.18	290.63	0.40	115.30	15.63	0.01	0.16	48.86	0.02	0.98	4.80	0.01	0.05
18	18.70	0.01	0.19	296.19	0.43	126.40	16.67	0.01	0.17	54.48	0.02	1.09	4.92	0.01	0.05
19	19.14	0.01	0.19	304.12	0.55	166.3	17.75	0.01	0.18	60.85	0.03	1.83	4.92	0.01	0.05
20	20.41	0.01	0.20	287.19	0.54	156.0	17.66	0.01	0.18	70.89	0.03	2.13	5.19	0.01	0.05
21	20.30	0.02	0.34	324.78	0.65	212.2	16.81	0.01	0.17	100.70	0.05	4.70	5.57	0.01	0.06
22	21.63	0.02	0.43	358.05	0.70	251.8	16.59	0.01	0.17	143.60	0.08	11.49	6.83	0.01	0.07
23	21.24	0.02	0.42	376.88	0.81	306.5	16.14	0.01	0.16	150.92	0.08	12.07	7.50	0.01	0.08
24	21.81	0.02	0.44	350.71	0.56	195.2	15.82	0.01	0.16	190.68	0.11	20.34	8.27	0.01	0.08
25	22.74	0.02	0.45	335.45	0.48	161.0	15.63	0.01	0.16	179.56	0.11	19.75	9.03	0.01	0.09
26	23.88	0.02	0.48	297.29	0.46	136.8	15.34	0.01	0.15	292.15	0.43	126.60	10.77	0.01	0.11
27	24.84	0.02	0.50	282.30	0.41	115.7	14.45	0.01	0.14	233.33	0.29	66.89	11.53	0.01	0.12
28	25.55	0.02	0.51	265.39	0.40	106.2	13.34	0.01	0.13	141.71	0.10	14.17	13.03	0.01	0.13
29	27.22	0.02	0.54	260.63	0.30	79.06	12.31	0.01	0.12	93.80	0.08	7.50	13.43	0.01	0.13
30	24.85	0.02	0.50	250.38	0.33	81.79	12.30	0.01	0.12	90.57	0.08	7.25	14.40	0.01	0.14
31	24.85	0.02	0.50	247.71	0.30	74.31	12.37	0.01	0.12	87.20	0.08	6.98	15.85	0.01	0.16
32	26.95	0.02	0.54	235.37	0.20	47.07	12.66	0.01	0.13	78.60	0.04	3.14	17.43	0.01	0.17
33	27.68	0.02	0.55	227.52	0.20	45.50	12.85	0.01	0.13	75.73	0.03	2.27	20.73	0.01	0.21
34	27.41	0.02	0.55	210.63	0.20	42.13	12.27	0.01	0.12	70.60	0.03	2.12	28.17	0.01	0.28
35	28.73	0.02	0.57	209.01	0.20	41.80	11.74	0.01	0.12	64.71	0.03	1.94	30.47	0.01	0.30
36	29.56	0.02	0.59	208.35	0.20	41.67	11.81	0.01	0.12	63.94	0.03	1.92	32.70	0.01	0.33
37	30.70	0.03	0.92	145.68	0.18	26.22	11.29	0.01	0.11	63.32	0.03	1.90	34.07	0.01	0.34
38	30.61	0.03	0.92	205.52	0.17	34.94	11.50	0.01	0.12	62.44	0.03	1.87	36.07	0.01	0.36
39	31.63	0.03	0.95	204.59	0.10	20.46	11.38	0.01	0.11	55.30	0.02	1.11	38.73	0.01	0.39
40	31.63	0.03	0.95	203.56	0.17	35.28	11.45	0.01	0.11	60.33	0.02	1.21	40.40	0.01	0.40
41	26.45	0.02	0.53	200.82	0.18	36.15	11.68	0.01	0.12	65.67	0.02	1.31	41.60	0.01	0.42
42	24.55	0.02	0.49	204.04	0.15	31.29	11.37	0.01	0.11	62.80	0.03	1.88	42.90	0.01	0.43
43	24.63	0.02	0.49	181.71	0.09	16.35	10.73	0.01	0.11	56.53	0.02	1.13	45.47	0.01	0.45
44	24.38	0.02	0.49	168.30	0.08	13.46	11.41	0.01	0.11	51.60	0.02	1.03	48.60	0.01	0.49
45	23.74	0.02	0.47	161.84	0.08	12.95	11.45	0.01	0.11	46.93	0.02	0.94	46.17	0.01	0.46
46	23.37	0.02	0.47	154.67	0.08	12.37	10.45	0.01	0.10	44.53	0.02	0.89	44.80	0.01	0.45

ISSN No:-2456-2165

47	23.11	0.02	0.46	148.39	0.08	11.87	8.90	0.01	0.09	42.23	0.02	0.84	43.80	0.01	0.44
48	23.45	0.02	0.47	145.60	0.08	11.65	7.06	0.01	0.07	40.37	0.02	0.81	41.77	0.01	0.42
49	22.14	0.02	0.44	140.54	0.08	11.24	6.46	0.01	0.06	38.27	0.02	0.77	38.40	0.01	0.38
50	22.63	0.02	0.45	130.78	0.08	10.46	5.73	0.01	0.06	36.57	0.02	0.73	38.03	0.01	0.38
51	22.34	0.02	0.45	127.82	0.08	10.23	5.11	0.01	0.05	34.40	0.02	0.69	37.50	0.01	0.38
52	22.30	0.02	0.45	125.78	0.08	10.06	4.60	0.01	0.05	30.33	0.02	0.61	36.20	0.01	0.36
53	21.45	0.01	0.29	110.78	0.04	4.43	3.60	0.01	0.04	25.60	0.02	0.51	33.40	0.01	0.33
54	21.81	0.02	0.44	105.40	0.04	4.22	2.43	0.01	0.02	22.80	0.01	0.23	30.13	0.01	0.30
55	21.27	0.02	0.43	99.27	0.04	3.97	3.23	0.01	0.03	20.50	0.01	0.21	29.33	0.01	0.29
56	21.30	0.02	0.43	94.07	0.04	3.76	3.73	0.01	0.04	19.23	0.01	0.19	28.50	0.01	0.29
57	20.08	0.02	0.40	79.67	0.03	2.39	3.83	0.01	0.04	18.43	0.01	0.18	27.27	0.01	0.27
58	20.67	0.02	0.41	70.30	0.03	2.11	5.00	0.01	0.05	18.17	0.01	0.18	25.17	0.01	0.25
59	20.63	0.02	0.41	59.97	0.03	1.80	7.70	0.01	0.08	17.67	0.01	0.18	23.43	0.01	0.23
60	20.33	0.02	0.41	50.37	0.03	1.51	8.17	0.01	0.08	17.63	0.01	0.18	22.63	0.01	0.23
61	20.56	0.02	0.41	47.80	0.03	1.43	5.33	0.01	0.05	16.56	0.01	0.17	19.40	0.01	0.19
62	19.56	0.02	0.39	30.37	0.02	0.61	4.47	0.01	0.04	16.86	0.01	0.17	18.53	0.01	0.19
63	18.20	0.01	0.18	27.17	0.02	0.54	3.77	0.01	0.04	16.36	0.01	0.16	17.77	0.01	0.18
64	16.67	0.01	0.17	24.70	0.02	0.49	3.87	0.01	0.04	15.45	0.01	0.15	16.63	0.01	0.17
65	15.26	0.01	0.15	19.07	0.01	0.19	3.67	0.01	0.04	15.78	0.01	0.16	14.77	0.01	0.15
66	13.34	0.01	0.13	16.57	0.01	0.17	3.37	0.01	0.03	15.44	0.01	0.15	13.67	0.01	0.14
67	10.74	0.01	0.11	15.47	0.01	0.15	3.20	0.01	0.03	15.05	0.01	0.15	12.73	0.01	0.13
68	10.30	0.01	0.14	14.60	0.01	0.15	2.83	0.01	0.03	14.85	0.01	0.15	11.60	0.01	0.12
69	10.09	0.01	0.10	13.73	0.01	0.14	2.50	0.01	0.03	14.79	0.01	0.15	10.73	0.01	0.11
70	9.64	0.01	0.10	12.70	0.01	0.13	2.43	0.01	0.02	14.26	0.01	0.14	9.60	0.01	0.10
71	8.56	0.01	0.09	12.33	0.01	0.12	2.30	0.01	0.02	14.41	0.01	0.14	8.53	0.01	0.09
72	8.30	0.01	0.08	11.67	0.01	0.12	2.00	0.01	0.02	14.27	0.01	0.14	8.30	0.01	0.08
73	7.50	0.01	0.08	10.63	0.01	0.11	1.80	0.01	0.02	13.67	0.01	0.14	6.43	0.01	0.06
74	6.52	0.01	0.07	10.40	0.01	0.10	1.57	0.01	0.02	10.70	0.01	0.11	5.27	0.01	0.05
75	5.51	0.01	0.06	9.63	0.01	0.10	1.50	0.01	0.02	9.67	0.01	0.10	5.30	0.01	0.05
76	4.38	0.01	0.04	9.43	0.01	0.09	1.40	0.01	0.01	8.40	0.01	0.08	4.70	0.01	0.05
77	4.04	0.01	0.04	8.60	0.01	0.09	1.30	0.01	0.01	7.87	0.01	0.08	4.27	0.01	0.04
78	3.82	0.01	0.04	8.30	0.01	0.08	0.97	0.01	0.01	6.77	0.01	0.07	3.70	0.01	0.04
79	3.30	0.01	0.03	7.80	0.01	0.08	0.57	0.01	0.01	6.23	0.01	0.06	3.63	0.01	0.04
80	2.30	0.01	0.02	7.47	0.01	0.07	0.30	0.01	0.00	3.30	0.01	0.03	3.13	0.01	0.03

Table 2:- Changes in daily Average Power generated from Sediment samples coupled to Microbial Fuel Cell during Bioremediation study

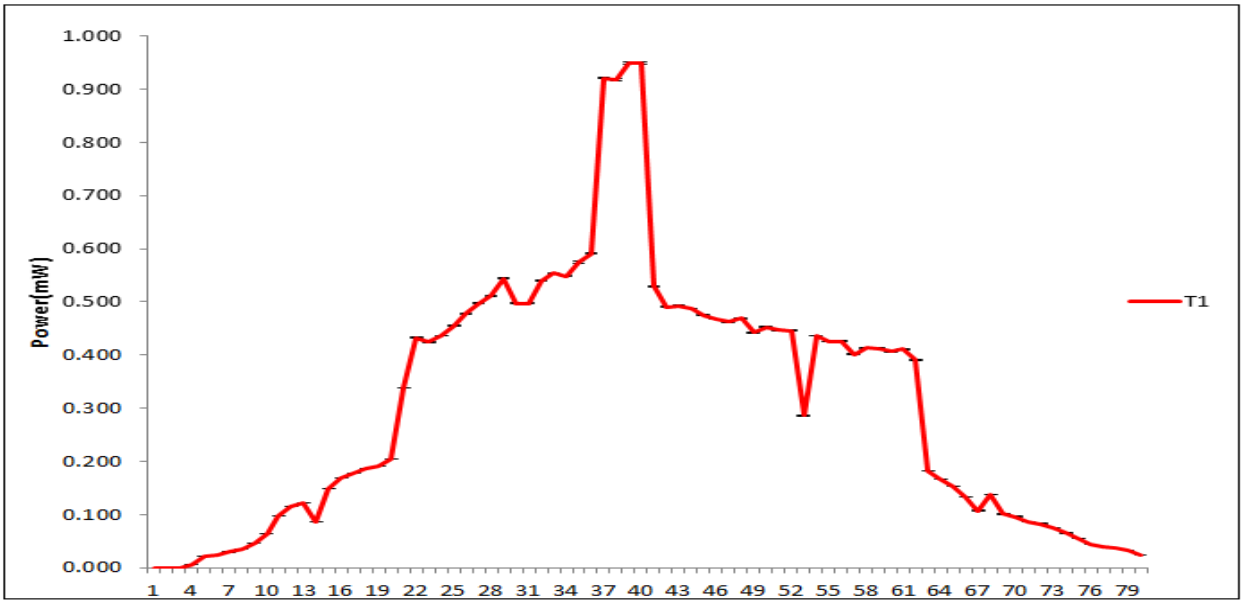

Fig. 3: Changes in Average Daily Power Generated with Time in Petroleum Hydrocarbon Contaminated Sediment (T_1) coupled to MFC during Bioremediation study

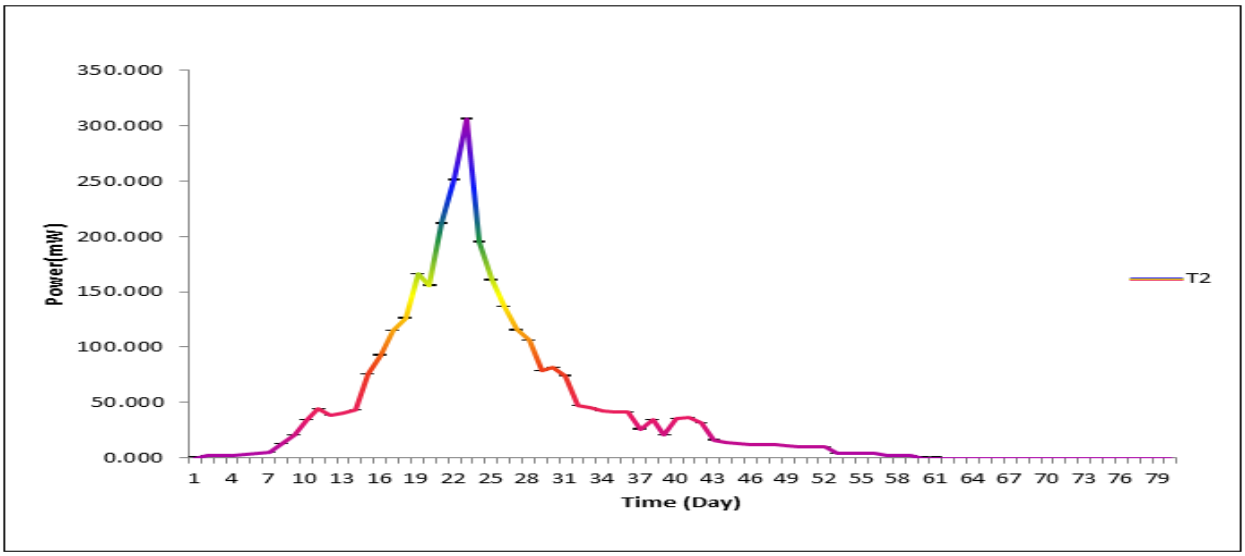

Fig. 4: Changes in Average Daily Power Generated with Time in Petroleum Hydrocarbon Contaminated Sediment (T_2) coupled to MFC during Bioremediation study

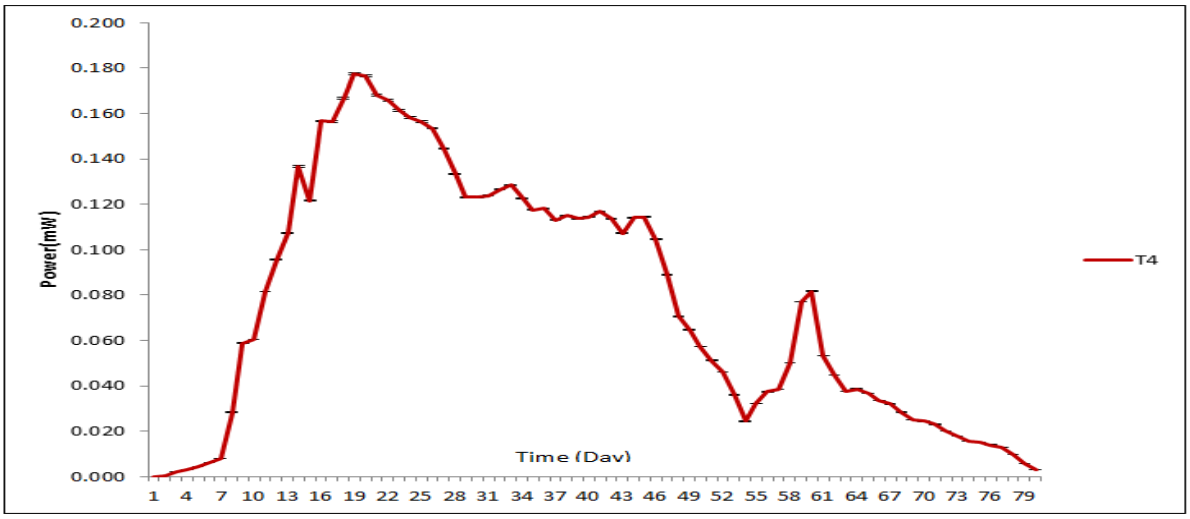

Fig. 5: Changes in Average Daily Power Generated with Time in Petroleum Hydrocarbon Contaminated Sediment (T_4) coupled to MFC during Bioremediation study

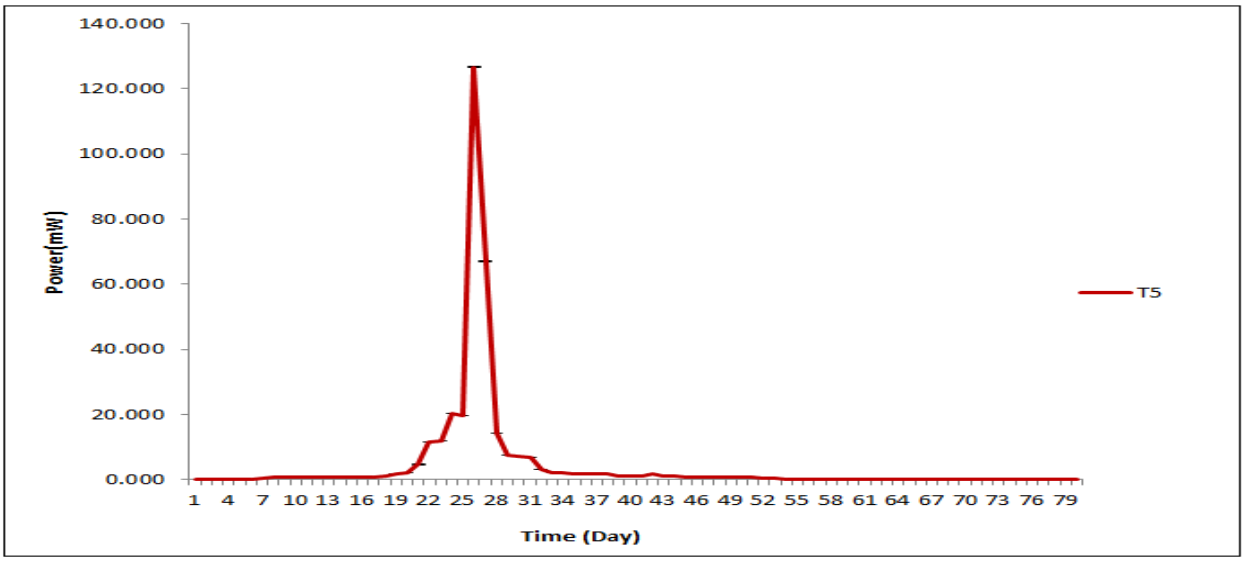

Fig. 6: Changes in Average Daily Power Generated with Time in Petroleum Hydrocarbon Contaminated Sediment (T_5) coupled to MFC during Bioremediation study

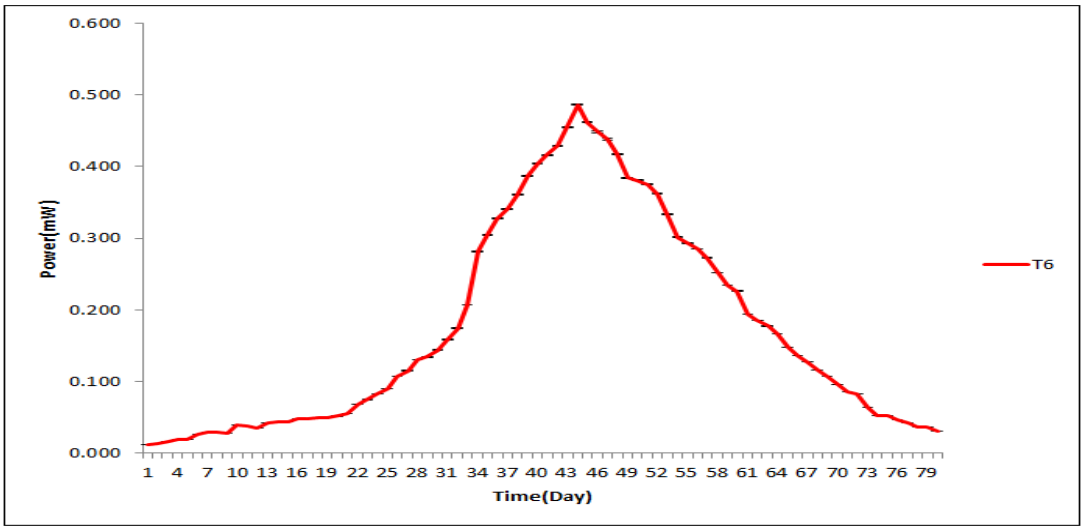

Fig. 7: Changes in Average Daily Power Generated with Time in Petroleum Hydrocarbon Contaminated Sediment (T_6) coupled to MFC during Bioremediation study

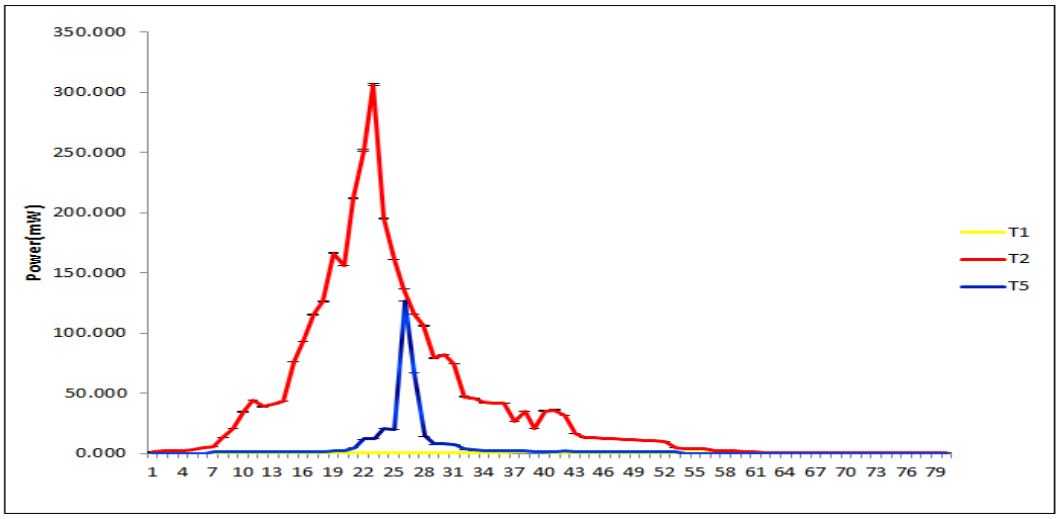

Fig. 8: Comparison of Changes in Average Daily Power Generated between T_1, T_2 and T_5 with Time in Petroleum Hydrocarbon Contaminated Sediment coupled to MFC during Bioremediation study

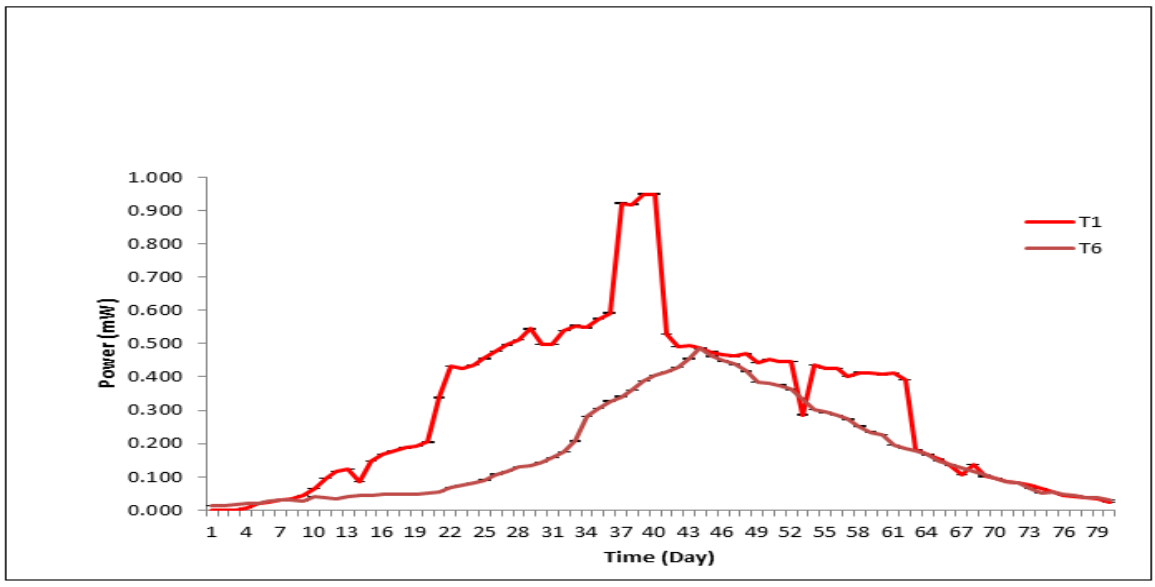

Fig. 9: Comparison of Changes in Average Daily Power generated between T_1, and T_6 with Time in Petroleum Hydrocarbon Contaminated Sediment coupled to MFC during Bioremediation study

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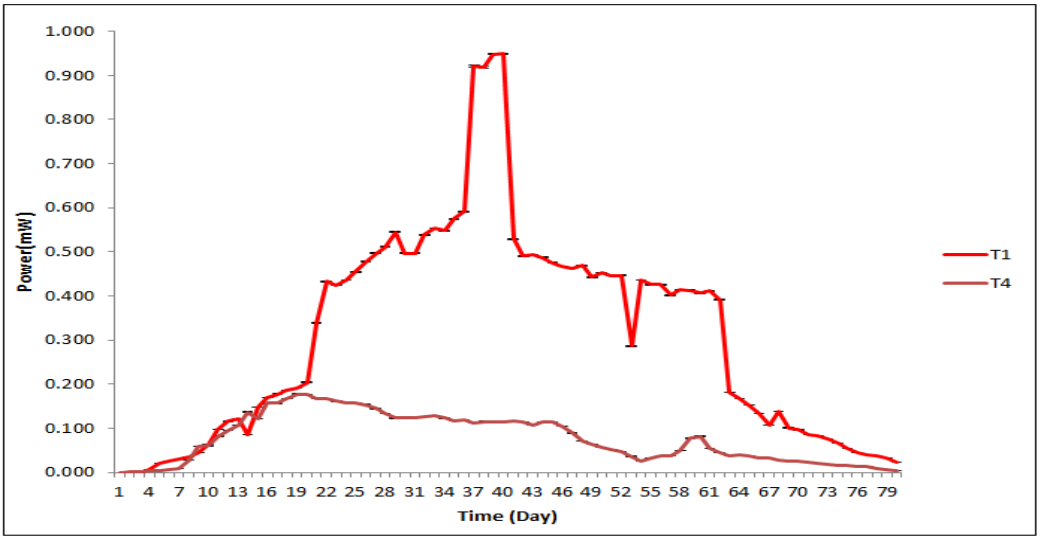

Fig.10: Comparison of Changes in Average Daily Power generated between T_1 and T_4 with Time in Petroleum Hydrocarbon Contaminated Sediment coupled to MFC during Bioremediation study

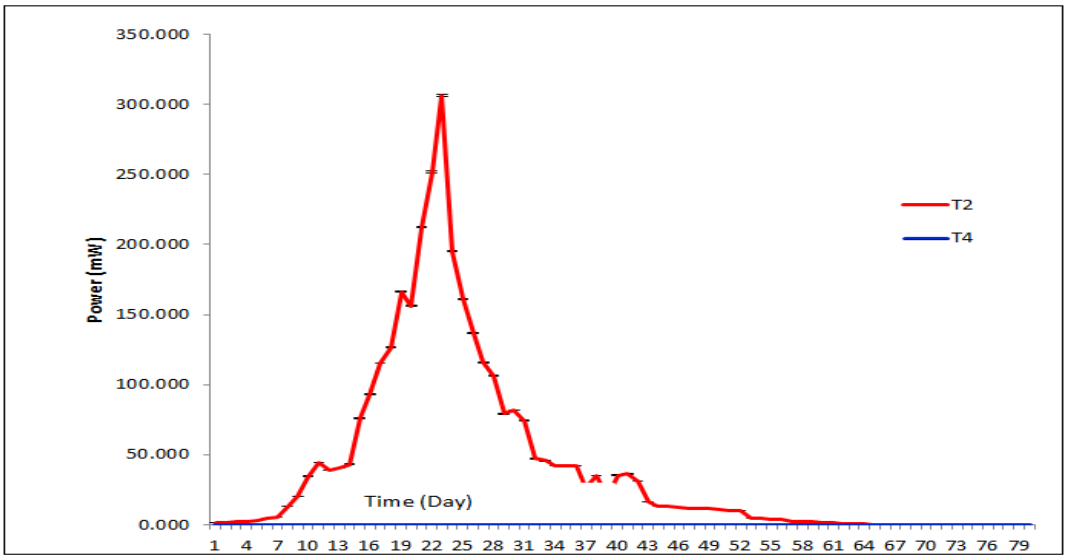

Fig. 11: Comparison of Changes in Average Daily Power generated between T_2, and T_4 with Time in Petroleum Hydrocarbon Contaminated Sediment coupled to MFC during Bioremediation study

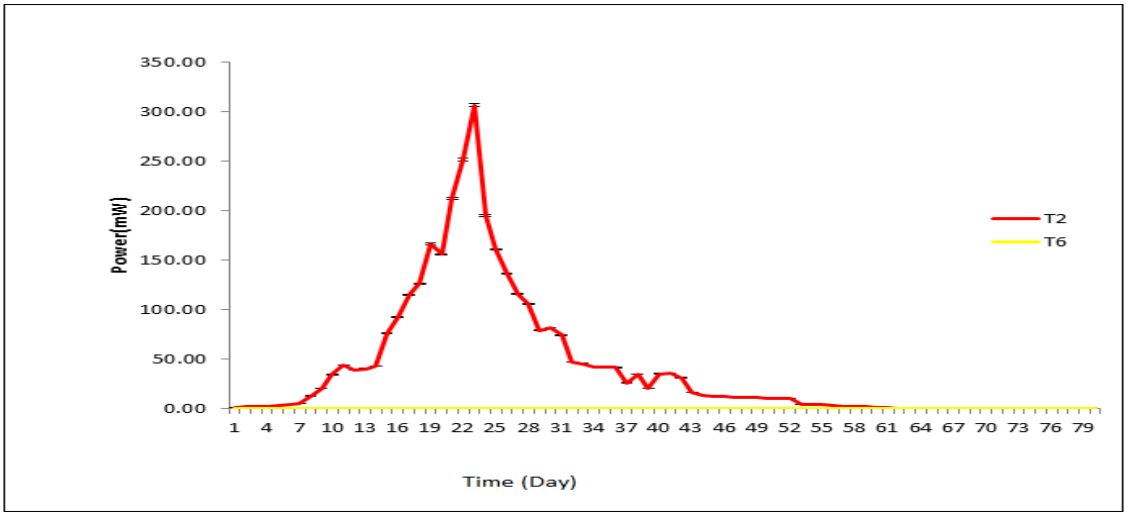

Fig. 12: Comparison of Changes in Average Daily Power generated between T_2 and T_6 with Time in Petroleum Hydrocarbon Contaminated Sediment coupled to MFC during Bioremediation study

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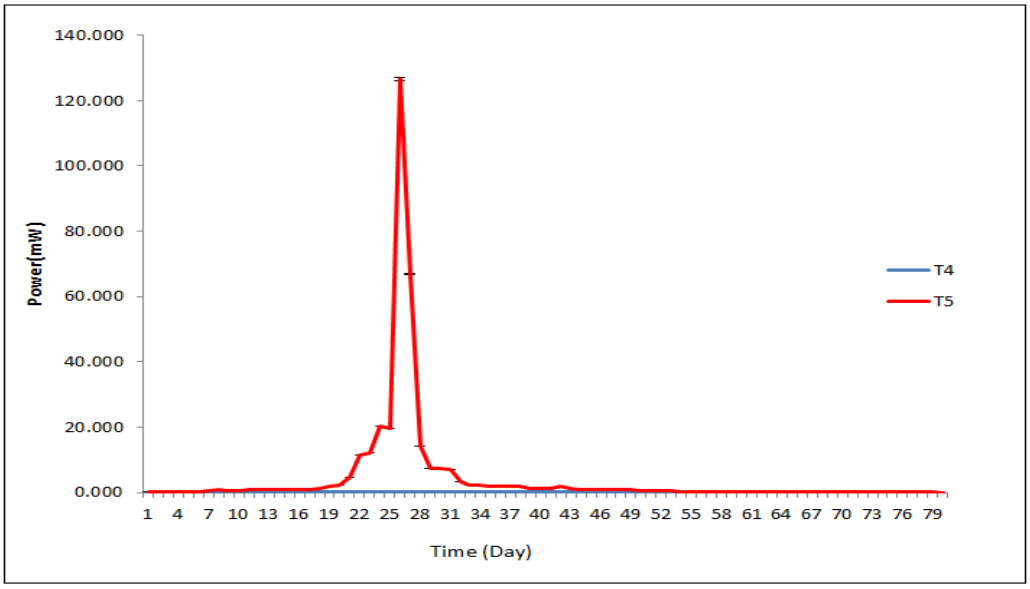

Fig. 13: Comparison of Changes in Average Daily Power generated between T_4 and T_5 with Time in Petroleum Hydrocarbon Contaminated Sediment coupled to MFC during Bioremediation study

Isolate ID	Gram Reaction	Catalase Test	Oxidase Test	Glucose Metabolism	O-F Test	Spore	Indole	Motility	Tentative identity
A1	+	-	-	+	F	+	-	+	*Bacillus sp.*
A2	-	+	+	-	F	-	-	-	*Enterobacter*
A3	-	+	-	+	F-O	-	-	-	*Bacillus sp.*
A4	-	+	+	+	O	+	-	+	*Enterobacter Sp.*
A5	+	+	+	-	F-O	-	-	+	*Bacillus sp.*
A6	+	+	+	+	O	-	-	+	*Bacillus sp.*
A7	+	-	-	+	F	+	-	+	*Bacillus sp.*
A8	+	+	-	+	O	+	-	+	*Bacillus sp.*
A9	-	+	+	+	F-O	-	+	-	*Serratia sp.*
A10	-	+	+	-	O	-	-	+	*Enterobacter sp.*

Table 3:- Biochemical Characterization of Distinct Hydrocarbon Utilizing Bacterial Isolates obtained from Sediment Samples during Bioremediation Study

+ = positive, - = negative, F= Fermentation, O= Oxidation

Isolate ID	Blastn Identity	% Identity Similarity	Accession Number
A_1	*Clostridium sporogenes* strain DSM 795, complete genome	99	MF623797
A_2	*Desulfobulbus propionicus* strain DSM 2032 16S ribosomal RNA gene, partial sequence	100	MF623798
A_3	*Ewingella americana* strain R12 16S ribosomal RNA gene, partial sequence	89	MF623799
A_4	*Bacillus amyloliquefaciens* strain MBE1283, complete genome	90	MF623800
A_5	*Helicobacter sp.* MIT 01-6242, complete genome	88	MF623801
A_6	*Alcaligenes faecalis* partial 16S rRNA gene, isolate KWW 84	85	MF623802
A_7	*Clostridium botulinum A str.* ATCC 19397, complete genome	99	nill
A_8	*Bacillus subtilis* strain J-5, complete genome	90	MF623803
A_9	*Klebsiella oxytoca* strain CAV1335, complete genome	93	MF623804
A_{10}	*Burkholderia cepacia* strain ATCC 49709 16S ribosomal RNA gene, partial sequence	97	MF623805

Table 4 :Molecular Characterization of Distinct Hydrocarbon Utilizing Bioelectrochemically Active Bacteria

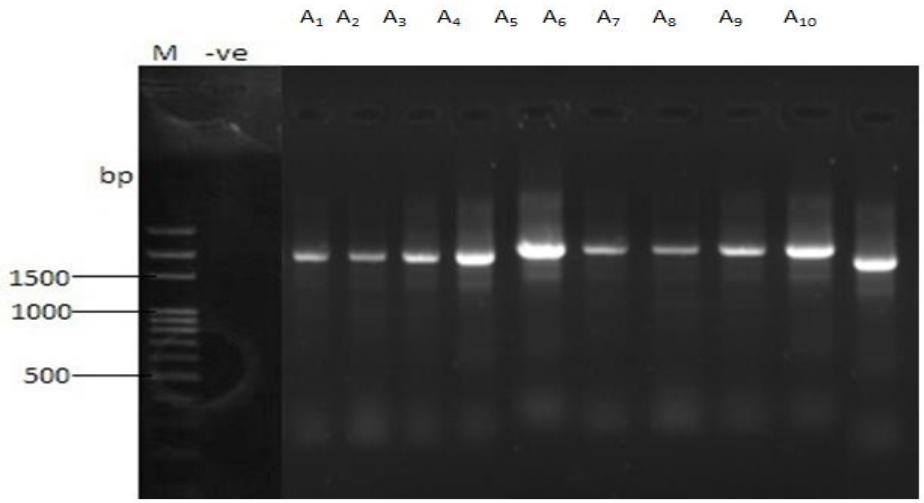

Fig.14:- Gel electrophoresis Photograph of PCR products as revealed by UV Transilluminator

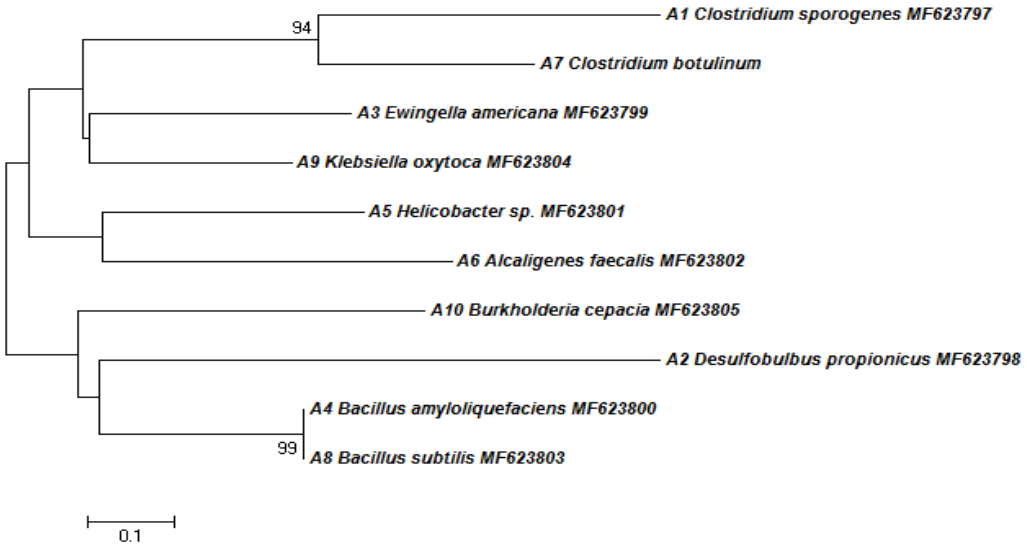

Fig. 15:- Phylogenetic Tree of isolated Bacteria from Sediment Samples (T_1-T_7)

V. DISCUSSION

In Fig .3, T_1 did not show any recordable value for voltage and current until day 4. This is as a result of heat sterilization of T_1 which might have exacted lethal effects on the microbial population in the system. The spore-forming microbes in the system survived the heat treatment and then developed into fully functional vegetative cells while the microbial population that are not heat resistant died or lost their metabolic viability and cellular integrity. This is supported by Pelczar *et al.* (2004) that numerous microbes have been identified as having resistant ability to heat sterilization. Bacteria spores are much more resistant than vegetative cells. *Bacillus stearothermophilus*, *Clostridium* sp. could withstand temperature of 121 ^{0}C heat for up to 12mins. Endospores of bacteria contain dipicolonic acid which could be responsible for heat resistance and calcium to heat and oxidizing agents. The maximum power generated in T_1 (Fig. 4.3) was 0.95 mW on day 39 and 40. Most microbes that could have interplayed metabolically might have died or mutated into another strain or better still, the gene initiating or responsible for rapid electrons release by the microbes during anaerobic oxidation of substrates might have been altered leading to loss of genetic information. On the other way, dead biomass might have contributed to internal resistance in the sediment leading to poor porosity in the sediment which can hamper rapid electron transfer. A decline in power output observed from day 41 to the end of bioremediation with lowest output value of 0.02 mW.

In T_2, 1.41 mW average power generated on day 1 followed with progressive increase in power output to 306.50 mW on day 23. A sharp decline of power output as observed from in Fig. .4 between day 23 and 24 to 195.2 mW may be due to internal resistance in the sediment hampering free electron transfer as well or a signal that substrates in the sediment is depleting rapidly. This followed a steady decline to 0.07 mW at the end of bioremediation. In T_4 containing Neutral red as mediator (Fig. 5). Power output began on day 4 as low as 0.01 mW with insignificant progressive increase with maximum power output of 0.18 mW on day 20 followed by decline in power from day 21 to as low as 0.01 mW on day 76 to 79. However, in T_5

containing m ethylene blue as mediator (Fig. 6), 0.12 mW of power output was generated on day 1 followed by progressive increase in power output. Maximum power output generated was 126.60mW with significant decline to 66.89 mW on day 27 and 14.17 mW on day 28 while a rapid decrease trend observed till day 80 as 0.03 mW. Comparing power output generated from T_4 containing neutral red and T_5 containing methylene blue as shown in Fig. 13, it can be deduced that T_5 generated more power output than T_4 due to difference in electron transfer ability by the mediators. This is in corroboration with Lohar *et al.* (2014) who reported 11.24 mW/m^2, 8.733 mW/m^2 for methylene blue and neutral red respectively as power density generated when methylene blue and neutral red were used as mediators in MFC in the course of electricity generation from diary waste water. Similarly, Taskan *et al.* (2014) observed 25.00 mW/m^2 and 20.00 mW/m^2 power density from MFC containing 100 µM methylene blue and Neutral red respectively where domestic waste water was used as substrate. But, the power generated from T_2 (306.50 mW) is of higher value than T_4 (0.18 mW) and T_5 (126.60 mW). This implies that T_2 contains microbial consortia that do not require artificial electron transfer mediators but sorely dependent on self–mediated electron transfer system through production of exogenous chemicals and nanowire which are used to shuttle electrons to the anode electrode as in agreement with Zhang *et al*. (2012).

More so, T_6 (Fig. 7 and Fig. .9) containing NPK 15-15-15 generated 0.01 mW power on day 1 and insignificantly increased to highest power output of 0.49 mW on day 44 and further decline as low as 0.03 mW at the end of bioremediation. Comparing the output of power from T_1 (0.95 mW) with T_6 containing NPK 15-15-15 (0.49 mW). It shows that, NPK 15-15-15 formulation as biostimulant (fertilizer) does not give significant power output due to negative or adverse effects of high K concentration on saltwater microbial population in the sample as explained by Kanaly *et al.* (2002).

Table 3 showed the hydrocarbon utilizing bacteria that were isolated from T_1 to T_7.

Clostridium sporogenes strain DSM 795 (MF623797) and *Clostridium botulinum* A str. ATCC 19397 were isolated from T_1 and T_5 sample. *Clostridium sp.* is known as anaerobic bacterium capable of forming spores. Clostridium spores are highly resistant to heat and may remain dormant (inactive) for some weeks before growth resumption. *Clostridium* has been reported to be capable of generating electricity and utilize petroleum hydrocarbon. Jiang *et al.* (2016) isolated eleven species of *Clostridium* including *C. sporogenes and C. botulinum* from contaminated soil with organic compounds used to generate electrical power in microbial fuel cell. This is in agreement with this investigation. Three (3) organisms were isolated from T_2. They are: *Desulfobulbus propionicus* strain DSM (MF623798), *Ewingella americana* strain R12 (MF623799), *Bacillus amyloliquefaciencs* strain MBE 1283 (MF623800)

D. propionicus has the metabolic potentialities to produce current and utilize organic substrates in sediments. Affirmation from Daighio *et al.* (2016) supports this study deduction, where *Desulfobulbus propionicus* was found to produce current from marine sediment contaminated with petroleum hydrocarbon in a reactor of a bioelectrochemical system in which electron transfer to the anodic surface was self-mediated by microbial nanowires of the organism. The isolation of sulfate reducing bacteria in this study showed that the sediment is rich in sulfates as supported with Table 1, showing the baseline physicochemical features of the sediment.

Two species of *Bacillus* were isolated from this study. They are *Bacillus amyloliquefaciencs* strain MBE 1283 (MF623800) from T_1 and Bacillus *subtilis* strain J-5 (MF623803) from T_5. Both species of *Bacillus* contributed to electrical power generation. This deduction is supported by Jude *et al.* (2015) in which *Bacillus amyloliquefaciencs* was isolated from mud/soil substrate contaminated with hydrocarbon contaminants resulting to remediation of petroleum hydrocarbon and concurrent electricity. *B. amyloliquefaciencs is* capable of producing biosurfactant containing both hydrophilic and hydrophobic moieties in their structure which can reduce both surface and intersurface tension, hence, facilitating emulsification process in petroleum hydrocarbon degradation.

Bioelectrochemcial potentials of *B. amyloliquefaciencs and B. subtilis* as shown in this study was also supported by Wang *et al.* (2015) where *Bacillus subtilis* and B. *amyloliquefaciencs* were screened for their ability to grow in liquid medium containing petroleum hydrocarbon compound: Diesel as a sole carbon.

Isolation of *Bacillus* by Ogugbue *et al.* (2015) from MFC in the course of generating electricity from swine waste water is also an indication that species of *Bacillus* associate with bioelectrochemical system, this is in agreement with this study. Similarly, Sabina *et al.,* (2014) employed *Bacillus subtilis* in microbial desalination cell of a bioelectrochemical system developed for producing green energy from organic wastes resulting to high level of efficiency, this corroborates this investigation.

Moreover, Olukunle *et al.* (2015) reported *E. Americana* as capable of hydrocarbon degradation by inoculating soil samples containing crude oil with Pure culture of *Ewingella Americana* which increased population of other bacteria consortia after inoculation due to the fact that petroleum components that might be toxic to other organisms were degraded by *Ewingella Americana*. It has been found that *Ewingella Americana* is capable of producing dehydrogenase, this enzyme aids direct electron transfer to electrode in MFC resulting to high current and low redox potential. This assertion corroborates this study that *Ewingella Americana* has contributed to hydrocarbon reduction and generation of electricity in sediment microbial fuel cell.

In another development, *Helicobacter* sp. MIT 01-6242 (MF623801) was isolated from T3 which does not contain MFC. *Helicobacter* has be identified as possessing metabolic potency of degrading polyaromatic and saturated hydrocarbons according to widdel *et al.* (2001). Much study

is still need to be done to specifically know the species of hydrocarbon that can be degraded by Helicobacter. But *Helicobacter* has not been well known among microbial corsortia in Bioelectrochmeical system. The isolation of *Helicobacter* sp. from T3 which does not contain MFC is still in agreement with available research study so far.

Also, *Alcaligenes faecalis* isolate KWW 84 (MF623802) was isolated from T4 sample. Ezikpe (2009) has reported on the ability of *Alcaligenes faecalis* to utilize chrysene and diesel oil and is also capable of inducing extracellular protein and carbohydrate with concomitant production of biosurfactant for industrial purpose and in bioremediation. Wang *et al*. (2015) noted *A. faecalis* is capable of using releasing electrons from substrates in electrode during denitrification and the production of hydrogen gas by *A. faecalis* in microbial fuel cell as reported by Rabaey *et al*. 2004 is in agreement with this study that Acaligenes can interplay with other microbes to synergistically remediate petroleum hydrocarbon in sediment and generate electricity but the presence of neutral red in T_4 does not really boost electron transfer to commensurate hydrocarbon degradation and electrical power production unlike T5 containing methylene blue.

In the same vein, *Klebsiella oxytoca* strain CAV1335 (MF623804) was isolated from T_6 sample. Klebsiella has the potency to biodegrade organic compounds including petroleum hydrocarbons and at the same time generate electricity. This is supported by Islam *et al*. (2016) where *Klebsiella oxytoca* was found to efficiently generate electricity from palm oil mill effluent (POME) The MFC was found to show maximum power density of 207.28 mW/m3 with continuous feeding of POME using microbes from anaerobic sludge (AS). The biodegradation ability of *Klebsiella oxytoca* as observed in this study is also in agreement with Chamka *et al.* (2011) in which *Klebsiella oxytoca* strain degraded crude oil in a Tunisian off-shore oil field and the GCMS analysis showed that *Klebsiella oxytoca* could utilize C_{13} to C_{30} aliphatic hydrocarbons.

Moreover, *Burkholderia cepacia* strain ATCC 49709 was isolated from T_7 sample. *Bukolderia* contributed to the degradation of petroleum hydrocarbon in this investigation as supported by Oyetibo *et al*. (2013) where *B. cepacia* was found to utilize crude oil and anthracene and the bacteria grew in hydrocarbon media amended with Nickel and Cobalt.

VI. CONCLUSION

Conclusively, employment of MFC technology, a bioelectrochemical system in environmental media remediation enhances electrical power generation for sustainable development. Heavily polluted saltwater sediment can serve as source of electron donors for millions of microbes in anoxic sediment. These microbes in anoxic sediments can transfer electron through direct or indirect system leading to simultaneous bioremediation and electricity generation. Numerous Microbial fuel cells could be connected in series or parallel and deployed into highly polluted sites where the power generated are harvested and stored until higher yield of power is achieved to power or motorize sophisticated devices.

Therefore, Governments at all levels should diversify into bioelectricity for power generation and sustainability. Sediment Microbial Fuel Cells with large electrodes could be buried into polluted Niger Delta Rivers for continual eco-friendly remediation, power generation and onsite biomonitoring of variables.

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