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PLANT MICROBIAL FUEL CELL AND RENEWABLE ENERGY FROM PADDY FIELD

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ABSTRACT

Plant microbial fuel cells (PMFCs) and microbial fuel cells (MFCs) are a new technology in renewable energy that generate bioelectricity by converting organic matter and biomass, facilitated by bacteria in anaerobic or microaerophilic conditions. PMFCs have garnered significant attention. In a laboratory setup, a prototype PMFC system was arranged with 11 buckets, each containing marshy soil from paddy fields, with a plant growing in each bucket. Bioelectricity generation was measured using a multimeter (in mV), along with temperature and pH, over a period of 66 days. The soil adhering to the electrodes was used for the isolation and characterization of bioelectricity-generating bacteria, which could be further harnessed for electricity production. Five bacterial isolates (D1, D2, D3, D4, and D5) were selected for their electricity-producing capabilities. These isolates were identified through morphological and biochemical analysis as Pseudomonas spp-1, Pseudomonas spp-2, Pseudomonas spp-3, Klebsiella spp., and Pseudomonas spp-4. The electricity-generating potential of these five isolates was studied in MFCs, using two different growth media: nutrient medium and succinate medium. Among them, Pseudomonas spp-4 demonstrated the highest electricity generation in the nutrient medium.

KEYWORDS: MFC, bioelectricity production, plant MFC.

INTRODUCTION

A **microbial fuel cell** (**MFC**), or biological fuel cell, is a bioelectrochemical system that drives an electric current by using bacteria and mimicking bacterial interactions found in nature.

The plant microbial fuel cell is a sustainable and renewable method for generating electricity**.** Plant MFCs (PMFCs) with living plants are way to get green energy (Moqsud et al. 2014, Strik et al. 2008). In PMFCs, plant roots directly fuel the electrochemically active bacteria at the anode by excreting rhizo deposits (Strik et al. 2011, D. Schamphelaire, 2008, Kaku et al. 2008, Timmers et al. 2010, Helder et al. 2010). When a paddy field is flooded, the soil immediately below the surface becomes anaerobic (Takai, 1969) and a community of anaerobic micro biota (comprised mainly of sulfate-reducing bacteria, iron-reducing bacteria and fermenting bacteria and methanogenic archaea) is established.

First a short classification of the underlying principles of microbial bio electrochemical systems (BESs) are given to coupling electron-donating reactions initially, workers used the spontaneous electron movement between electronegative bio anodes. The electro positive abiotic cathodes in microbial fuel cells to generate electric current. Operated MFCs with bio cathodes at which bacteria catalyze the electron transfer from the cathode to electro positive terminal electron acceptors, such as oxygen or nitrate.

Bacteria capable to transference electrons to metals are key agents in biogeochemical metal cycling, subsurface bioremediation, and oxidation processes. these bacteria have grown attention as the transfer of electrons from the cell surface to conductive materials can be used in several applications. The integration of biomolecules with electronic elements to yield functional devices attracts substantial research efforts because of the basic fundamental scientific questions and the potential practical applications of the systems. The research field gained the buzzword ‘‘bioelectronics’’ aimed at highlighting that the world of electronics could be combined with biology and biotechnology [Willner & Hoffmann, 2002].

Microbial fuel cells are devices that exploit microbial catabolic activities to generate electricity from a variety of materials, including complex organic waste and renewable biomass. In MFCs, microbes utilize organic compounds as energy and carbon sources. In order to generate energy for growth, organics are decomposed, and chemical energy is released that is (fermentation). In addition, high-energy electrons released from organics are

transferred to oxidized chemicals that is (electron acceptors, such as molecular oxygen) to conserve electrochemical energy that is (respiration). In microbial cells, electrons released from organic matter are first captured by intercellular electron-shuttling compounds, such as nicotinamide adenine dinucleotide (NAD) and subsequently transferred to electron acceptors via respiratory electron-transport chains. If a mechanism is present by which electrons released from organics can be transferred from any step in the intercellular electron transfer pathway to an extracellular electrode (i.e., anode), then microbial oxidation of organics can be coupled to electricity It has been suggested that MFCs have many possible future applications; these include water treatments coupled to energy recoveries, portable fuel cells, biosensors, and in-situ energy sources. So in future this green technology can be adapted to sites of phytoremediation where plant systems are used to treat waste waters which may be coupled to bioelectricity generation, and can provide a cost effective energy option. During course of this investigation bioelectricity production by plant MFC will be studied & effect of various parameters for enhancing bioelectricity will be studied & microorganisms responsible for electricity generation will be isolated & characterized.

Plate 1.1: Paddy Field.

Methods

I) **Studies of bioelectricity generation with paddy MFC**

In this study the soil samples and paddy plants were collected from Lonavala paddy field. after collection of sample eleven buckets were used for the test set up of PMFCs as seen in the plate 2.1. Out of eleven buckets 10 buckets were prepared with the same soil without mixing any compost or organic fertilizer.

The control bucket was prepared with same soil but without the paddy plant, all the plants were always kept submerged in water.

In each of the buckets the electrodes like cathode (Al) and anode (Cu) were placed the length of this electrode was 5 cm. the cathode was placed in the surface of each bucket and anode was inserted up to the bottom of the bucket then electric wire was connected to anode and cathode.

The Bioelectricity generation was measured in each bucket individually with the help of Multimeter this electric current was measure in mV and in all the 11 buckets temp. And pH was also measured.

II) Isolation and characterization of bacteria from MFC

Firstly, soil samples were serially diluted and spread plated on nutrient agar plates and these plates were incubated at 37^0C for 24 hrs. After incubation colonies having different colony characters were studies for colony morphological and biochemical characters.

Maintenance of cultures

Bacterial cultures were maintained on sterile nutrient agar slants and stored in a refrigerator (28^0C.)

Detection of current formation

Nutrient broth & Succinate broth were prepared to check the efficiency of current formation. Both media were inoculated separately with isolated cultures and then incubated. After every 24 hrs. Of incubation current formation was measured using digital Multimeter.

Bioelectricity generation with Bacterial cultures

The isolated microorganisms were grown in nutrient broth & succinate broth with appropriate nutrient conditions and electricity was measured for five days after every 24 hrs.

1) All growing cultures of Nutrient broth & succinate broth were separately transferred in ice tray.
2) After that in this ice tray, in each of the ice tray the electrodes like cathode (Al) and anode (Cu) were placed the length of this electrode was 2 cm.
3) The cathode was placed in the surface of each ice tray and anode was inserted up to the bottom of the ice tray then electric wire was connected to anode and cathode.
4) The Bioelectricity generation was measured in each ice tray individually with the help of Multimeter this electric current was measured in mV.

Plate 2.1: Laboratory Setup of Paddy Field Plants.

Laboratory setup of cultures

RESULTS AND DISCUSSIONS

Bioelectricity generation with plant MFC

In this study, soil samples and paddy plants were collected from a paddy field in Lonavala. A total of 11 buckets were used to set up the PMFCs, as shown in Plate 2.1. Ten of the buckets were filled with the same soil, without any compost or organic fertilizer, and each contained one paddy plant. The control bucket was prepared with the same soil but without a paddy plant, and all buckets were kept submerged in water throughout the experiment.

In each bucket, electrodes were placed: a cathode (aluminium) positioned at the surface and an anode (copper) inserted at the bottom. Both electrodes were connected via electric wires. The length of each electrode was 5 cm. Bioelectricity generation in each bucket was measured individually using a multimeter, recording the electric current in millivolts (mV). Additionally, temperature and pH were monitored in all 11 buckets.

Daily readings of electricity generation were recorded over 66 days for all buckets, including the control, as shown in Table 3.1. The results indicated that, compared to the control bucket, the buckets with paddy plants consistently produced higher current. In the control, the minimum current was 245 mV, the maximum was 390 mV, and the average over 66 days was 349.74 mV.

In contrast, with the paddy plants, the minimum current was 288 mV, the maximum reached 976 mV, and the average was 705.41 mV. When all 10 MFCs were connected in series, the current generation increased significantly, reaching 7–8 V, which was sufficient to light four LED bulbs simultaneously. The pH ranged between 5.7 and 6.1, while the temperature varied from 24°C to 31°C**.**

Day	PH	Temp.	Current in mV										
			Control	Plant-1	Plant-2	Plant-3	Plant-4	Plant-5	Plant-6	Plant-7	Plant-8	Plant-9	Plant10
1.	6.0	30	372	385	387	215	249	249	250	249	215	387	372
2.	6.0	31	363	692	678	250	251	250	249	250	249	215	335
3.	5.8	29	374	784	750	290	293	290	290	246	246	335	338
4.	6.0	30	378	700	699	298	294	294	294	250	250	346	346
5.	6.4	29	377	696	648	335	338	338	338	290	238	338	338
6.	6.0	30	371	923	916	916	456	335	335	335	335	335	338
7.	6.0	30	369	906	870	897	854	335	338	335	338	335	335
8.	5.9	28	300	904	321	862	869	388	364	385	365	377	346
9.	6.0	30	372	897	903	265	685	366	363	373	372	388	373

10.	6.0	29	378	855	288	351	890	880	904	894	908	924	907
11.	6.0	30	377	929	876	729	905	902	966	876	902	917	917
12.	6.0	30	378	889	872	639	882	845	872	862	889	903	873
13.	6.0	28	369	876	330	361	343	338	353	871	328	328	228
14.	6.0	29	379	878	790	890	860	790	876	808	876	784	750
15.	6.0	29	372	880	860	790	776	782	750	890	872	700	760
16.	6.0	27	360	870	880	780	790	776	789	880	870	750	770
17.	5.8	28	376	976	883	788	771	782	779	893	883	788	794
18.	6.0	30	360	833	832	774	708	758	775	871	898	774	745
19.	6.0	27	284	629	694	898	679	560	807	546	639	642	594
20.	6.0	27	295	630	680	880	670	530	803	500	630	640	580
21.	5.7	28	270	640	690	870	669	555	801	508	640	432	555
22.	6.0	27	270	587	605	874	772	466	786	502	660	660	495
23.	6.0	28	245	496	762	810	799	507	744	637	587	554	450
24.	5.7	27	247	559	767	809	800	515	742	636	586	555	451
25.	5.7	28	247	496	763	874	799	466	801	500	639	642	745
26.	5.8	27	245	496	762	809	903	709	803	403	883	642	770
27.	6.0	28	247	496	767	874	799	515	786	636	586	432	555
28.	6.0	29	284	559	763	810	772	555	742	637	640	555	495
29.	6.0	30	372	880	870	779	833	870	880	870	833	779	870
30.	6.0	29	360	870	880	793	880	880	870	833	880	870	880
31.	5.8	28	376	976	870	880	793	793	880	880	880	880	883
32.	5.7	30	372	880	833	870	870	880	793	793	833	883	801
33.	6.0	27	376	870	833	880	880	833	870	833	870	801	870
34.	6.1	30	284	640	880	976	976	870	976	870	779	870	779
35.	5.7	28	295	833	870	870	880	976	880	976	870	880	870
36.	6.0	30	376	880	976	833	976	833	976	880	880	833	976
37.	6.0	30	360	860	900	820	960	800	972	800	820	803	960
38.	5.7	28	360	860	870	833	880	800	800	800	800	833	870
39.	6.1	30	376	833	833	870	833	833	833	833	833	870	833
40.	6.0	27	360	880	880	870	870	870	870	880	870	833	833
41.	5.8	30	284	870	870	880	833	779	779	793	800	801	801
42.	5.7	29	295	833	833	870	793	779	779	793	793	779	779
43.	6.0	30	300	850	833	860	860	779	793	793	779	793	793
44.	6.1	30	360	870	870	833	833	640	640	640	793	640	779
45.	6.0	28	376	860	880	860	833	635	630	645	779	649	788
46.	6.1	24	360	880	873	863	840	650	635	647	750	660	780
47.	6.0	25	376	860	650	670	700	630	625	640	700	620	720
48.	6.0	26	370	862	666	679	750	625	680	650	680	620	780
49.	5.8	26	372	855	670	650	772	630	690	656	622	615	615
Day	**PH**	**Temp.**					**Current in mV**						
			Control	**Plant-1**	**Plant-2**	**Plant-3**	**Plant-4**	**Plant-5**	**Plant-6**	**Plant-7**	**Plant-8**	**Plant-9**	**Plant10**
50.	5.7	27	370	865	656	660	780	635	695	670	670	615	670
51.	5.8	25	378	880	690	665	782	680	685	690	687	620	680
52.	6.0	24	380	887	695	680	790	682	670	696	690	627	690

53.	6.0	25	382	895	700	687	760	690	678	670	695	630	685
54.	5.8	29	380	890	700	686	677	670	690	635	695	698	680
55.	5.7	24	385	880	720	685	670	675	684	630	690	690	675
56.	5.9	29	380	885	725	680	678	678	680	625	694	680	674
57.	6.0	24	382	886	729	690	675	680	685	628	697	677	670
58.	6.0	27	370	880	730	689	670	675	690	627	698	678	680
59.	6.1	28	375	890	735	685	679	670	695	628	680	675	679
60.	6.0	27	378	880	734	680	680	680	688	620	690	680	684
61.	5.8	28	380	884	730	684	685	685	670	629	684	682	686
62.	5.9	27	384	886	739	685	687	690	680	630	685	687	688
63.	6.0	25	385	870	742	680	690	685	700	632	700	690	690
64.	6.0	24	390	890	750	682	695	690	701	634	695	697	692
65.	6.1	25	380	895	745	687	698	680	690	630	680	680	692
66.	6.0	26	385	872	752	685	697	670	695	600	670	650	680

When the current generation of all the plants was plotted against time with the control 1, it can be seen from figure 3.1 that for control bucket it ranged between 245 – 390mV, and for all the buckets PMFC it was away above 600 mV showing the effectiveness of PMFCs which, when used in the field could generate enough current to the farmer in case of emergency.

As can be seen from table 3.2 and fig.3.2 always higher than that of the control for 66 days for which the study was underneath.

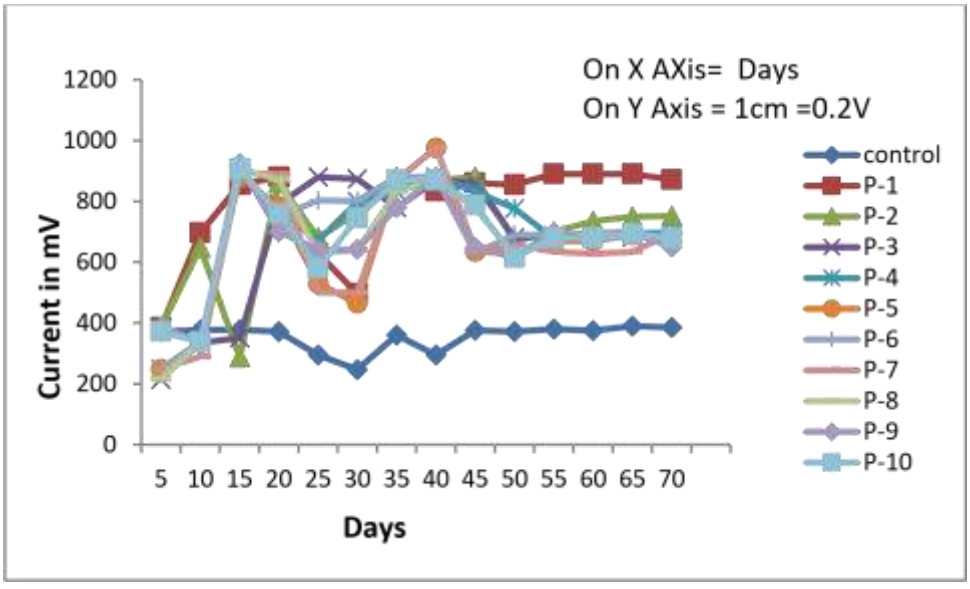
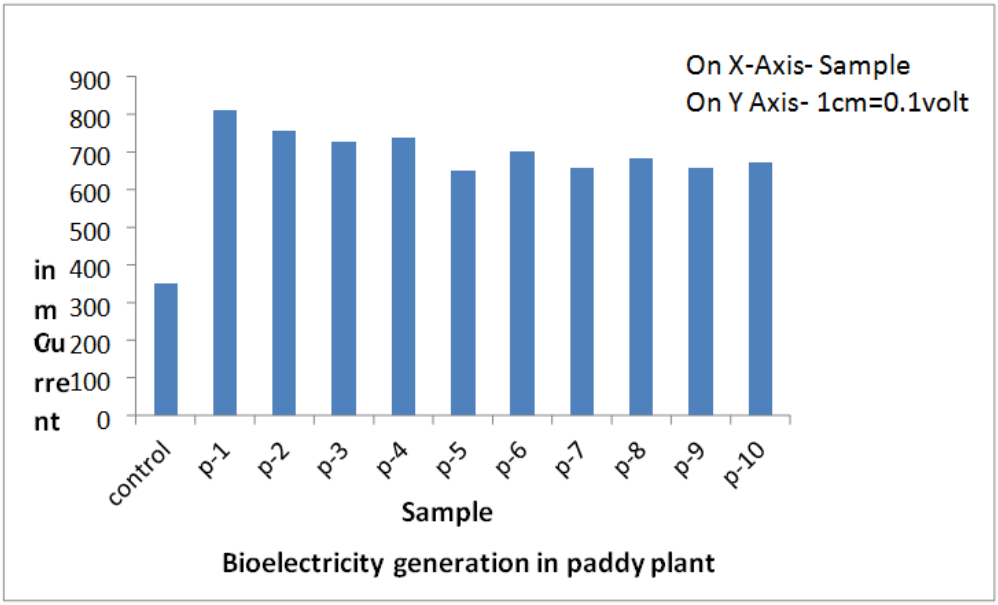


Fig. 3.1: Bioelectricity generations in paddy plant (MFC).

Table 3.2: Average current reading with paddy plants MFC (Average reading for all plants up to 66 days)



II) Isolation, Identification & Characterization of Bacteria

After the PMFC were run constantly to produce electricity, the electrodes were removed and scraped in saline suspension to isolate the bacteria responsible for electricity generation. In these studies 5 different cultures were isolated, which were characterized, based on their colony characteristics, morphological and biochemical characteristics and referring to the Bergey's Manual of Determinative Bacteriology the 5 isolates.

III) Bioelectricity generation with Bacterial cultures:

The isolated microorganism were grown in nutrient broth & succinate broth with appropriate nutrient conditions and electricity was measured for five days every 24 hrs.

Table 3.6: Bioelectricity generation from MFCs by isolated bacterial culture in nutrient broth

The isolated organisms were separately inoculated in nutrient broth. Firstly current generation was measured at 0 hrs. And kept for incubation at 37^0C for 24hrs. After the incubation of 24 hrs. Measured the electricity of cultures with the help of Multimeter. Bioelectricity was measured for five days every 24 hrs.

Table 3.6: Bioelectricity generation from MFCs by isolated bacterial culture in nutrient broth.

Sr. No.	Hrs	Current in mV					
		0	**24**	**48**	**72**	**96**	**120**
1)	**Control**	102	106	100	120	115	125
2)	**D-1**	100	666	600	677	660	600
3)	**D-2**	112	703	712	773	754	677
4)	**D-3**	115	850	823	782	801	703
5)	**D-4**	125	764	802	800	800	764
6)	**D-5**	120	712	742	771	760	800

From table 3.6 and figure 3.3 all the 5 bacterial isolates were capable of producing bioelectricity up to 5 days which generally increased from the first day. The average current generation for control was **113 mV** whereas for **D1 – 640.6 mV, D2 – 723.8 mV, D3 – 791.8 mV, D4 – 786 mV, D5 – 757 m**V. The current generation jumped up after first 24 hrs which remained more or less consistent for next 4 days, the setup and multimeter reading can be seen in plate no. 3.1. Similar results were observed when all the organisms were grown in succinate broth but, the electricity generation was slightly higher than that, when the isolates were grown in nutrient broth as can be seen from table 3.6, figure 3.3, plate 3.1

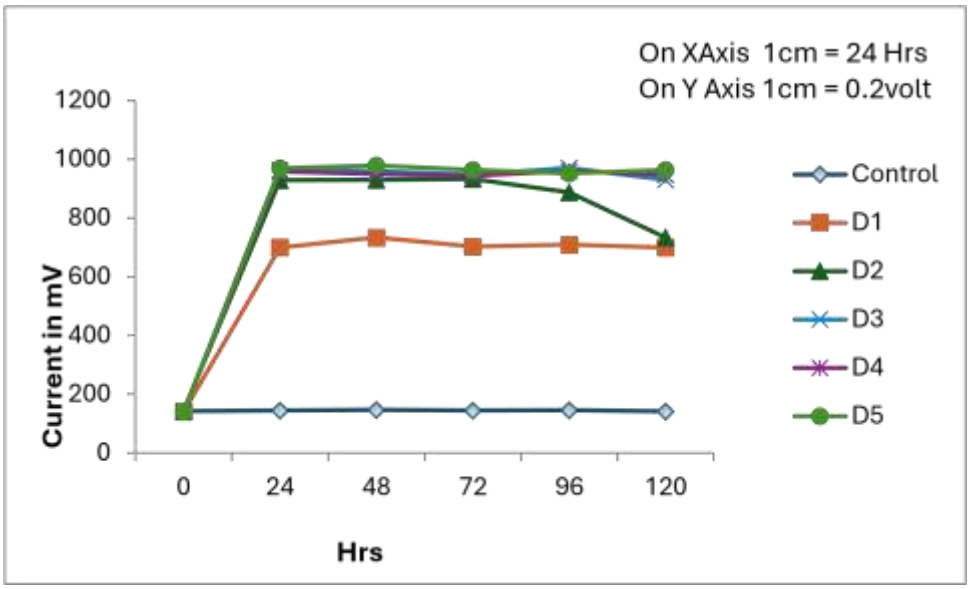


Fig. 3.3: Bioelectricity generation from MFC by isolated Bacterial culture in nutrient broth.

Plate 3.1: Electricity Production by Bacteria in nutrient broth.

Table No. 3.7: Bioelectricity generations in microbial fuel cell by isolated bacterial cultures in Succinate Broth.

Sr. No.	Hrs	Current in mV					
		0	**24**	**48**	**72**	**96**	**120**
1	**Control**	143	145	147	145	146	142
2	**D-1**	140	700	734	703	710	700
3	**D-2**	143	929	930	933	887	734
4	**D-3**	141	964	960	948	971	930
5	**D-4**	142	958	950	944	960	948
6	**D-5**	141	969	979	964	952	964

The isolated organisms were separately inoculated into succinate broth. Initial current generation was measured at 0 hours, followed by incubation at 37°C for 24 hours. After incubation, the bioelectricity produced by the cultures was measured using a multimeter. This process was repeated every 24 hours for five days.

As shown in Table 3.7 and Figure 3.4, all five bacterial isolates demonstrated the ability to generate bioelectricity over the five-day period, with an increase observed from the first day. The average current generation for the control was 145 mV, while the readings for the isolates were as follows: D1 – 709.4 mV, D2 – 882.6 mV, D3 – 954.6 mV, D4 – 952 mV, and D5 – 965.6 mV. A significant increase in current generation was observed after the first 24 hours, which then remained relatively stable over the next four days. The setup and multimeter readings are illustrated in Plate 3.1.

Similar results were observed when all the isolates were grown in succinate broth; however, electricity generation was slightly higher compared to the initial readings, as shown in Table 3.7, Figure 3.4, and Plate 3.2.

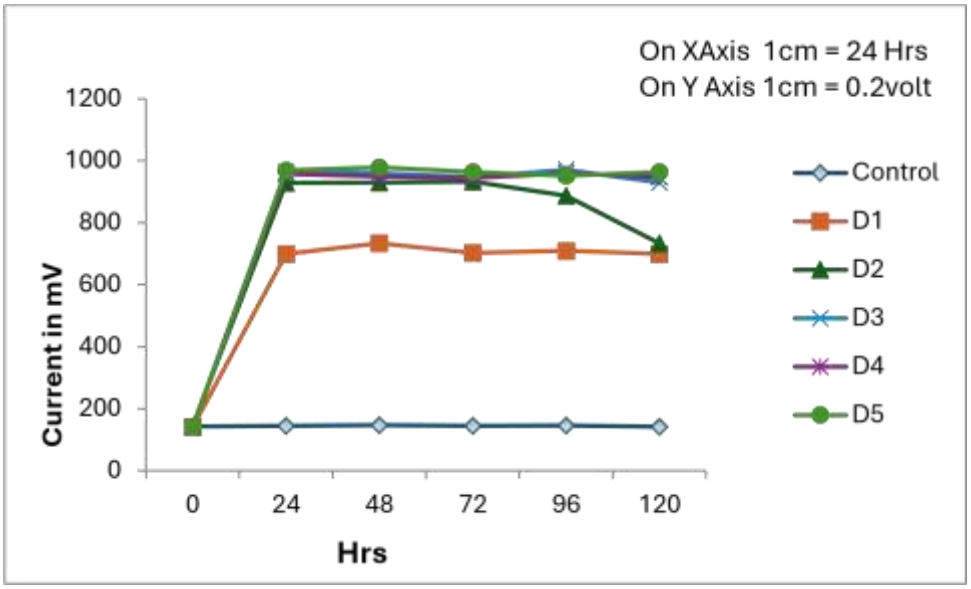


Fig. 3.4: Bioelectricity generation in microbial fuel cell by isolated bacterial cultures in Succinate Broth.

Plate 3.3 Electricity Production by Bacteria

In this experiment, the promising bacterial isolates were inoculated into two different media: nutrient broth and succinate broth. After every 24 hours of incubation, both the increase in bacterial growth and the rise in current generation were observed. The maximum current was recorded at 24-hour intervals.

Subsequently, 250 ml of each bacterial culture was transferred into separate ice trays. Each tray was fitted with a cathode (aluminium) and an anode (copper), which were connected in series using electric wires. The starting point of the cathode wire and the end point of the anode wire were then connected to an LED bulb using attachment clamps. Upon completing the setup, the LED bulb successfully lit up, as illustrated in Figure 3.3.

SUMMARY

In this investigation, electricity production was studied using bacteria isolated from paddy field soil in Lonavala, with paddy plants as the starting material. Bioelectricity generation in the PMFC setup was measured individually in 11 buckets, using a multimeter to record electric current in millivolts (mV). Additionally, temperature was measured using a thermometer, and pH was determined with pH paper. These readings were recorded daily over a period of 66 days, as detailed in Table 3.1, revealing the bacteria's ability to produce electricity.

The electrodes at the bottom of each bucket were washed with saline solution, and this washing solution served as the starting material for the isolation and identification of electricityproducing bacteria. From this screening process, five bacterial isolates were obtained. These isolates were characterized based on biochemical properties and morphology and were preserved on sterile nutrient agar slants.

To further evaluate their potential, these electricity-producing bacteria were inoculated into two different media. After 24 hours, the electricity production was measured, successfully demonstrating electricity generation by lighting up an LED bulb.

CONCLUSION

The PMFCs demonstrated bioelectricity generation using paddy plants collected from Lonavala, Dist. Pune. In the PMFC setup, the minimum control current was 245 mV, and the maximum was 390 mV, with a mean current generation of 349.74 mV over 66 days. In comparison, with paddy plants, the minimum electricity generation was 288 mV, the maximum reached 976 mV, and the average was 705.41 mV. When all 10 MFCs were connected in series, the electricity output significantly increased to 7–8 V, which was enough to power four LED bulbs simultaneously.

Among the five bacterial isolates from the paddy fields, Pseudomonas spp-4 exhibited the highest electricity production compared to the other isolates. Further research can explore the factors influencing electricity generation for optimization.

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