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BIOELECTRICITY PRODUCTION FROM *LYSINIBACILLUS SPHAERICUS* DMS-3 ISOLATED FROM SWINE WASTE

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ABSTRACT:

Microbial fuel cell (MFC) is a promising source of energy for future. In the present study, Swine waste was used for the electricity production. It produced a maximum of 330mV and 240mA for 10 days. The biofilm of swine waste from MFC was taken and serial dilution was performed and cultured for isolation of bacteria. Out of five isolates obtained, *Lysinibacillus sphaericus* DMS-3, a gram positive, rod shaped bacterium produced maximum electricity in MFC. The voltage and current generation was 510mV and 260mA respectively. The electrochemical activity of the bacteria was checked by cyclic voltammetry experiment and it has been confirmed by the oxidation and reduction peaks.

Keywords: *Lysinibacillus sphaericus*, swine waste, Microbial fuel cell, Electrochemically active bacteria, Cyclic voltammetry.

[I] INTRODUCTION:

Microbial fuel cell (MFC) technology is the promising approach for generating bioelectricity from biomass using microbes. The current energy demand has alarmed the entire world and this has created the interests in MFCs among scientists as a way to generate bioenergy or hydrogen from biomass without causing any environmental pollution problems. The type of microbe in the anodic chamber of an MFC, fuel cell configuration and operating conditions affects certain factors like Power output and Coulombic efficiency. [22]. This technology converts the energy stored in organic compounds to electrical energy through the catalytic reactions of microorganisms. This has grabbed the attention of many researchers in the field of science [3, 6 & 12]. MFCs operated using mixed bacterial cultures achieve greater power densities than those with pure cultures. In a recent experiment, however, a MFC showed high power generation using a pure culture, but the same result was not achieved using acclimated mixed cultures [16]. Bacterial population analysis of the microorganisms that exist in MFCs has so far exposed a huge variety in composition [1].

In most MFCs, the electrons from the anode diffuse through a separating membrane and reach the cathode to combine with protons and oxygen

provided from air; to provide water as the resulting product [8,11]. Chemical oxidizers, such as ferricyanide or Mn (IV), can also be used although these must be replaced or regenerated [15,18].

A real development was made when some microbes were found to transfer electrons directly to the anode [5,7]. The microbes are functionally stable and yield high charge efficiency [17]. *Geobacteraceae sulferreducens* [4], *Shewanella putrefaciens* [8], *Rhodoferax ferrireducens* [5] and *Geobacter metallireducens* [11] are all electrochemically active and can develop a biofilm on the anode surface and transfer electrons directly to the electrode.

In this study we have focused on using swine waste in the anodic compartment for the bioelectricity production. The main significance of the study is to generate bioelectricity from swine waste and also to reduce the environmental pollution caused by it. The biofilm from MFC was serially diluted and *Lysinibacillus sphaericus* was isolated and checked for the bioelectricity generation. This is the first report on using this bacterium isolated from swine waste for the bioenergy production.

[II] MATERIALS AND METHODS:

2.1 Construction of MFC:

The Cell is made out of an inert Plexiglas material each chamber with the dimension of 3.5cmX3.5cmX13cm. The chambers were connected with a 1.2 cm diameter, 5cm long plastic pipe with two provisions to fill salt bridge. The place where the rod connects with the cell is sealed properly to avoid leakage. The carbon electrodes of 2cm diameter and 16cm long were initially treated with 0.1N HCl overnight before using for the experiment and cleaned with 0.1N NaOH after the completion of experiment. The anode compartment has been filled with 300mL of the sample (swine waste) and 100mL of substrate (Vegetable waste). The cathode compartment was filled with distilled water which acted as air cathode.

2.2 Sample collection:

Swine waste sample was collected from Postgraduate Institute of Animal Sciences, Kattupakkam and the filtrate was prepared using 500mL of Sterile Distilled water.

2.3 Isolation and characterization of *Lysinibacillus sphaericus* DMS-3:

The biofilm was taken from the anode of MFC after 10 days and serial dilution was done and cultured for isolation of bacteria. Totally five bacteria were isolated from sample of dilution 10^{-6},10^{-7} and 10^{-8}. Among the five isolates tested, *Lysinibacillus sphaericus* produced the maximum voltage and current. The bacterium was characterized by gram staining followed by biochemical tests and 16srRNA sequencing was performed for the positive isolate using the primers: Forward – 8F (5'–GAGTTTGATCATGGCTCAG-3') Reverse–1495r(5'-CTACGGCTACCTTGTTACG-3').

The obtained sequence of culture has been deposited in the Genbank with accession number JX012235.

2.4 MFC with pure culture of *Lysinibacillus sphaericus* DMS-3*:*

Microbial fuel cell has been setup with anodic compartment filled with 500ml nutrient broth inoculated with 210CFU/ml pure culture of *Lysinibacillus sphaericus* DMS-3 and cathodic compartment filled with distilled water. The voltage and current were measured for 10 days and the results were shown in Figure 3.

2.5 Cyclic Voltammetry Experiment:

To confirm the presence of electrochemical activity of *Lysinibacillus sphaericus* DMS-3, the electrodes were taken after 15 days from MFC and the cyclic voltammograms were recorded in phosphate buffer pH – 7.0 (61.5ml of 1M Dipotassium hydrogen phosphate and 38.5ml of Potassium dihydrogen phosphate were taken and

made up to 1 litre with distilled water).The redox potential was measured in the presence of phosphate buffer pH – 7.0 in the absence of microorganisms by cyclic voltammetry using a PC4/750 potentiostat (Gamry Instruments).

[III] RESULTS:

3.1 MFC model:

In this study, swine waste has been tried out for the bioelectricity production. Figure 1 shows the MFC model with swine waste in the anode chamber.

Figure 1: MFC developed with swine waste in anode chamber

3.2 Bioelectricity generation from swine waste:

Swine waste has produced 330mV and 230mA on the tenth day. Figure 2 shows the gradual increase of voltage from 100mV to 330mV. The increase in current production (230mA) on the tenth day has been shown in Figure 3.

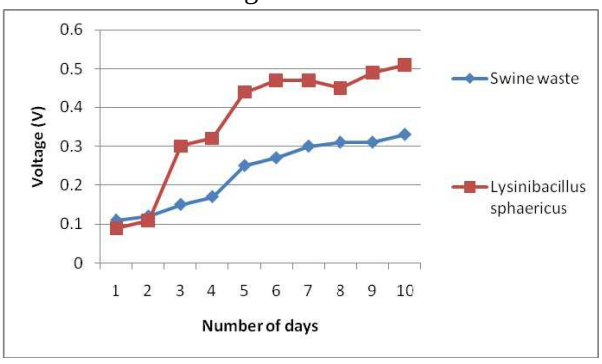

Figure 2: Voltage production of swine waste and *Lysinibacillus sphaericus* DMS-3

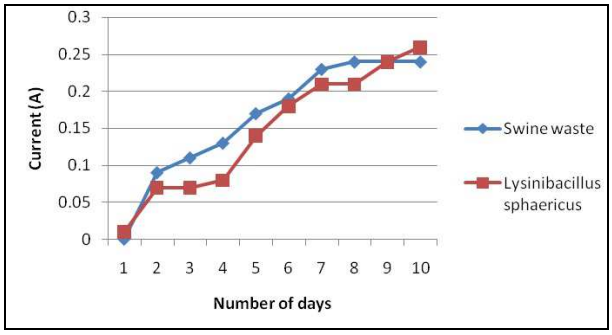

Figure 3: Current generation of swine waste and *Lysinibacillus sphaericus* DMS-3

3.3 Bioelectricity production by *Lysinibacillus sphaericus* DMS-3:

Lysinibacillus sphaericus DMS-3 inoculated in nutrient broth in anode chamber has produced a maximum of 510mV and 260mA. Figure 2 and 3 shows the voltage and current production of the bacterium.

3.4 Cyclic Voltammetry of *Lysinibacillus sphaericus* DMS-3:

The experiment has revealed that the oxidation of the substrate has initiated and the electrons have been released in the anode. The CV obtained showed oxidation and reduction peaks with an apparent redox potential of around 10 mV against the Ag/AgCl reference electrode. The oxidation and reduction peaks were observed and Eap was -0.34mV and Ecp was -0.25mV.

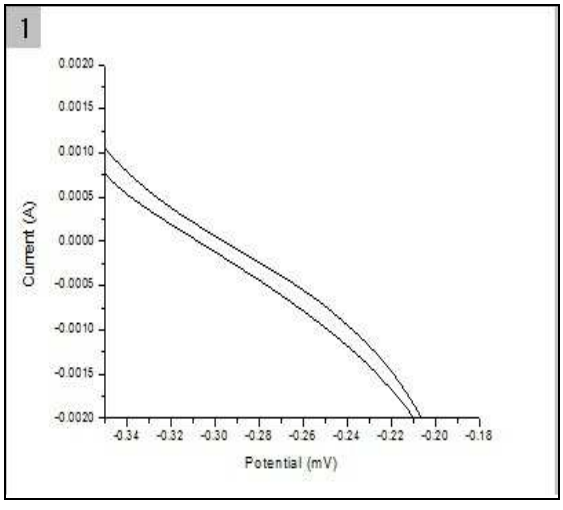

Figure 4: Cyclic voltammogram of *Lysinibacillus sphaericus* DMS-3

[IV] DISCUSSION:

MFC with different sources like domestic wastewater (10), sugar industry effluent (13), Paneer whey (2), artificial wastewater, Biodiesel production waste (20), potato wastewater, activated sludge (21), Dairy manure wastewater and composite chemical wastewater (19) have been used so far for generating electricity. The present study has been focused on using swine waste for the bioelectricity production.

Knight *et al*, 2008 (9) used *Aeromonas hydrophila* ATCC7966 in MFC was grown under anaerobic conditions with ferric citrate and reported that the electrochemical activity was observed in the organism. *Clostridrium sp* immobilized on a gold electrode were subjected to the electrochemical experiment and the cyclic voltammograms obtained were similar to a plateau. The anodic peaks at -0.16 and 0.44V were observed at a scan rate of 50mV s^{-1}. The redox molecules found in the outer membrane helps to record the cyclic voltammograms (13).

In the earlier reports, the direct electron transfer has been proved by performing cyclic voltammetry. Presence of two pairs of redox peaks has been recorded at 0.09 and 0.38V. Ferricyanide reductase and Lactate dehydrogenase are the responsible enzymes for these kind of peaks obtained in voltammograms (14).

The present study reveals that *Lysinibacillus sphaericus* DMS-3 isolated from swine waste is electrochemically active and it can be used for the bioelectricity production in future. The study also revealed that the bioelectricity can be produced by using swine waste which in turn may help in reducing the pollution.

[V] CONCLUSION

The work demonstrates the electrochemical nature of *Lysinibacillus sphaericus* isolated from swine waste. The maximum voltage production observed on the tenth day was 330mV and current production was 240mA when swine waste was used. On the other hand, *Lysinibacillus sphaericus* gave 510mV and 260mA on the same time period. The pure culture showed the maximum electricity production.

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