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EVALUATION OF ELECTRICITY GENERATION BY MICROBIAL FUEL CELL FROM HYPERSALINE INDIAN SODA LAKE

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ABSTRACT

India has faced challenge for more electricity generation and alternative for the traditional sources and due to the lack and depletion of primary sources. Microbial Fuel Cells (MFCs) is an alternative source of the sustainable and renewable energy.The objective of this study was to investigate the electricity generation from the haloalkalophilic bacterium isolated from Lonar soda crater (India). Aerobic, haloalkaliphilic bacteria were isolated and characterized from sediment and water samples. A total twenty eight bacterial culture were isolated, of which ARS4 was selected for the further MFC was investigated. The two-chamber MFCs were used to conduct experiments. The MFC was constructed and measure the electricity generation after various intervals, 387mV was electricity was generated after 1h, but after 48h the electricity generation dramatically decreased to 229mV. The effect of salt on MFC was also studied, NaCl enhanced electricity generation compared to KCl, indicating requirement of NaCl for bacterium ARS4 MFC. Supplement of glucose, increased electricity generation was found to be increases (170mV). Our results also suggest that seeking for and isolating novel bacteria that are more halophilic and alkaliphilic from Lonar crater could be a new strategy to generate bioelectricity from MFCs.

KEYWORDS:*Microbial Fuel Cells, Lonar soda crater, Electricity*

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INTRODUCTION

In related with global warming effects and decreasing limited fossil fuel sources have promote to the search for alternative sources for energy production[1]. Generally biomass products tested for energy generation include a wide range of growth plants, crops and wastes[2]. In this context, microbial fuel cells (MFCs) can serve as an alternative to land based methods for energy generation. The cultivation of microorganisms has several advantages over other alternatives- they require less low cost nutrient, have higher growth rates and less expensive energy production.The microbes are able to degrade the organic content to produce the electricity which is known as microbial fuel cell (MFCs). The microbes act as catalyst that degrades the organic manure resulting in the generation of electricity.It is specific designs that cause microbial metabolic or enzyme catalytic activity into electricity by using electrochemical technology[3].The MFC are potential for immediately capturing the electrons bring into existence when electrochemically unique bacteria degrade the organic substrates. Microbial fuel cell have become the dynamic due to the generate energy in the electric state or hydrogen from renewable sources of organic and inorganic substance by these activity MFC also can used in the bioremediation in the polluted soil and water.. The MFC are cells, producing electric power by utilizing microorganisms, instead of isolated enzymes, to assist redox reactions. The conventional MFC is a two chamber system, consistingofanode and cathode chambers that are separatedby a proton exchange membrane[4].MFCs have operational and functional advantages over the technologies currently used for generating energy from organic matter[5]. These approaches encourage, studying the microbial physiology, energy metabolism under the extremophilic environment, making MFC unique technique for studying the extracellular electron transfer system in mixed microbial communities and pure cultures. Some scientific investigation was studied for Shewanella sp. for the Electricity generation as MFC Some scientific investigation was studied for Shewanella sp.[6] Over the past decade, The most extensively studied has been execute MFCs that used electrochemically active bacteria (EAB) and these were enable to convert carbon substrates into electric pawar Over the past decade, The most extensively studied has been execute MFCs that used electrochemically active bacteria (EAB) to convert carbon substrates into electrical current and power[7-8]. Many EAB have the inherent ability to reduce metal content in theirnatural condition by performing extracellular electrontransfer[9-10]. These EAB naturally occurring environments in the world, such as ocean, lake and river[11-12]. The electricity production has not previously been linked to the metabolic activity of haloalkaliphiles or other salt-tolerant microorganisms. However, electricity generation by anaerobic bacteria and anoxic sediments from hypersaline soda lakes was investigated by Miller and Oremland[13].Soda lakes are a specific type of salt lake with high to extremely high carbonate alkalinity (pH from 9 to 11) and a moderate to extremely high salinity. They are spread all over the world, but located mostly in inland salt lakes, arid and semi-arid areas where the evaporative climate favors accumulation of salts in local depressions. These equally extreme conditions make soda lakes a unique ecosystem. In the last decade, special attention has been given to the investigation of the microbial communities in soda lakes using traditional isolation methods and molecular biology techniques. But very few studies were investigated related to the electricity generation from MFCs from soda lake. The alkaline Lonar Lake in Buldhana district of Maharashtra is unique ecosystem and wonder in the India (latitude 19º58', Longitude 76º36'). The lake water is alkaline having an average pH of 9.5- 10. Lonar Lake is a closed lake without any outlet and unique due to its salinity, alkalinity and biodiversity. Due to the uniqueness, the lake has evoked much scientific value among researchers. The ecology and diversity of an soda lake was studied and extensively reviewed for their biotechnological potential from all over the world. The microbial diversity of saline lakes has been studied primarily by focusing on the isolation and characterization of individual organisms with potential industrial applications[14-18].Many previously unidentified MFCs have been discovered in environmental samples. However, due to the difficult and time consuming procedures required to isolate such species, potentially useful EABs native to the environment most likely remain undiscovered[19]. As far as Indian soda lakes are concerned, a culture-dependent and independent approach has been applied to analyze bacterial diversity[20]. But MFCs studies for the electricity production have not previously been studied from haloalkaliphiles or other salt-tolerant microorganisms from the Lonar soda lake. The aim of the present study was to get insight into the species composition of microbial communities for electricity generation from Lonar crater.

MATERIALS AND METHODS

Enrichment and isolation of microorganisms

Enrichment of water samples and sediment samples were carried out in various enrichment media. All flasks were incubated at 37°C on a rotary shaker (100 rpm) for 48h. After enrichment, the organisms were isolated on respective media agar plates and incubated at 37°C for 24h. Well isolated and morphologically distinct colonies from these plates were transferred on the respective medium slants and maintained as stocks.

Identification of the bacterial culture

Bacterial cultures were examined for their cultural, morphological character, and standard biochemical test were performed according to Bergey's Manual of systematic bacteriology.

Cultural condition

The culture was retrieved by streaking on bioluminscent agar plates and incubated at 37^{o}C. For MFC operation 2-3 isolated colonies were inoculated in 100 ml of bioluminscent Broth and incubated at 37^{o}C at 160 rpm in 48 hrs in shaking conditions.

Enrichment of culture

The 100 mL bioluminscent media prepared and inoculated with culture and incubated for 48h at 37°C.

All the flasks were incubated at RT on arotary shaker (120 rpm) for 48h. After enrichment, these cultures serve as a MFC for the electricity generation.

MFC assembly design and component

Electrode

Carbon electrode (Copper) of dimension 15cm×0.2cm were used at both the ends of cathode and anode and tightly fixed with the containers containing medium, culture and distilled water.

Cathodic chamber

The cathode chamber of the MFC was made up of 0.5 liter plastic bottles filled with distilled water as catholyte.

Anodic Chamber

0.5 liter plastic bottles were used for this purpose. The bottles were surface sterilized by washing with 70% ethyl alcohol and followed by UV exposure for 15 minutes. Then 100 ml of previously enriched culture of bacteria was added in sterile soil.

Salt bridge

The salt bridge was prepared by dissolving 3% agarose in 2M NaCl. The mixture was boiled for 2 minutes and poured in PVC pipes (dimension 10 × 3 cm) under aseptic condition.The salt bridge was properly sealed and kept in refrigerator for proper settling. Two holes were made in the lower side of bottles for the insertion of salt bridge.

Circuit Assembly

Two chambers were internally connected by salt bridge and externally the circuit was connected with copper wires which were joined to the two electrodes at its two ends and to the multimeter by another two ends.

Measurement of potential difference and current

The potential difference generated by the Fuel Cell was measured by using multimeter from HAOYUE -DT830D.

MFC operations

All the components of MFC were connected i.e. via salt bridge internally and with externally with wires to the multimeter. The pure colony were aseptically transferred in 100 ml specific broth and incubated at 37° C at 160 rpm for 48 hours. The bottles were surface sterilized prior to operation of MFCs by 70% alcohol and exposed to UV radiation for 15 minutes.The salt bridge was sealed inside the holes under aseptic conditions. Sterile soil was poured in the anodic chamber and ARS4 culture broth was mixed in sterile soil and prepared liquid suspension. Then in cathodic chamber sterile distilled water was poured. The MFCs was operated at room temperature. The MFC set up was kept at static conditions. The varied carbon source and salts concentration was one by one tested isolates for their ability to generate potential difference. The MFCs was run up to 48 hrs and the voltage was recorded at every 1h,24h,48h interval in all the cases.

RESULTS AND DISCUSSION

The alkaline Lonar Lake is a unique basaltic rock meteorite impact crater. Lonar Lake is a one such soda Lake in which the indigenous microflora is present, and such microbial flora has ability thrives in alkaline condition[21]. Total twenty eight bacterial isolates obtained in the isolation exercise, cultural, morphological characteristics and standered biochemical test were performed for of all the isolates. This was done to avoid several identical isolates from the total isolates. Out of twenty eight, seven bacterial cultures were selected for the electricity generation, out of seven one bacterial isolate ARS4 was selected for the further MFC studies. In the present study, the 48h alkaline pH (10) culture was prepared and aseptically pours into anodic chamber which contain sterile soil.. Similar study was performed with MFC *B. selenitireducens* by Miller and Oremland[13].Electricity can be generated directly from sewage sludge with a microbial fuel cells (MFCs), combining degradation of organic matter and MFC for the generation of bioelectricity and the degradation of sewage sludgeorganic matter under the alkaline condition studied by yuan*et al.*[22]. In the present study, the 48h alkaline pH (10) culture was prepared and aseptically poured into anodic chamber which contain sterile soil. After the preparation of sediment slurry, MFC assembly was constructed MFC was constructed and measure the electricity generation after various intervals such as 1h, 24h and 48h. the electricity generate 387 and 290 mV after 1h and 24h interval respectively, but after 48h the electricity generation was dramatically decrease about 41% (229mV).Velasquez-Orta*et al.*[23] evaluated the performance of MFCs using two different types of algae as substrates- *Chlorella vulgaris* (a microalgae) and *Ulvalactuca* (a macroalgae). One of the first pure cultures to be studied as an oxidation catalyst in MFCsystems was *Shewanellaoneidensis*[6,24].Salinity effect on the microbial fuel cell performance was investigated. The 4% of Nacl was added into the MFC, after the addition of NaCl, the electricity generation was found to be decrease. But as the incubation period was increase the electricity was also increases (1h, 19mV), after 24h and 48h the electricity was 111mV and 224mV generated respectively. The contractory result was observed by Luo *et al.*[25], after the addition of NaCl on electricity generation was decreases. Muralidharan *et al.*[26] was studied on the impact of salt concentration on electricity production in microbial hydrogen based salt bridge fuel cells. The best of our knowledge, these MFC was most efficient for electricity generation. Parkash *et al.*[27] studied the impact of various salt and concentration on the electricity generation based on dual chambered MFC. They revealed that the KCl salt bridge was efficient electricity generating that the NaCl. The 4% of KCl was added into the MFC, after the addition of KCl, the electricity generation was found to be decrease. But as the incubation period was increase the electricity was also increases (1h, 4mV), after 24h and 48h the electricity was 104mV and 117mV generated respectively. But in the present study, the effect of salt on MFC, Nacl was the prominent electricity generation than the KCl, indicating a bacterium ARS4 was required NaCl for the MFC.Tarte *et al.*[28]were studies on effect of NaCl and Glucose on generation of electricity from waste water with the different combinations of glucose and NaCl. In the present studies, bacterium ARS4 also supplemented with the glucose, after the addition of glucose, the electricity generation was found to be increases. When the incubation period was increase the electricity also increases (1h, 119mV), after 24h and 48h the electricity was 168mV and 170mV generated respectively.

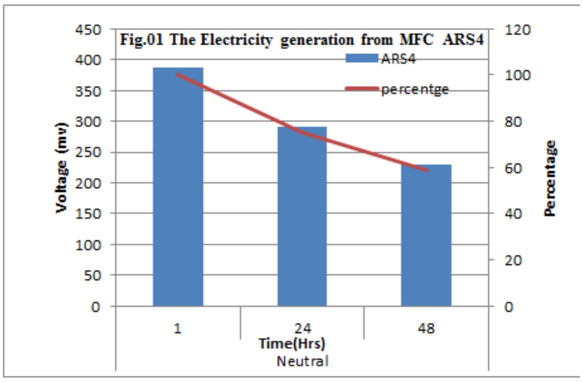

Fig.01 The Electricity generation from MFC ARS4

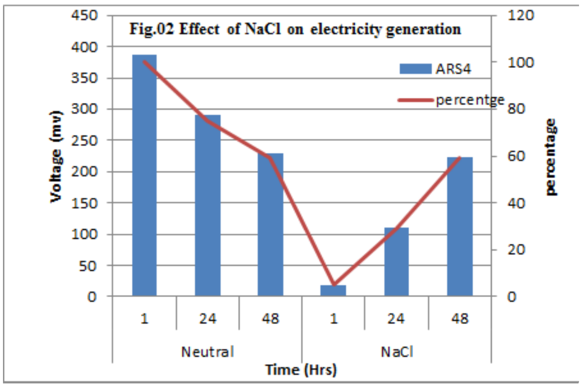

Fig.02 Effect of NaCl on electricity generation

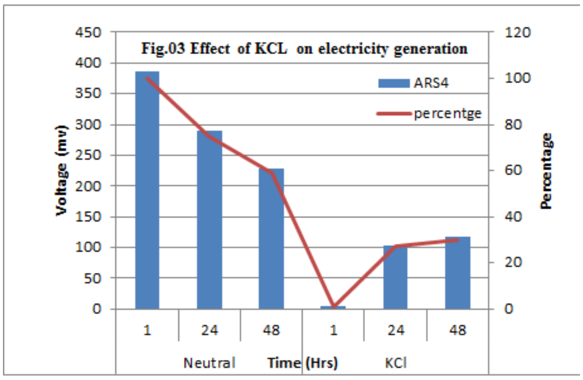

Fig.03 Effect of KCL on electricity generation

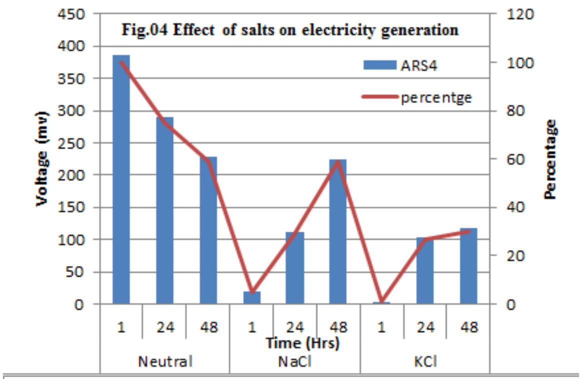

Fig.04 Effect of salts on electricity generation

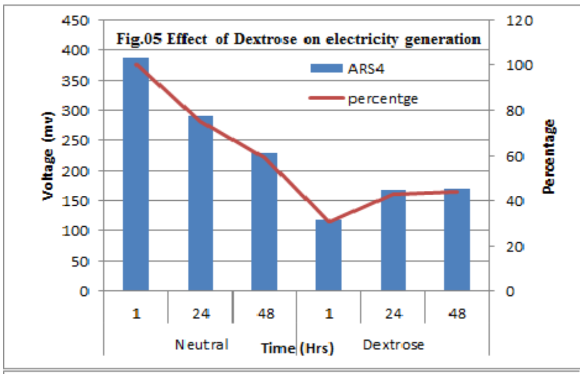

Fig.05 Effect of Dextrose on electricity generation

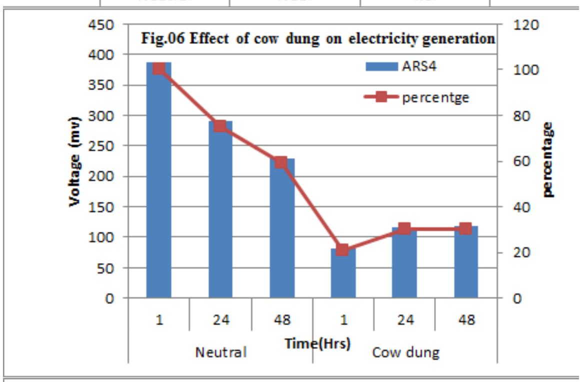

Fig.06 Effect of cow dung on electricity generation

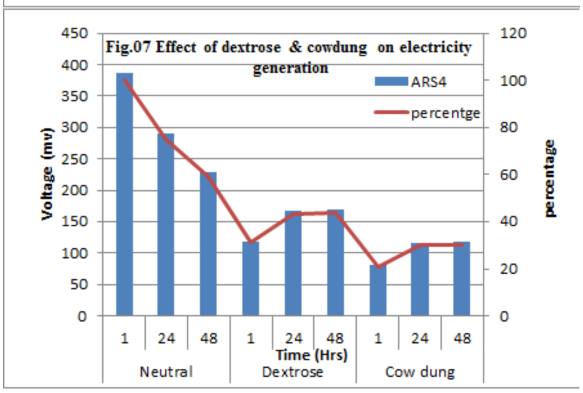

Fig.07 Effect of dextrose & cowdung on electricity generation

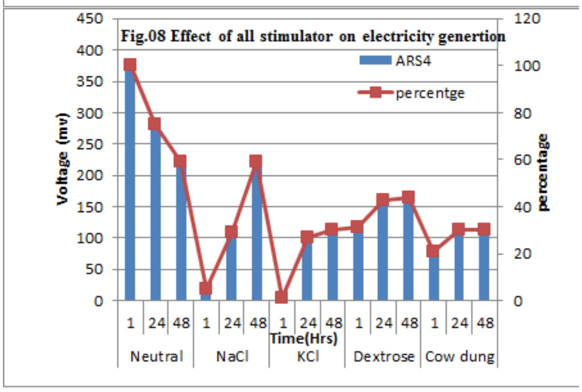

Fig.08 Effect of all stimulator on electricity genertion

The various studies have been performed such as the effect of different carbon sources sugars, starch, and cellulose, salt, MFCs From waste water, and substrate for MFC's include human, animal, and industrial waste water[29]. Evaluation of electricity generation from animal based wastes in MFCs has also been studied[30]. In the present study, use of animal manure as the organic fuel and its bacteria as biocatalyst in the anode chamber along with water as catholyte for the production of bioelectricity generation by MFCs. The bacterium ARS4 also supplemented with the cow dung as organic fuel, after the addition of sterile cow dung suspension, the electricity generation was found to be increased. When the incubation period was increased the electricity also increases (1h, 81mV), after 24h and 48h the electricity was 116mV and 118mV generated respectively. Similar finding was studied by Kumar *et al* [31]explored the usage of cow dung as anolyte against sewage water as catholyte.

CONCLUSION

A total twenty eight bacterial isolates obtained in the isolation exercise, cultural, morphological characteristics and standard biochemical test were performed for of all the isolates. Out of twenty eight, seven bacterial cultures were selected for the electricity generation; out of seven ARS4 was selected for the further MFC studies. In this study, double chamber MFC using bacterium ARS4 was used as biocatalyst. Anode chamber was kept up in batch condition for the addition of sugar, cow dung and in cathode chamber salt was added and maintained at continuous condition. The MFC was constructed and measure the electricity generation after various intervals, 387mV was electricity was generated after 1h, but after 48h the electricity generation was dramatically decrease upto 229mV.The effect of salt on MFC, NaCl was enhance electricity generation than the KCl, indicating a bacterium ARS4 was required NaCl for the MFC and salt, sugar, and cow dung had an even greater effect on execute the electricity generation by MFCs. Our results alsosuggest that seeking for and isolating novel bacteria that are more halophilic and alkaliphilic fromLonar crater could be a new strategy to generate bioelectricity from MFCs.

CONFLICT OF INTEREST

Conflict of interest declared none.

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