Advances in Biological Research 7 (5): 136-140, 2013
ISSN 1992-0067

DOI: 10.5829/idosi.abr.2013.7.5.1109

Construction of Double Chambered Microbial Fuel Cell (MFC) Using Household Materials and *Bacillus megaterium* Isolate from Tea Garden Soil

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Abstract: The current study was carried out for the isolation and screening of potential bioelectricity generating bacteria from tea garden soil samples and also to construct an indigenous microbial fuel cell (MFC) using house hold wastes. *Bacillus megaterium* was found to the best isolate for the production of bioelectricity, out of a total of 25 bacterial isolates from soil samples collected from Lepetkata Tea Estate of Dibrugarh district of Assam. The isolate was identified on the basis of staining techniques and biochemical characteristics. Two chambered MFC was constructed using two poly acrylic containers of 500 ml volume each. The two chambers were connected using an agar salt bridge and carbon rods were used as electrodes. The electricity generated by the isolate was compared using glucose and fructose as sole carbon source in minimal media. The maximum voltage was found to be 440 mV in presence of glucose as sole carbon source after 84 hrs of incubation at room temperature. The voltage was further increased up to 698 mV after the media was supplemented with 1.5% (w/v) yeast extract, which would have served as additional source of vitamin to the bacteria to proliferate. During the entire study, the experimental set up was allowed to incubate at room temperature and occasional shaking was done manually, hence no external electricity was required. With all the above features the isolate *Bacillus megaterium* was found to be a good source of bioelectricity.

Key words: Bioelectricity · MFC · *Bacillus megaterium* · Tea garden soil

INTRODUCTION

The global energy demand is increasing with exponential growth of population. Unsustainable supply of fossil fuels and the environmental concerns like air pollution and global warming associated with the use of fossil fuels are acting as major impetus for research into alternative renewable energy technologies. The high energy requirement of conventional sewage treatment systems are demanding for the alternative treatment technology which will require less energy for its efficient operation and recover useful energy to make this operation sustainable. In past two decades, high rate anaerobic processes such as up-flow anaerobic sludge blanket (UASB) reactors are finding increasing application for the treatment of domestic as well as industrial wastewaters. Microbial fuel cell (MFC) is a promising technology for simultaneous treatment of organic wastewater and bio energy recovery in the form of direct electricity, which has gained much interest in recent years [1, 2]. Microbial fuel cells (MFCs) are devices that use bacteria as the catalysts to oxidize organic and inorganic matter and generate electricity [3, 4].

Clostridium acetobutylicum, *Clostridium thermohydrosulfuricum, Saccharomyces cerevisae* etc. are well known microbes used for the production of bioelectricity [5, 6, 7]. The marine microalgae *Isochrysis* sp., *Nanochloropsis* sp., *Dicarteria* sp., *Chaetoceros calcitrans, Pavlova* sp., *Synecocystis* sp., *Dunaliella* sp., *Cholorella salina, Tetrasilmis gracilis* etc. also showed the capability of producing electricity when cultured in microbial fuel cell system [8, 9].

The state Assam, located in North-East India is well known for its biodiversity across the world. There are so many micro flora and fauna are known to be existing in the region which are found to be having various economical and commercial importance. Currently the research in the field of exploration of native microbial strains for the production of bioelectricity in the region is found to be very limited with the evidence of scientific publication.

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In our current study, we have considered tea garden soil as a source of potential microorganisms capable of generating electricity, considering the fact that the soil would have provided favorable conditions for the survival of cellulose degrading bacteria. Further, effort has been made for the construction of cost effective indigenous microbial fuel cell (MFC) using household materials.

MATERIALS AND METHODS

Isolation and Screening of the Potential Bioelectricity Generating Bacteria from Tea Garden Soil Samples: Tea garden soil samples were collected from Lepetkata Tea Estate, located in Dibrugarh District of Assam. Samples were brought to the Lab in sterile air sealed packets to retain the moisture. Soil samples were serially diluted upto 10^{-8} dilutions using 0.5% saline water and plated on cellulose agar medium (0.05% KH_2PO_4, 0.025 % $MgSO_4$, 0.2% cellulose, 1.5% agar and 0.2% gelatin). Colonies obtained were sub-cultured subsequently to obtain pure culture. Pure cultures obtained were inoculated in minimal media (0.8% Glucose, 0.3% KH_2PO_4, 0.6% K_2HPO_4, 0.5% NaCl, 0.2% NH_4Cl and 0.01% $MgSO_4$) broth and incubated at 36°C ± 2 for 48 hrs at 135 rpm (Sartorius Stedim-Certomat BS-1 Shaker Incubator, Germany Gmbh).

Identification of the Potential Isolate: The identification was done on the basis of various staining procedures and biochemical tests prescribed by Bergey's Manual of Systematic Bacteriology *IVth Edition* [10].

Construction of Microbial Fuel Cell: A double chambered microbial fuel cell (MFC) was constructed using waste material. 500 ml polyacrylic jars were used for the construction of the two chambers (cathodic and anodic chamber) and were connected using an agar salt bridge (3% KCl agar) with a length and diameter of 5 cm and 0.5 cm respectively. The anodic and cathodic chambers were filled with Basal minimal salt media and freshly prepared 100 mM Phosphate buffer (pH 7) respectively. 15cm long carbon electrodes were used in each chamber. The electrodes measured a diameter of 1 cm. The containers were kept air tight during the entire incubation period. Schematic representation of the MFC has been shown in the Fig. 1.

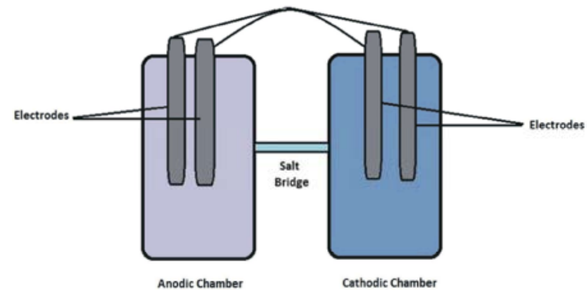

Fig. 1: Schematic representation of a Microbial Fuel Cell (MFC)

Formulation and Standardization of Media: The media filled in the anodic chamber was designed using the results of the biochemical characterization. Basal's Minimal Salt Media was used where glucose was used as the main carbon source as the glucose utilization test for the bacterial strain was positive and the other sugars either had a negative result or a delayed reaction. Minimal media with different carbon sources 0.8% of glucose, fructose, lactose, maltose and starch (soluble) were taken in different conical flasks to check the highest absorbance after the inoculation. Isolate was incubated in 100 ml minimal media taken in 250 ml Erlenmeyer flask maintained at 36°C ± 2 for 48 hrs at 135 rpm. The absorbance was taken at 660 nm against suitable blank.

Comparison of Media with Reference to the Production of Electricity: The voltage produced by the microbial fuel cell was measured regularly. This voltage was used to compare the electricity generation between the cell containing glucose as the sole carbon source and the cell containing fructose as the carbon source. The voltage was measured to give a comparative analysis of the efficiency of the microbial culture to degrade the constituents of the media. All the media used in the study were purchased from HiMedia India Pvt. Ltd. and all the chemicals and reagents were purchased from Merck India Pvt. Ltd.

RESULTS

The microbes were isolated from tea garden soil and were characterized. A total of 25 isolates were obtained from 3 (three) tea garden soil samples. The isolate S 23 was found to be showing maximum cell density of 0.89 (data not shown) after 48 hrs of incubation. The potential isolate S 23 was considered for further studies. The potential isolate was identified as *Bacillus megaterium* on the basis of various staining methods and biochemical characterization prescribed by Bergey's Manual of Systematic Bacteriology, *IVth Edition* [10]. The biochemical characteristics of the isolate were shown in Table 1. Biochemical characterization was carried out using commercially available kits HiBacillus™ KB013.

Table 2: Absorbance of the media containing different carbon sources which were measured after 48 hrs of incubation

Carbon Source	Absorbance at 660nm
Glucose	0.061
Fructose	0.041
Lactose	0.002
Maltose	0.046
Starch	0.063

Fig. 2: The MFC constructed in the lab attached with a multimeter

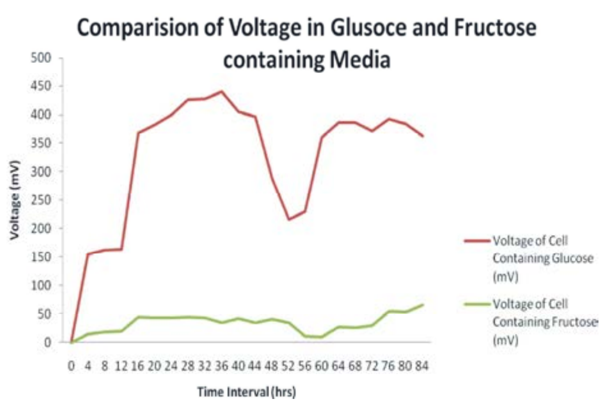

Fig. 3: Graphical representation of Voltage measured after 48 hrs of initial incubation for cells containing glucose and fructose at an interval of 4 hrs

Double chambered MFC was constructed using waste material (Fig. 2). The bacterial strain was inoculated in the Anodic chamber and incubated for 7 days at room temperature. The voltage generated was measured at an interval of 4 (four) hrs after 48 hrs of initial incubation.

Standardization of the media was carried out using Minimal salt media supplemented with different carbon sources in separate conical flasks to check the highest absorbance after the inoculation. The carbon sources

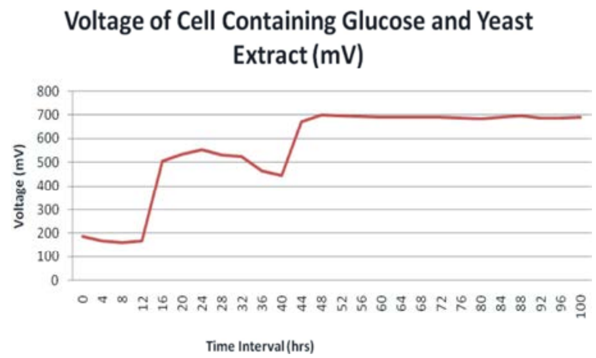

Fig. 4: Graphical representation of Voltage measured for cells containing glucose supplemented with and Yeast Extract at an interval of 4 hrs

used were glucose and fructose, maltose, lactose and starch (soluble). The absorbance measured at 660 nm and the values obtained were tabulated (Table 2). Glucose was found to the most suitable carbon source followed by fructose, as the isolate was found to be showing maximum absorbance in the medium containing glucose as sole carbon source.

The maximum voltage was found to be 440 mV and 66 mV after 84 hrs and 128 hrs of incubation using glucose as sole carbon source respectively (Fig. 3). The voltage was again found to be increasing up to 698 mV after 48 hrs of incubation using glucose and yeast extract as sole carbon source and external vitamin source respectively (Fig. 4).

DISCUSSION

The current study was carried out to screen potential bioelectricity generating bacteria from tea garden soil, considering the probability to obtain highly efficient microbes to fulfill the objectives, as the North-East India is well known for its biodiversity. The potential isolate was identified as *Bacillus megaterium* on the basis of biochemical characteristics and staining techniques. Though most of the previous literature shows *Clostridium butyricum* [11], *Saccharomyces cerevisae* [12], *Proteus vulgaris* [13], *Shewanella putrefaciens, Geobacter sulfurreducens*, *Geobacter metallireducens* and *Rhodoferax ferrireducens* [14], *Clostridium acetobutylicum, Clostridium thermohydrosulfuricum* [5, 15], *Isochrysis* sp., *Nanochloropsis* sp., *Dicarteria* sp., *Chaetoceros calcitrans, Pavlova* sp., *Synecocystis* sp., *Dunaliella* sp., *Cholorella salina, Tetrasilmis gracilis,* [8], *Shewanella sp.* [16, 17, 18], *Klebsiella sp.* [19], *Corynebacterium sp.* [20], *Enterobacter cloacae* [21] and *Lactococcus lactis* [22] as a potential strain for the production of bioelectricity, but our currents study shows that *Bacillus megaterium* as the best isolate out of a total 25 isolates. Moreover the voltage was found to be increasing rapidly upto 698 mV after the media was supplemented with yeast extract (Fig. 4).

This feature is certainly the most 'green' aspect of microbial fuel cells. Electricity is being generated in a direct way from biowastes and organic matter. This energy can be used for operation of the waste treatment plant, or sold to the energy market. Furthermore, the generated current can be used to produce hydrogen gas. Since waste flows are often variable, a temporary storage of the energy in the form of hydrogen, as a buffer, can be desirable.

As previously reported, in anaerobic processes the yield of high value electrical energy is only 1/3rd of the input energy during the thermal combustion of the biogas. While recuperation of energy can be obtained by heat exchange, the overall effective yield still remains of the order of 30% [23].

A microbial fuel cell has no substantial intermediary processes. This means that if the efficiency of the MFC equals at best 30% conversion, it is the most efficient biological electricity producing process at this moment. However, this power comes at potentials of approximately 0.5 Volts per biofuel cell. Hence, significant amounts of MFCs will be needed, either in stack or separated in series, in order to reach acceptable voltages. If this is not possible, transformation will be needed, entailing additional investments and an energy loss of approximately 5%.

Another important aspect is the fact that a fuel cell does not as is the case for a conventional battery- need to be charged during several hours before being operational, but can operate within a very short time after feeding, unless the starvation period before use was too long to sustain active biomass.

During the current study, the voltage generated by the isolate was found to be almost constant around 690 mV for a longer duration of time (100 hrs). With all these features, the strain *Bacillus megaterium,* isolated from tea garden soil of Lepetkata Tea Estate, located in Dibrugarh district of Assam was found to be a potential strain for further studies.

CONCLUSIONS

In the future, the amount of low-power devices implanted in the human body will significantly expand. These devices need long term, stable power provision. To provide this power, a MFC can be used. Two possibilities exist: enzymatic and microbial fuel cells. In enzymatic fuel cells, the potential difference is created by the use of two electrodes with different enzymatic reactions, creating a potential difference based on the reaction redox potential. Micro-organisms have the advantage of providing longer term stability than enzymes immobilized onto a surface.

Conflict of Interest: The authors declare that they have no conflict of interest and do not have any financial relationship with the organization that sponsored the research in the manuscript.

ACKNOWLEDGEMENTS

The authors acknowledge Director, Centre for Studies in Biotechnology, Dibrugarh University for providing all the facilities to carry out the study and DBT-MHRD, Govt. of India for funding.

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