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A Membrane Less, Single Chamber Microbial Fuel Cell for Waste Water Treatment

K. Anantha Narayanan, Stalin Selvaraj and MeeraParthasarathy
Centre for Nanotechnology and Advanced Biomaterials, SASTRA University, Thanjavur 613-401, Tamil Nadu, India

Corresponding Author: MeeraParthasarathy, School of Chemical and Biotechnology, SASTRA University, Thanjavur-613-401, Tamil Nadu, India

ABSTRACT

The electrochemical activity of four different species of microbes isolated from sewage water was established. The bacteria were screened on EMB medium post isolation in order to differentiate and characterize them. The metallic green colonies in EMB were confirmed to be (1a) *Escherichia coli*, The (1b) pink and the (1c) purple colonies were Lactose consuming organisms. The colorless colonies (1d) are organisms that do not consume lactose. Glass wool and Glass Beads were used in the mediator less, single chambered MFC instead of the proton exchange membrane. The catholyte consists of standard NaCl as the electrolyte. The anolyte consists of nutrient broth enriched with 80% artificial sewage water. Graphite electrodes were used as both cathode and anodes in this study. The maximum power density from the *E. coli* (1a) fed MFC was 12.94 mW m^{-2}, from the Lactose consuming (1b) fed Microbial Fuel Cell (MFC) was 184.36 mW m^{-2}, from the lactose consuming (1c) fed MFC was 298.47 mW m^{-2} and from the non-lactose consuming (1d) fed MFC was 400.92 mW m^{-2}. The present study demonstrates the Immense ability of the lactose consuming (1b) and (1c) as a means of monitoring Lactose and hence a microbial sensor in monitoring Diary industry effluent (1c) enjoys a potential application in waste water treatment plants that are coupled with current generation.

Key words: Microbial fuel cell, glass wool, polarization curve, waste water, membrane less

INTRODUCTION

Microbial fuel cells are electrochemical systems where the biological activities of microorganism are used to convert chemical energy directly into electricity. Bacteria that transfer electron directly onto the surface of the electrodes, without the use of an external mediator are known as Electrochemically Active Bacteria (EAB) (Kim *et al*., 2006). The most commonly used membrane is nafion (Chae *et al*., 2008). EAB are grown in the anodic chamber oxidizing a substrate which acts as the indirect electron donor. The electrons are transferred to the anode surface through the membrane bound proteins residing in the bacterial cell membrane. Studies have shown that the membrane protein cytochrome-C is said to have a direct involvement in the electron trafficking pathway (Kim *et al*., 2006). While the protons make their way through the cation exchange membrane, the electrons deposited onto the anode, simultaneously take an external circuit path to reach cathode where a reduction reaction take place (most commonly Oxygen reduction or Ferricynaide reduction). Trapping the electrons for harnessing power as they travel through the

external circuit makes them a subset under sustainable electricity generation systems. Such an ability of the microorganism is exploited in various engineering applications like Biosensors (Lorenzo *et al*., 2009), Waste water treatment (Ghangrekhar and Shinde, 2007) and power generation. MFC enjoys the special status of producing useful power from waste matter (Cha *et al*., 2010). In this era of increasing economic and energy strain on pollution control and waste treatment systems, there is an immense demand for an alternate technology which is economically effective and as well as stringent in terms of energy consumption. Thus the pollution control industry has taken a new shape with sudden emergence of interest in sustainable and clean source of power with emphasis on minimal carbon foot print.

Coupling an MFC with waste water treatment can be a sustainable source of pollution management system that aids in energy recovery and in effective removal of organic sludge. They are a cost effective alternate to the conventional pollution control strategies that are phenomenally expensive. Since a major part of the chemical energy of the waste water is directly converted into electricity, these processed involves a simultaneous decrease in the generation of excess sludge (Jang *et al*., 2004). Studies involving energy production using MFC fed with waste water from different sources has established them as one of the most inexpensive sources of energy. The use of a cationic membrane, an expensive metal coated electrode, synthetic growth media are some factors that makes the scaling up of an MFC a rather complicated issue. The selection of anode inoculums is an important parameter in MFC working. In this study, a mediator less and membrane less MFC (Du *et al*., 2011; Jang *et al*., 2004; Ghangrekhar and Shinde, 2007; Kim *et al*., 2007) was constructed. The anode was inoculated using organisms isolated from sewage water keeping in mind the potential impact these systems have on environmental friendly pollution control cum power generation processes. The economic design focused on coupling a waste water run electrical power production device where expensive additions like cation exchange membrane and catalyst coated cathode were avoided.

MATERIALS AND METHODS

Materials: The inorganic salts used in the artificial sewage water preparation were purchased from HiMedia Labs, Mumbai. The graphite electrodes used in the study were furnished by Rajkart enterprises, Chennai. The acrylic sheets used for making the MFC were bought at Prakash Acrylics, Chennai. A resistance box (Model AS-5S, Century Technologies, India) and source meter (Keithley 2601 System Source meter) were used for taking the polarization readings. Nutrient broth, nutrient agar and Eosin Methylene Blue (EMB) agar were purchased from HiMediaLabs, Mumbai. The sewage water was filtered to remove all macroscopic contaminants and any possible larvae or other biological vectors before any further experimental processing. The glass beads (purchased from The Madras Scientific, Chennai) and glass wool (purchased from The Madras Scientific, Chennai) were thoroughly sterilized in steam and 70% ethanol prior to use in every run of MFC. All the reagents used were of analytical grade.

Methods

Isolation and culturing of microorganisms: To isolate the possible electrochemically active bacteria from waste water, anaerobic sludge was collected from a continually flowing sewer source (Kiely *et al*., 2011; Jiang *et al*., 2010) in SASTRAs main campus. The sample was filtered to remove the possible macroscopic biological agents that may be potentially harmful. The undiluted stock sample (10°dilution) was subjected to serial dilution technique with sterile distilled water. The 10^{-6}

dilution was screened for microorganisms on an EMB medium. The secondary screening procedure involved growing the different colonies obtained on EMB, on Nutrient Agar plates and comparing their different colony morphologies. Gram staining (HiMedia Labs, Mumbai) was done to determine the shape and for further differentiation.

Prior to their use in the MFC, the isolated bacteria were cultured in 500 mL conical flasks with 200 mL nutrient broth as the substrate. They were allowed to grow till their mid log phase on a shaker at 140 rpm before enriching it with the Artificial Sewage Water (ASW).

Microbial fuel cell design: The MFC used in our study was a single chambered, mediator less and membrane less setup. The anode and cathode compartments were connected by Glass wool and glass beads which were used instead of the cation exchange membrane. A constant electrode separation distance was maintained at 18 cm. Plain graphite rods of dimension 8×2×2 cm were used as the electrodes, no corrections (Scott *et al*., 2007; Sun *et al*., 2011) or alterations were done to the electrodes. Anolyte and catholyte had a volume of 1200 mL. The catholyte was a 0.1 M solution of NaCl. Modifications to the cathode system were avoided (Mohan *et al*., 2008; Raghavulu *et al*., 2009; Fornero *et al*., 2010). The anolyte consisted of 80% Artificial Sewage Water (ASW) (Mathuriya and Sharma, 2010) and 20% Nutrient Broth with the microorganism in their mid log phase of their growth.

The electrical circuit was closed with a variable resistance box (Model AS-5S, Century Technologies, India). The current and power outputs were directly monitored using a source meter (Keithley 2601 System Source meter). The power density was measured along with current density by varying the external resistance from 1Ω to xx Ωover a period of 10 min at regular time intervals.

Pre run procedures: ASW was prepared according to the composition of Mathuriya and Sharma (2010), but however instead of glucose as the substrate, dextrose was used in our study. It is to be noted that different substrates have different effects on the performance of the MFC run (Chae *et al*., 2009). The ASW and the Catholyte (0.1 M NaCl) were sterilized in an autoclave (121°C and 15 Psi) before use in the MFC run. The electrodes were cleaned and washed with sand paper and 70% ethanol. The acrylic MFC setup, glass wool and glass beads were sterilized with steam and 70% ethanol. The glass wool was discarded after every MFC run and was not reused. Proper safety measures were taken while handling glass wool.

MFC run: The anode chamber of the setup was batch fed with ASW enriched by nutrient broth (80:20 v/v) containing the corresponding microbes in their mid-log phase. The electrodes were connected to a variable resistance box through copper wires. The polarization data i.e., power and current across different external resistance was measure at regular intervals over a period of 72 h using the Keithley 2601 System Source meter, under an applied potential equal to the open circuit potential at the time of measurement. Polarization curves were plotted for the four different MFCs fed by the respective isolated bacterial species.

Statistical analysis: All the data were analyzed using Microsoft Excel 2003 and Origin 6.0.

RESULTS

Microbiological aspect: Four different colored colonies, namely metallic green (1a), pink (1b), purple (1c) and colorless (1d) were observed on EMB plates. They were further differentiated on

Table 1: Cultural characteristics of the four different species (1a-1d)

Name	Nature	Color of colonies on EMB	Inference
1a	Gram negative/rods	Metallic green	Lactose consuming
1b	Gram negative/rods	Pink	Lactose consuming
1c	Gram negative/circular	Purple	Lactose consuming
1d	Gram negative/rods	Colorless	Non-lactose consuming

Table 2: Table maximum power density extracted from polarization curves in MFC inoculated with the four different bacterial species (1a-1d)

	Power density (mW m^{-2})			
Time (h)	1a	1b	1c	1d
0	12.94	184.36	9.36	53.47
24	Data not available	122.60	175.10	44.05
48	7.95	95.42	298.50	400.92
72	Data not available	13.57	2.16	Data not available

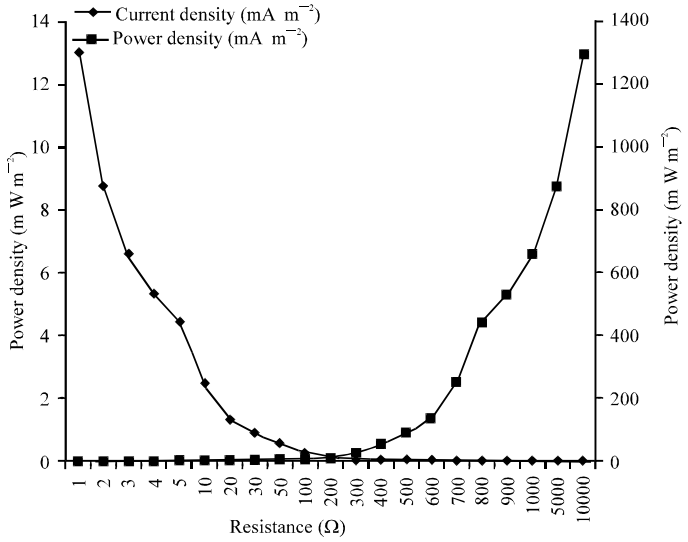

Fig. 1: The polarization plot for MFC fed with 1a

Nutrient agar plates based on the differences in their colony morphologies. Further microbiological studies revealed the following details (Table 1).

Electrochemical monitoring: The electrochemical activity of the inoculums from the anaerobic sludge was tested by running four different MFCs with the respective bacterial species (1a-1d) as the inoculums in the anode chamber. The voltage drop between the electrodes, Power generated, current and internal resistance were measured at regular intervals. The values reported in Table 2 shows the power and current density generated that were monitored at regular intervals. Polarization curves which were recorded periodically during the MFC operation by varying external resistance, showed a maximum power density of 12.939 mW m^{-2} at a current density of 1.297 A m^{-2} in (1a) run MFC (Fig. 1), 184.36 mW m^{-2} at 4.608 A m^{-2} in (1b) (Fig. 2), 298.5 mW m^{-2} at 3.508 A m^{-2} in (1c) (Fig. 3) and 400.92 mW m^{-2} at 8.024 A m^{-2} in (1d) (Fig. 4).

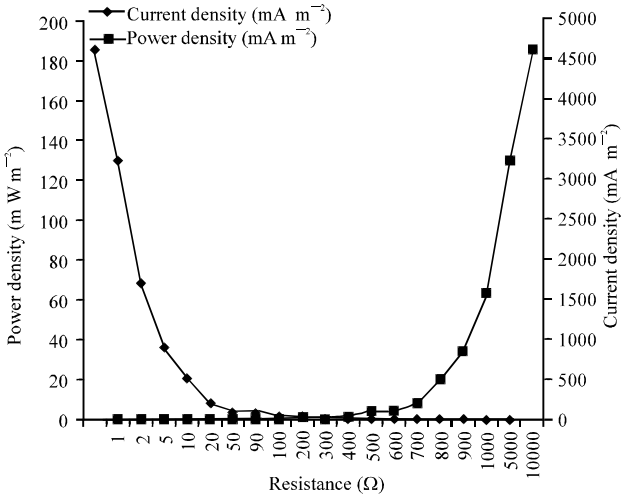

Fig. 2: The polarization plot for MFC fed with 1b

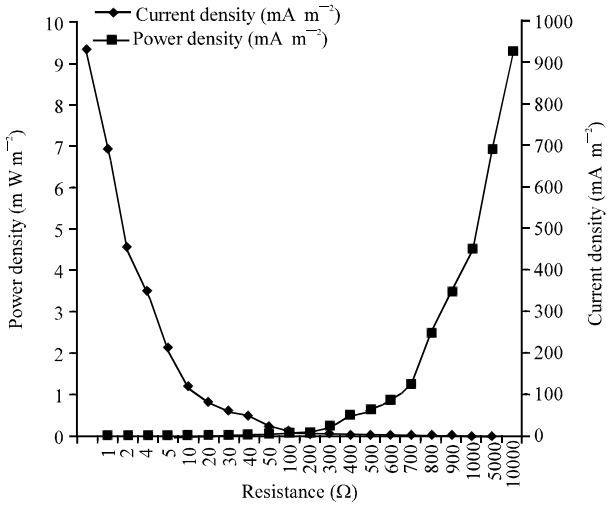

Fig. 3: The polarization plot for MFC fed with 1c

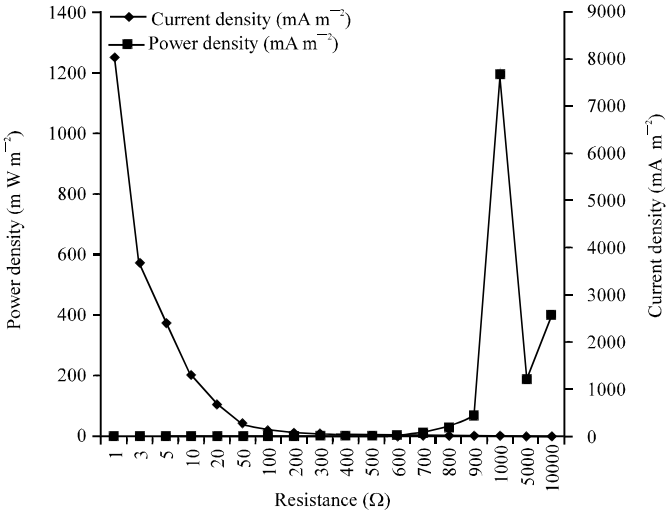

Fig. 4: The polarization plot for MFC fed with 1d

DISCUSSION

It is evident from the data in Table 2 that the four different MFC's gave a maximum power output at different time of operation. It may be attributed to that fact that the four different (1a, 1d) bacteria have significant differences in the time taken to reach a particular phase of the growth curve, once the mother inoculums of mid log phase is introduced into the anode with ASW. The above inference is an added support to the fact that the MFC performance is a function of the growth curve phase of the bacteria (Wang *et al*., 2010).

The power density values obtained in literature have been reported in the range of 30 mW m^{-2} to 3750 mW m^{-2} (Scott *et al*., 2007; Hmidet *et al*., 2009) whereas the setup used in our study gave a maximum power density of 400 mW m^{-2}. The decreased internal resistance due to usage of glass wool and glass beads can be attributed to the moderately high power density. But however the implementation of a batch operated MFC, absence of any novel cathode modifications (Forneroet *et al*., 2010; Lefebvre *et al*., 2008; You *et al*., 2008, 2009) and the low oxygen diffusion rate into the catholyte (lack of oxygen pumping system) are some of the reasons that support the not very high power output obtained. The operating conditions have a tremendous impact on the MFC operation (Martin *et al*., 2010). Scott *et al*. (2007) made use of a low-cost hydrophilic membrane (Min *et al*., 2005) to replace costly proton exchange membranes which explains the low power density. On the other hand the MFC design of Hmidet *et al*. (2009) has low electrode spacing coupled with high specific electrode surface area. The continuous feeding of carbon source and the MFC operation at an intermittent high flow explains the phenomenally high power.

The gram negative nature of the isolated EAB suggests that the absence of a thick peptidoglycan layer in the cell membrane has a tremendous influence on the electron transport to the nearby electrodes from the intracellular space. Thus indicating the possible role played by the peptidoglycan layer in the electron trafficking pathway, this still remains to be investigated completely.

The reasonable electrochemical activity of 1b and 1c finds them a potential use in making Biosensor. Being lactose consuming organisms, they can be exploited in constructing a MFC coupled lactose biosensor device to determine the lactose levels in diary processing effluents and Milk industry waste water. The power output being a function of the substrate concentration will be one of the important parameter in such a biosensor to monitor the lactose concentration since lactose is the main substrate for 1b and 1c.

In our study presented here, the electrochemical activity of four different species of gram negative bacteria was established. The high current density of the 1d run MFC shows promising possibilities of being used in electricity generation plants. The electrochemical activity of an EAB is a direct measure of the amount of sludge it can reduce. Being isolated from sewage sludge, these 1a-1d bacteria can be coupled with waste water (Kiely *et al*., 2011; Cha *et al*., 2010; Du *et al*., 2007; Oh *et al*., 2010) processing plant with the aim of reducing the organic sludge, which is carried out without any cost and also accompanied by power generation. Such multifunctional traits of MFC make it a very happening field of research currently (Pant *et al*., 2010). Based on our preliminary set of experiments, we propose the use of membrane less and mediator less MFC as pollution free power production system that is not economically demanding.

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