ELECTRICITY GENERATION IN MEDIATORLESS MICROBIAL FUEL CELL USING AGROBACTERIUM TUMEFACIENS SU-11 HAVING LACTOSE AND DAIRY WASTE AS CARBON SOURCE

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ABSTRACT

Bioelectricity generation can be done in MFC. In this study, Agrobacterium tumefaciens SU-11 two electrodes with surface area of 2.376 cm² and 69.5325 cm² and two different length salt bridge were used for electricity generation in mediator less microbial fuel cell. Two different concentrations of lactose i.e. 2% and 3 % lactose were used as carbon source. More electricity generation was found at 2 % lactose and 2.376 cm² electrode used MFC. The organism was able to produce around 450 mV while dairy wastewater was used as carbon source.

Keywords: Electricity Generation, Microbial Fuel, Agrobacterium, Tumefaciens, Carbon Source.

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1. INTRODUCTION

World energy demand is increasing day by day. Production of energy using the renewable feed stocks help in producing energy in sustainable manner and also reduce pollution. When the energy is derived out of wastewater it gives us two advantage as water can be recycled after treatment and also reduces the cost of water treatment [1]. MFC (microbial fuel cell) is a system where electrical energy is produced by biochemical reaction performed by microorganisms [2]. MFC can be used to produce energy from wastewater, thus this technology becomes revolution as two purposes are solved, one is wastewater treated and other is getting energy out of it [3]. Mohan et al [3] used mixed consortium and produced more bioelectricity. Using the mixed consortia act synergistically as the byproduct of one organism can be used by the next organism. Sometimes, it might be reason for less energy production as the other organism diverts the metabolism and no hydrogen is produced [3].

A microbial fuel cell (MFC) is consisting of anode and cathode chamber, where it is partitioned using a salt bridge or proton exchange membrane (PEM). Biochemical reaction of bacteria happens at anode and electrons generated in the reaction is transferred through a circuit to generate electricity, proton moves through the PEM to anode and forms water [4,5]. Park and Zeikus [6] incorporated dyes like neutral red and metals (such as Mn^{4+} , Fe^{3+}) to the graphite anodes and investigated. Wastewater treatment is possible with MFC [7,8]. In this work, microorganism was isolated from garden soil of Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu – 600 119, India and utilized for electricity generation in MFC having lactose /dairy waste water in anode chamber and also to evaluate electrode surface area and the length of salt bridge for better electricity generation.

2. MATERIALS AND METHOD

2.1. Isolation of Bacteria

Soil sample was subjected for serial dilution and pour plate was performed on minimal media. After 2 days of incubation at 37°C, pure culture obtained was inoculated into 50 ml of the minimal media. The above procedures were repeated three times in order to stimulate an enrichment culture. After the enrichment steps, the culture broth was streaked on nutrient agar for storage.

2.1.1. Isolation and identification of bacteria

The organism was identified by its biochemical characteristics as well as by 16SrRNA sequencing.

2.2. Microbial Fuel Cell Construction with Salt Bridge Set up and Bioelectricity Generation

The “H” shaped microbial fuel cell consists of an anaerobic anode chamber and an aerobic cathode chamber. It was made using the autoclavable plastic bottles of capacity 500 mL, which are being connected using salt bridge made of agar (saturated with NaCl) (two lengths were used, they were 16.5 cm length and 11.5 cm length). Graphite rods acted as electrodes in

both the chambers. Anaerobic condition in the anode chamber was maintained and the cathode chamber was being aerated using aerator pumps (Fig.1). Two electrodes with surface area of 2.376 cm^2 and 69.5325 cm^2 were used. Two different concentration of lactose was used in this study, i.e. 2 % lactose and 3 % lactose in the minimal media used by Samrot et al [9].

Figure 1 Microbial Fuel Cell Salt Bridge Set Up

2.3. Electricity Generation from Dairy Wastewater

2.3.1. Collection of Sewage Sample

Sewage sample was collected from Aavin, Sholinganallur, and Chennai. The sample was collected in a sterile glass container and brought to the lab and stored in 4°C till it used.

2.3.2. Electricity Generation in MFC with Salt Bridge

Collected dairy wastewater was sterilized by autoclaving and added into the anode chamber of salt bridge MFC with the electrode of size which yielded more electricity in this study was chosen. Potential difference in mV was noted for 400 h at regular time interval.

2.3.3. Substrate Analysis

BOD and COD estimation (open reflux method) was carried out with initial sample and final sample to determine the activity of microorganism. The detection of the carbohydrate was done by DNS method, Protein concentration was estimated using Lowry's method [12]. Estimation of minerals like chloride, fluoride, nitrate, iron, free chlorine and hardness of water were done using multi parameter water testing kit (HIMEDIA WT023).

3. RESULTS AND DISCUSSION

A pure culture of was isolated and it was being sequenced by 16SrRNA gene analysis, it was confirmed to be *Agrobacterium tumefaciens* (Genbank number: JQ756125).

Electricity generation by the microbe was analysed in salt bridge set up. The organism was found to produce constant potential difference in the range of 700mV to 750mV when it was incubated with 2% lactose in MFC with 16.5 cm long salt bridge. This was higher than the electricity produced by the organism in MFC with 11.5. Here the electrode size was kept constant as 2.376cm^2 (Fig.2). With the constant electrode surface area of 69.5325cm^2 and varied salt bridge length (11.5 and 16.5 cm) did not have any impact on potential difference produced by the organism (Fig.3). With lactose 3% and electrode surface area of 2.376 cm^2 , organism was found to generate constant potential difference in MFCs with varied length of salt bridge (11.5cm and 16.5cm) (Fig.4), but it was found to be fluctuating in electrode surface area of 69.5325cm^2 in both the salt bridges (11.5cm and 16.5cm) (Fig.5).

Figure 2 Electricity generation by *A. tumefaciens* in mediatorless MFC with 2% lactose (varying salt bridge length and constant electrode size of 2.376 cm²)

Figure 3 Electricity generation by *A. tumefaciens* in mediatorless MFC with 2% lactose (varying salt bridge length and constant electrode size of 69.5325 cm²)

Figure 4 Electricity generation by *A. tumefaciens* in mediatorless MFC with 3% lactose (varying salt bridge length and constant electrode size of 2.726 cm²)

Electricity Generation in Mediatorless Microbial Fuel Cell Using *Agrobacterium Tumefaciens* SU-11 Having Lactose and Dairy Waste as Carbon Source

Figure 5 Electricity generation by *A.tumefaciens* in mediatorless MFC with 3% lactose (varying salt bridge length and constant electrode size of 69.5325 cm²)

Table: 1 Analysis of Biochemical parameters of dairy waste

Sl No:	Test	Values(mg/L)	
		Before(0 hours)	After(400 hours)
1.	REDUCING SUGAR	49.313	24.2
2.	PROTEIN	250	90
3.	BOD	19.5090	6.7977
4.	COD	40	0
5.	CHLORIDE TEST	40	30
6.	FLOURIDE TEST	0.5	10
7.	NITRATE TEST	10	9
8.	IRON TEST	0.3	0.3
9.	RF CHLORINE TEST	0.0	0
10.	HARDNESS OF WATER	150	140

Thus, electrode with surface area of 2.376 cm² was chosen for the further study with treatment of dairy waste. In this study, the bacterium used was found to produce around 450 mV, where Parkash et al [10] could produce 0.4V using the dairy waste. The reducing sugar concentration was estimated for wastewater before and after the set up was run. It showed that the concentration of reducing sugar was reduced which showed that the microbes have utilized it for their survival. The COD and BOD of the sample was also estimated and the values were found to be reduced in the final sample compared to initial sample and the values are shown in Table 1. Sanjay et al [11] found that the treatment of dairy wastewater was found

to be reducing the BOD and COD of the sample. Table 1 shows that the protein content of the sample was reduced after the set up was run, which means the microbes have utilized the protein for their growth and thus the protein amount was found to be reduced in the sample. Mineral analysis was done with the initial samples and final samples, but the difference was not much significant (Table 1).

4. CONCLUSION

Two electrodes with surface area of 2.376 cm^2 and 69.5325 cm^2 and two different length salt bridge were used to find the electricity generation in mediator less microbial fuel cell having lactose as carbon source. It was found that 2 % lactose and 2.376 cm^2 electrode utilized MFC gave maximum output. Even the organism used in this study was capable of producing electricity from dairy waste which was around 450 mV.

AUTHOR'S CONTRIBUTION

All the authors involved in idea creation and made intellectual contribution to the work. All the authors involved in manuscript preparation.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

DATA AVAILABILITY

N/A

ETHICS STATEMENT

No animals or humans are used in this study. Hence, the work does not require ethical clearance.

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